CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 16(4)
2024

Technical University of Cluj Napoca
U.T.Press Publishing House

Carpathian Journal of Food Science and Technology

Print : ISSN 2066-6845
Online : ISSN 2344-5459
ISSN-L 2066-6845

Vol. 16, Nr.(4) 2024

UNIVERSITATEA TEHNICĂ DIN CLUJ-NAPOCA

Technical University of Cluj Napoca
U.T.Press Publishing House

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY
ISSN-L 2066 -6845

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CONTENT

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

ASSESSING OF MAIZE-BASED SNACKS FORMULATED WITH WHOLE AND SEEDLESS WHITE GRAPE POMACE

Silvia Mironeasa[1], Mădălina Ungureanu-Iuga[2,3]✉, Costel Mironeasa[4]

[1] Faculty of Food Engineering, Ştefan cel Mare University of Suceava,13th University Street, 720229, Suceava – Romania

[2] Institute of Advanced Studies, Integrated Research, Development and Innovation Center for Advanced Materials, Nanotechnologies and Distributed Manufacturing and Control Systems (MANSiD), "Ştefan cel Mare" University of Suceava, 13th University Street, 720229 Suceava, Romania

[3] Mountain Economy Center (CE-MONT), National Institute of Economic Research (INCE), Romanian Academy, 49 Petreni Street, 725700, Vatra Dornei, Romania

[4] Faculty of Mechanical Engineering, Automotive and Robotics, Ştefan cel Mare University of Suceava, 13th University Street, 720229 Suceava, Romania

✉ *madalina.iuga@usm.ro*

https://doi.org/10.34302/crpjfst/2024.16.4.1

Article history:
Complete by editor

Keywords:
White grape pomace
Corn snacks
Extrusion process
Optimization

ABSTRACT

Grape pomace is a winery by-product with a high biological value that can be valorized in snacks production. This work aimed to explore the impact of white grape pomace type, seedless (SGPW) and with seeds (GPW) and addition level (10-40%) on the chemical, antioxidant, color, texture, and sensory acceptability of maize snacks obtained through extrusion by means of Response Surface Methodology. Furthermore, the optimal addition level for each grape pomace type was selected and the optimal samples were characterized from a molecular point of view. The results showed that the protein, lipids, fiber, ash, total polyphenols, and antioxidant activity increased proportionally with the addition level, while the cutting force and L* decreased. The optimal samples were found to contain 29.66% SGPW or 29.22% GPW and exhibited enhanced nutritional profile and antioxidant activity compared to maize snacks used as control. The acceptability of the product was satisfactory (>7 score), which confirmed the opportunity to include these ingredients in extrusion to obtain functional snacks. The molecular characterization of the optimal samples revealed changes in absorbances intensities and the presence of additional compounds compared to the control. These results can be of real interest for producers who want to develop novel products with functional benefits and for consumers aware of a healthy diet.

1. Introduction

Extruded corn-based snacks are popular among consumers, and their consumption has raised exponentially worldwide over recent years (Asif et al., 2023). Even if they gained wide-ranging acceptability in all age groups of consumers, these snacks are often lacking protein and fiber, having a deficiently nutritional content (Ačkar et al., 2018). Also, they present a high glycemic index due to high starch and sugar content. The World Health Organization (WHO) has recommended reducing the sugar and carbohydrate contents in such products with high glycemic index. One way to combat the negative consumers health effects of snacks is the addition of vegetable byproducts, recognized as sources of valuable ingredients.

Extrusion technology is promising in developing customized ready-to-eat foods like snacks products, and it has future potential to

incorporate functional ingredients, and can contribute to sustainable food production by utilizing byproducts of food industries (Mandliya et al., 2024). Researchers have explored the incorporation of various vegetable byproducts into extrudates. One of this is grape pomace generated by the wine industry which possesses several health and technological benefits (Iuga and Mironeasa, 2020; Lavelli et al., 2016). The chemical composition of the different anatomical parts of the grape byproducts, of the species *Vitis Vinifera* L. depends on the variety, cultivation system, soil, and climatic conditions (Radulescu et al., 2023; Iuga et al., 2019), and also on the winemaking process, the pomace obtained after mechanical pressing having a different composition than that obtained after fermentation. The chemical composition of whole grape pomace differs from that of seedless grape pomace. Whole grape pomace is considered a source of dietary fiber, consisting from cell walls polysaccharides, such as hemicelluloses and cellulose, and of pectin and lignin; it also contains protein, fat, minerals, and bioactive compounds such as phenols (Beres et al., 2016; Iuga and Mironeasa, 2020). Various phenolic fractions were identified in matrix of the grape pomace fibers, which makes fiber have antioxidant properties (Saura-Calixto, 1998; Zhu et al., 2015). After wine making, approximately 70% of the phenolics remain in pomace, depending on the variety, the most important being tannins, phenolic acids, anthocyanins, and resveratrol (de la Cerda-Carrasco et al., 2015; Sousa et al., 2014). Grape seeds constituents are represented by fiber, oils, proteins, phenolic compounds, minerals, vitamins, sugars, organic acids, etc. The compounds with antioxidant properties are more abundant in grape seeds than in grape peels and the main phenols identified are flavanols, procyanidins, and phenolic acids (Iuga and Mironeasa, 2020; Tang et al., 2018). The nutritional value of maize-based snacks can be improved via the addition of bioactive compounds, fibers, and other beneficial nutrients of the white grape pomace. Studies on the addition of vegetable pomace into extruded products revealed that raised pomace levels beyond a certain level had a negative impact on the expansion quality parameters (Altan et al., 2009; Bibi et al., 2017; Selani et al., 2014). Recent studies indicated that chokeberry pomace added at a 20% level determined an increase in the content of dietary fiber, ash, total phenolic compounds, phenolic acids, flavonoids, flavanols, anthocyanins, and antioxidant activity, and didn't worsen the physical properties, hardness, and expansion ratio (Gumul et al., 2023). The incorporation of 10% tomato pomace in corn-based extrudates caused a significant reduction of fat and carbohydrate contents of the extrudates, whereas protein, ash, and fiber were remarkably enhanced (Jabeen et al., 2022). The radial expansion ratio decreased at 15% inclusion of cherry pomace into corn starch extrudates, but the extrusion process did not reduced the total phenolic content (Wang et al., 2017).

Consequently, with the tendency of healthier gluten-free snacks and sustainable development, the partial substitution of maize flour with grape pomace using an extrusion cooking process represents a proper alternative to formulating new snacks. This work aimed to develop newly expanded snacks by extrusion cooking of mixtures from maize flour and whole and seedless white grape pomace at different ratios and evaluate the products from nutritional and sensorial points of view.

2. Materials and methods

2.1. Materials

2.2.1. Samples

The white grape pomace obtained from a Romanian research institute was conditioned to obtain a moisture content of <10%. For seedless grape pomace (SGPW) the seeds were extracted manually from the whole pomace (GPW). Maize flour was provided by a Romanian producer. SGPW or GPW (10-40%) was mixed with maize flour and the moisture was adjusted to 15% (wet basis).

A laboratory single-screw extruder (Kompakt extruder KE 19/25, Brabender,

Duisburg, Germany) was used for extrusion. The barrel has a 19 mm diameter, a length-to-diameter ratio of 25:1, and a 2 mm nozzle diameter. Snacks were obtained at a constant feeding speed of 24 rpm and a screw speed of 150 rpm. The temperatures in the four zones were 50°C, 95°C, 175°C, and 180°C. After cooling (16 h) the final product was packed in polyethylene bags.

2.2. Methods

2.2.1. Chemical profile

The chemical profile of the snacks was determined according to standard protocols as follows: ash content was measured according to SR ISO 2171:2023, protein content following SR EN ISO 20483:2014, and lipid content using SR 91:2007. Total dietary fiber was determined with a Megazyme kit (K-TDFR-200a 04/17) according to the AACC 32-05.01 guidelines.

2.2.2. Total polyphenols content (TPC) and DPPH antiradical activity

The extract was prepared by mixing the sample (1g) with a solution containing 70% acetone, 28% water, and 2% acetic acid (v/v/v) (10 mL), followed by ultrasonication (1h), and the supernatant was retained. Two extractions were performed and the liquid phases obtained were mixed.

The total polyphenol content (TPC) was measured by the Folin-Ciocalteu method (FAO/IAEA, 2000). The extract (0.2 mL) was mixed with distilled water (0.8 mL), Folin–Ciocalteu reagent 1N (0.5 mL), and sodium carbonate 20% (2.5 mL). Samples were kept in the darkness for 40 min and the absorbance was measured at 725 nm using a Shimadzu 3600 UV-Vis-NIR spectrophotometer (Tokyo, Japan), with gallic acid (GAE) used as a standard.

The antiradical activity (DPPH) was measured by mixing 0.5 mL extract with 0.5 mL of 80% methanol and was added to 5 mL of DPPH solution. After keeping the mix in the darkness at 25°C for 30 min, the absorbance was read at 517 nm, with gallic acid as a standard.

2.2.3. Rapid (RDS), slowly (SDS) digestible and resistant (RS) starch

The measurements of rapid, slowly digestible, and resistant starch were done according to the international AOAC 2017.16 method, using a Megazyme kit (K-DSTRS; Megazyme, Bray, Ireland).

2.2.4. Snacks color

The color properties (lightness – L*, red or green hue – a*, yellow or blue hue – b*) were measured using a CR-400 chromameter (Konica Minolta, Tokyo, Japan).

2.2.5. Texture maximum force at cutting

The cutting force (CF) of the snacks was measured using a TVT 6700 texturometer (Perten Instruments, Hägersten, Sweden), with a Warner-Bratzler shear blade probe. The measurement was made at a speed of 1 mm/s until the sample was completely broken.

2.2.6. Sensory acceptability

The acceptability of the snacks was investigated by 65 semi-trained panelists. Explanations about the 9-points scale used in the evaluation and a brief training were made before product tasting. Water was used as a neutralizer before each test.

2.2.7. FT-IR spectra

The molecular profile of the optimal snacks was assessed by Fourier transform infrared (FTIR) spectroscopy using a Thermo Scientific Nicolet iS20 spectrophotometer (Waltham, MA, USA) in attenuated total reflection (ATR) mode. The spectra were collected in the 4000 to 400 cm^{-1} range, with a resolution of 4 cm^{-1} and 32 scans.

2.2.8. Experimental design and statistics

The effects of the addition level (A) at 4 levels (10, 20, 30, 40%) and type of white grape pomace (B) at 2 levels (seedless - SGPR and whole - GPR) on maize snacks characteristics (protein, ash, lipids, fibers content, TPC, DPPH, SDS, RDS, RS, L*, a*, b*, acceptability and cutting force) were investigated by using response surface methodology (RSM) by means of a D-optimal design. The actual and coded factors values of the design are shown in Table 1. Three repetitions for each experiment were included.

The experimental data for each response was fitted to a polynomial cubic or quadratic regression equation. Model adequacy was evaluated by using a sequential *F*-test, coefficients of determination (R^2), adjusted coefficients of determination ($Adj.\text{-}R^2$), and significant probabilities. The significance of the coefficients of the models was evaluated by using ANOVA at a confidence level of 95%.

The experimental design and the optimization were carried out by using Stat-Ease Design-Expert software (trial version). To determine the optimal levels of the factors, multiple response analysis was applied to the fitted predictive models, and the desirability function was utilized. For this purpose, the following conditions were established for the responses: the protein, ash, a* and b* were kept in range, the lipids, fiber, TPC, DPPH, SDS, RS, acceptability, and L* were maximized, and the RDS and cutting force were minimized.

Table 1. Actual and coded values of the factors

Run	Actual		Coded	
	A-type	B-level (%)	A-type	B-level
1	SGPW	40	{ -1 }	1.000
2	SGPW	20	{ -1 }	-0.333
3	SGPW	30	{ -1 }	0.333
4	GPW	10	{ 1 }	-1.000
5	SGPW	20	{ -1 }	-0.333
6	GPW	30	{ 1 }	0.333
7	SGPW	20	{ -1 }	-0.333
8	GPW	30	{ 1 }	0.333
9	GPW	20	{ 1 }	-0.333
10	GPW	10	{ 1 }	-1.000
11	GPW	40	{ 1 }	1.000
12	SGPW	10	{ -1 }	-1.000
13	GPW	30	{ 1 }	0.333
14	SGPW	30	{ -1 }	0.333
15	GPW	40	{ 1 }	1.000
16	GPW	20	{ 1 }	-0.333
17	SGPW	40	{ -1 }	1.000
18	SGPW	30	{ -1 }	0.333
19	SGPW	10	{ -1 }	-1.000
20	GPW	20	{ 1 }	-0.333
21	SGPW	10	{ -1 }	-1.000
22	SGPW	40	{ -1 }	1.000
23	GPW	40	{ 1 }	1.000
24	GPW	10	{ 1 }	-1.000

XL STAT was used for means comparison between the optimal and control samples by using ANOVA with the Tukey test ($p < 0.05$).

3. Results and discussions

3.1. Influence of factors on snacks quality

The influence of factors on the responses studied is presented in Table 1. The models used for data fitting were suitable, with a R^2 value >84%. DPPH antiradical activity, RS, and CF were fitted to the quadratic model, while the other responses (protein, ash, lipids, fibers, TPC, RDS, SDS, acceptability, L*, a*, and b*) were fitted to the cubic model. All the models proposed had a significance level of $p < 0.01$.

Table 2. ANOVA results for the cubic or quadratic model fitted for the extruded snacks properties

Factor	Protein (%)	Ash (%)	Lipids (%)	Fibers (%)	TPC (mg GAE/g)	DPPH (%)	RDS (%)	SDS (%)	RS (%)	Acceptability	L*	a*	b*	CF (g)
Const.	9.35	1.39	1.73	14.38	31.71	94.51	36.06	2.46	3.23	7.04	57.68	6.86	12.12	631.63
A-Type	0.22**	-0.04**	0.51**	0.25**	3.15**	0.05	0.82**	0.85**	0.94**	-0.01	-1.36**	0.42**	0.38**	30.02*
B-Level	1.11**	0.69**	1.07**	8.61**	-0.02	0.63*	6.41**	-0.07	-0.26**	0.62**	-1.63**	0.12	0.14	-166.26**
AB	0.14**	0.05**	0.33**	0.07	2.76**	-3.15**	-2.15**	-0.17**	0.18**	-0.06*	-0.55**	0.27**	0.54**	61.04**
B^2	-0.02	-0.13**	0.12**	-1.09**	-8.09	-4.42**	1.65**	0.06**	-0.23**	-0.14**	-0.18	-0.14*	0.28**	56.60
AB^2	-0.23**	0.05**	-0.17**	-0.73**	-8.01**		-1.61**	0.04*		0.08*	1.40**	-0.05	0.12	
B^3	-0.19**	-0.23**	-0.01**	-0.13	3.98**		-1.98**	-0.26**		-0.52**	-2.06**	0.68**	-0.67**	
***p*-value**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R^2	0.99	0.99	0.99	0.98	0.98	0.91	0.98	0.99	0.99	0.86	0.98	0.97	0.95	0.84
Adj.-R^2	0.99	0.99	0.99	0.98	0.98	0.87	0.97	0.99	0.99	0.82	0.98	0.96	0.94	0.81

* - significant at $p < 0.05$, ** - significant at $p < 0.01$

Figures 1-5 confirmed that the predicted data generated by the RSM cubic and quadratic models align well with the actual experimental data, demonstrating a satisfactory correlation between the two. Consequently, the developed models are suitable for predicting the optimal composite extruded snacks properties based on the factors considered.

The addition level of white grape pomace resulted in significant increases in protein, ash, lipids, fibers, TPC, and DPPH proportional with the amount used. The experimental data *vs.* the predicted ones for these responses are displayed in Figure 1. The results are in agreement with previous research which highlighted the enrichment in protein, ash, and especially fiber of wheat snacks as the amount of grape pomace was higher (Alshawi, 2024). The nutritional composition of grape pomace depends on the variety and the presence of seeds (Martins et al., 2017). The increase in dietary fiber content is due to the grape pomace composition formed mainly by fibers like cell wall polysaccharides and lignin (Martins et al., 2017). Pérez-Jiménez et al. (2008) stated that the dietary fiber from grape pomace has a reduction effect on the lipid profile and blood pressure much higher compared to other fiber sources like oat, psyllium due to the synergistic effect of fiber with antioxidant compounds.

Factor A (pomace type) and B (level) influenced significantly ($p < 0.05$) the protein, ash, lipids, and fibers, while their interaction has effects only on protein, ash, and lipids content (Table 1). TPC was significantly affected by A and the interactions between factors, while the DPPH antioxidant activity was influenced only by B factor and the interaction with A. Mildner-Szkudlarz et al. (2012) reported a tenfold increase of polyphenols and a considerably higher DPPH scavenging activity in biscuits enriched with 30% grape pomace. The authors affirmed that the presence of phenols like gallic acid and catechin from grape pomace determined the improvement of antioxidant activity since these compounds have a great antioxidant power (Mildner-Szkudlarz et al., 2012).

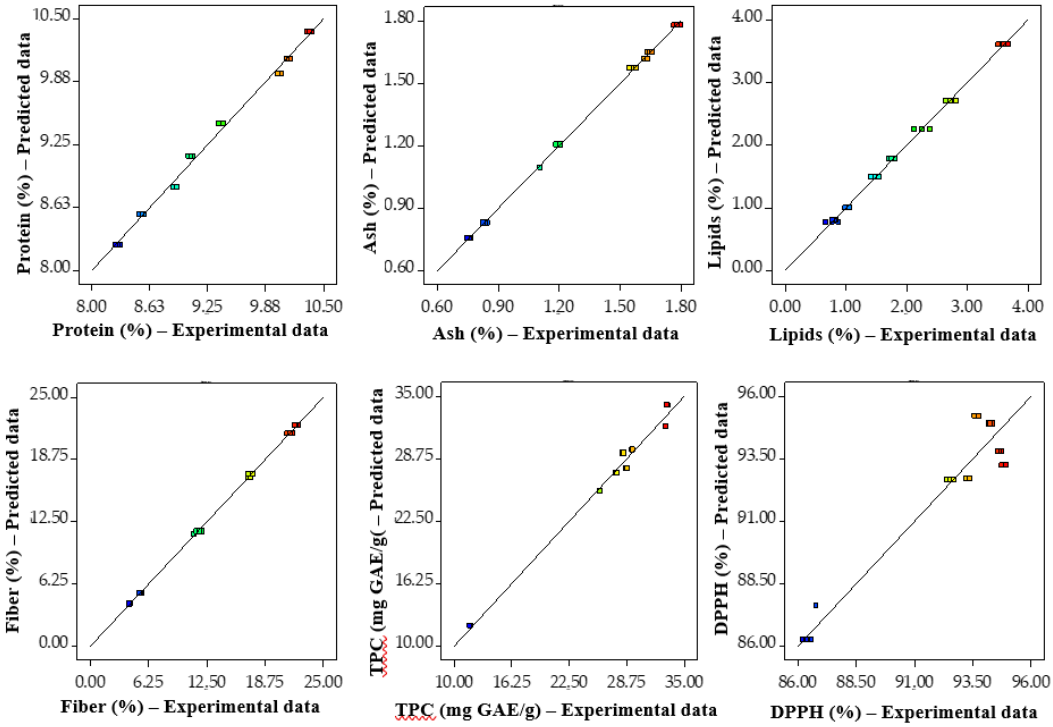


Figure 1. Predicted *vs.* experimental values for the proximate composition, total polyphenols and antioxidant activity

The strong antioxidant activity of gallic acid is given by the inductive effects of its 3 hydroxyl groups (Sánchez-Moreno et al., 1999). Grape pomace polyphenols accomplish the structural criteria for strong antioxidant activity because they contain "either a 3-OH group on an unsaturated C ring, a 2,3-double bond with the 3-OH group and 4-one in the C ring, or an ortho-OH substitution pattern in the B ring, where the OH groups are not glycated" (Rice-Evans et al., 1996). Grape pomace level led to the decrease of SDS and RS content, while the RDS increased. Both factors and their interaction affected ($p < 0.05$) RDS and RS variation, while SDS was not influenced by the addition ($p > 0.05$) level (Table 1). The experimental data *vs.* the predicted ones for starch fractions are displayed in Figure 2. The changes in starch fraction content can be attributed to interactions between polyphenols and starch (Camelo-Méndez et al., 2017). Polyphenols from grape pomace can modify the starch structure and thus its digestibility can be modified (Chi et al., 2017; Sun Miao, 2020).

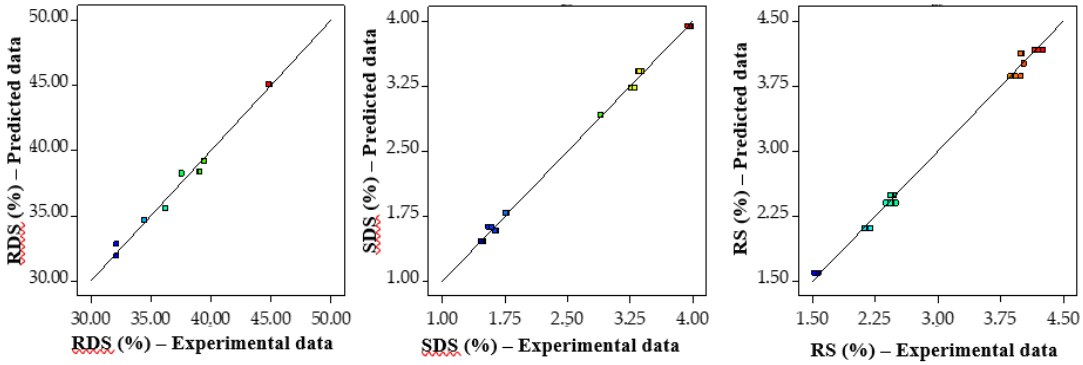


Figure 2. Predicted *vs.* experimental values for the starch fractions

All the color parameters (L*, a*, and b*) were significantly changed ($p < 0.05$) depending on the grape pomace type and the interaction between factors, while the addition level influenced only L* (Table 1). L* and b* values decreased proportional with the addition level increase, while a* increased. Figure 3 represents the dependence of the experimental values *vs.* the predicted ones. Bender et al. (2017) reported intensification of darkness and changes of a* and b* values when white seedless grape pomace was added to muffins. Final product color is influenced by polyphenol oxidase activity, quantity of polyphenols, pH, and ionic linkage strength (Manoj Kumar et al., 2019).

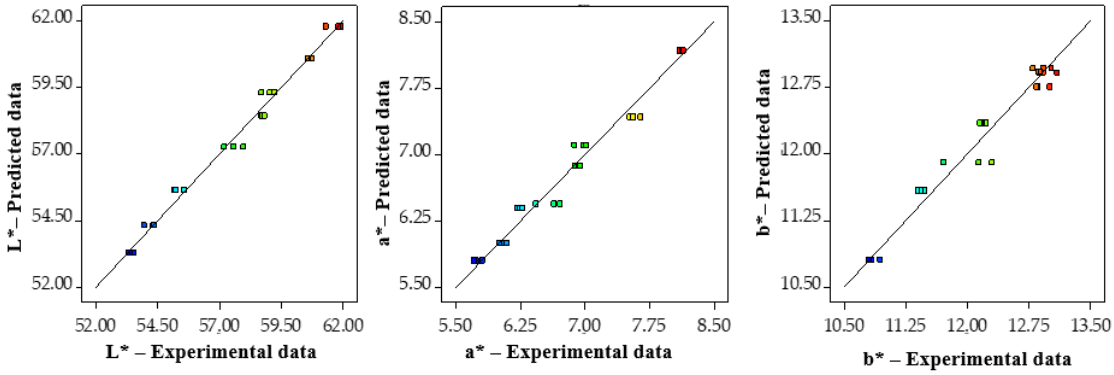


Figure 3. Predicted *vs.* experimental values for the color properties

B factor and the interaction between A and B determined significant variations ($p < 0.05$) of acceptability and CF (Table 1). Grape pomace type (A) has not a significant effect on acceptability ($p > 0.05$). At addition levels up to 30%, the acceptability increased with the raise of the amount incorporated. CF registered a decreasing trend depending on the addition level. A previous study reported a linear reduction of the cut force of rice snacks when cashew apple pomace was added (Preethi et al., 2021). It was stated that the texture of snacks depends on the moisture content, cavity space, sample diameter, compactness of pores, and pore wall strength (Pandiselvam et al., 2019; Preethi et al., 2021). The structural integrity of corn snacks could have been impacted by the interactions between grape pomace and protein which determined changes in cutting force values.

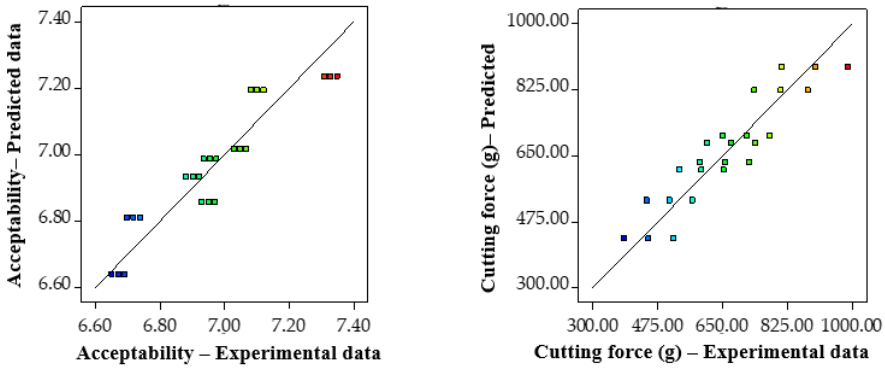


Figure 4. Predicted *vs.* experimental values for the acceptability and cutting force (CF)

Our results regarding product acceptability were in line with those obtained by Kakaei et al. (2019) for corn snacks with pomegranate seeds. They found an increase in overall acceptability probably due to the flavor compounds from grape pomace and/or to the aromatic substances resulting from the Maillard reactions (Kakaei et al., 2019).

The chemical components (protein, ash, lipids, and fibers) were positively correlated among them (Figure 5) and with RDS content. L* and cutting force were negatively correlated with the chemical components listed above.

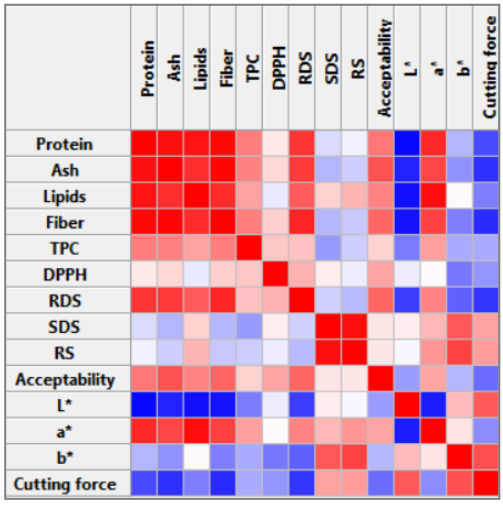


Figure 5. Correlations between the variables (red – positive correlations, blue – negative correlations)

3.2. Optimization

To obtain the optimal addition level for each type of grape pomace, the desirability function was applied. The optimization process revealed that 29.66% SGPW and 29.22% GPW are suitable doses to produce a good quality snack (Table 2). The content of protein, ash, lipids, fiber, SDS, RS, and DPPH was enhanced in the optimal samples compared to the control. The highest TPC level was observed in the O_GPW optimal sample. These results were expected since grape pomace is rich in fibers and polyphenols with antioxidant activity compared to maize flour (Beres et al., 2016; Iuga and Mironeasa, 2020).

Table 2. Characteristics of the optimal samples (predicted values) *vs.* the control

Property	O_SGPW		O_GPW		M	
	Mean	SD	Mean	SD	Mean	SD
Level (%)	29.66	-	29.22	-	0.00	-
Protein (%)	9.44^{b}	0.06	9.91^{a}	0.06	7.61^{c}	0.03
Ash (%)	1.61^{a}	0.01	1.54^{b}	0.01	0.45^{c}	0.01
Lipids (%)	1.48^{b}	0.08	2.64^{a}	0.08	0.35^{c}	0.05
Fiber (%)	16.74^{a}	0.24	16.94^{a}	0.24	0.46^{b}	0.03
TPC (mg GAE/g)	27.80^{b}	0.88	34.44^{a}	0.88	28.85^{b}	0.04
DPPH (%)	95.20^{a}	1.07	93.50^{a}	1.07	38.04^{b}	0.10
RDS (%)	38.15^{a}	0.60	38.05^{a}	0.60	30.03^{b}	0.03
SDS (%)	1.62^{b}	0.05	3.24^{a}	0.05	0.88^{c}	0.03
RS (%)	2.11^{b}	0.07	4.13^{a}	0.07	1.21^{c}	0.01
Acceptability	7.23^{a}	0.08	7.17^{a}	0.08	-	-
L*	58.49^{b}	0.36	55.75^{c}	0.36	78.48^{a}	0.12
a*	6.39^{b}	0.15	7.40^{a}	0.15	0.71^{c}	0.03
b*	11.60^{c}	0.18	12.71^{b}	0.18	21.75^{a}	0.20
CF (g)	536.36^{b}	66.06	636.48^{b}	66.06	1625.43^{a}	147.86

Mean values followed by different letters are significantly different ($p < 0.05$)

The control snack was lighter and had lower a* and higher b* values compared to the optimal samples. These color changes depend on the pigments of the ingredients added and on their chemical composition, especially sugars and amino acids which promote Maillard and other browning reactions. The control sample presented significantly higher cutting force compared to the optimal ones. The presence of fiber from grape pomace likely has a major impact on snacks structure, leading to a lower force needed to break the sample (Różańska-Boczula et al., 2023).

3.3. Characterization of the optimal samples

The characterization of the optimal and control samples from a molecular point of view is presented in Figure 6. Control snacks exhibited the lowest absorbances. The highest absorbances were observed for the O_GPW sample. Compared to the control, the optimal samples exhibited additional peaks at 1106 and 2856 cm^{-1}. The peak at 2856 cm^{-1} is given by the stretching vibration of CH_2 and could indicate the presence of grape pomace cutin, waxes, and cutan (Nogales-bueno et al., 2017). In the study of (Nogales-bueno et al., 2017), the peak at 2924 cm^{-1} was attributed to the C–H stretching vibration of grape pomace structure, while the band at 1741 cm^{-1} was assigned to the stretching vibration of carbonyl (C = O). The protein presence was indicated by the peaks at the protein bands 1647 and 1544 cm^{-1} (Amador-Rodríguez et al., 2019).

The following absorption bands were observed: "Amide II (an N-H bending vibration couples to C-N stretching) (1480–1575 cm^{-1}); N-H bending vibration of primary amines (1580–1650 cm^{-1}); Amide I absorption (predominantly the C=O) (1600–1700 cm^{-1}), and the C=O stretching of triglycerides or alkali ester (pectin) (1745–1740 cm^{-1})" (Amador-Rodríguez et al., 2019), similar with previous reported results. The presence of pectin and cellulose from grape pomace was indicated by the peaks at 1015 cm^{-1} given by the C-O and C-C stretching vibration and 1245 cm^{-1}

given by the C-O stretching vibration (Amador-Rodríguez et al., 2019).

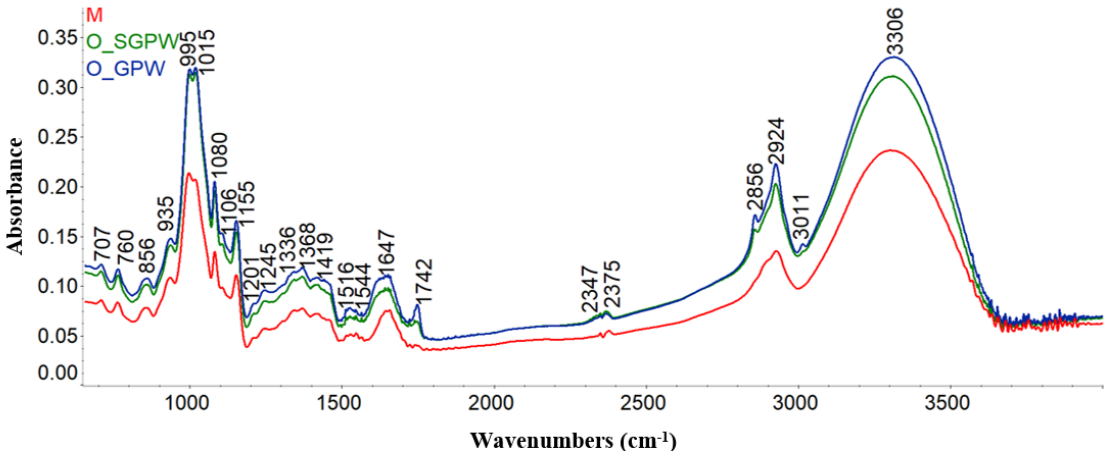


Figure 6. FT-IR spectra of the optimal and control samples

4. Conclusions

Grape pomace valorization in considerable amounts in maize snacks was proved to be feasible since the optimal samples containing 29.66% SGPW or 29.22% GPW presented good acceptability and enhanced nutritional profile. Compared to the maize snacks, the products enriched with grape pomace showed higher nutrients, total polyphenols, and antioxidant activity which may be associated with health benefits. The color and texture of the snacks changed due to the characteristics of the ingredients added, but the acceptability of the product remained in good limits (>7 from 9 points). In conclusion, this paper demonstrated that seedless and whole grape pomace derived from white grape variety processing for wine can be used as an ingredient in snacks production. Further researches regarding the impact of these ingredients on the rheological properties of the mixtures could be assessed.

5. References

Ačkar, Đ., Jozinović, A., Babić, J., Miličević, B., Balentić, J. P., Šubarić, D. (2018). Resolving the problem of poor expansion in corn extrudates enriched with food industry by-products. *Innovative Food Science Emerging Technologies*, 47, 517–524.

Alshawi, A. H. (2024). Enriching wheat flour with grape pomace powder impacts a snack's chemical, nutritional, and sensory characteristics. *Czech Journal of Food Sciences*, 42(4), 243–250. https://doi.org/10.17221/103/2024-cjfs

Altan, A., McCarthy, K. L., Maskan, M. (2009). Effect of extrusion cooking on functional properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. *Journal of Food Science*, 74(2), E77–E86.

Amador-Rodríguez, K. Y., Silos-Espino, H., Valera-Montero, L. L., Perales-Segovia, C., Flores-Benítez, S., Martínez-Bustos, F. (2019). Physico-chemical, thermal, and rheological properties of nixtamalized creole corn flours produced by high-energy milling. *Food Chemistry*, 283, 481–488. https://doi.org/10.1016/j.foodchem.2019.01.044

Asif, M., Khan, M. K. I., Khan, M. I., Maan, A. A., Helmick, H., Kokini, J. L. (2023). Effects of citrus pomace on mechanical, sensory, phenolic, antioxidant, and gastrointestinal index properties of corn extrudates. *Food Bioscience*, 55, 103012.

Bender, A. B. B., Speroni, C. S., Salvador, P. R., Loureiro, B. B., Lovatto, N. M., Goulart, F. R., Lovatto, M. T., Miranda, M. Z., Silva, L. P., Penna, N. G. (2017). Grape Pomace Skins and the Effects of Its Inclusion in the Technological Properties of Muffins.

Journal of Culinary Science and Technology, 15(2), 143–157. https://doi.org/10.1080/15428052.2016.1225535

Beres, C., Simas-Tosin, F. F., Cabezudo, I., Freitas, S. P., Iacomini, M., Mellinger-Silva, C., Cabral, L. M. C. (2016). Antioxidant dietary fibre recovery from Brazilian Pinot noir grape pomace. *Food Chemistry*, 201, 145–152.

Bibi, S., Kowalski, R. J., Zhang, S., Ganjyal, G. M., Zhu, M. J. (2017). Stability and functionality of grape pomace used as a nutritive additive during extrusion process. *Journal of Food Processing and Technology*, 8(7). https://doi.org/10.4172/2157-7110.1000680

Camelo-Méndez, G. A., Agama-Acevedo, E., Tovar, J., Bello-Pérez, L. A. (2017). Functional study of raw and cooked blue maize flour: Starch digestibility, total phenolic content, and antioxidant activity. *Journal of Cereal Science*, 76, 179–185. https://doi.org/10.1016/j.jcs.2017.06.009

Chi, C., Li, X., Zhang, Y., Chen, L., Li, L., Wang, Z. (2017). Digestibility and supramolecular structural changes of maize starch by non-covalent interactions with gallic acid. *Food and Function*, 8(2), 720–730. https://doi.org/10.1039/c6fo01468b

de la Cerda-Carrasco, A., López-Solís, R., Nuñez-Kalasic, H., Peña-Neira, Á., Obreque-Slier, E. (2015). Phenolic composition and antioxidant capacity of pomaces from four grape varieties (Vitis vinifera L.). *Journal of the Science of Food and Agriculture*, 95(7), 1521–1527.

Gumul, D., Berski, W., Zieba, T. (2023). The influence of Fruit Pomaces on Nutritional, Pro-Health Value and Quality of Extruded Gluten-Free Snacks. *Applied Sciences*, 13(8), 4818. https://doi.org/10.3390/app13084818

Iuga, M. and Mironeasa, S. (2020). Potential of grape byproducts as functional ingredients in baked goods and pasta. *Comprehensive Reviews in Food Science and Food Safety*, *19*(5), 2473-2505. https://doi.org/10.1111/1541-4337.12597

Iuga, M., Mironeasa, C., & Mironeasa, S. (2019). Oscillatory rheology and creep-recovery behaviour of grape seed-wheat flour dough: effect of grape seed particle size, variety, and addition level, *Bulletin UASVM Food Science and Technology*, 76(1), 40–51. https://doi.org/10.15835/buasvmcn-fst:2018.0020.

Jabeen, A., Naik, H., Jan, N., Hussain, S. Z., Amin, T., Rafiq, A. (2022). Studying the effect of tomato pomace incorporation on physicochemical, nutritional, and storage characteristics of corn-based extrudates using response surface approach. *British Food Journal*, 124(11), 3705–3723. https://doi.org/10.1108/BFJ-05-2021-0483

Kakaei, K., Noshad, M., Nasehi, B., Hojjati, M., Beiraghi-Toosi, S. (2019). Optimization of Physicochemical Characteristics of Corn-Based Extruded Snacks Containing Pomegranate Seed Powders. *Nutrition and Food Sciences Research*, 6(1), 35–40.

Lavelli, V., Torri, L., Zeppa, G., Fiori, L., Spigno, G. (2016). Recovery of winemaking by-products for innovative food application. *Italian Journal of Food Science*, 28, 542–564.

Mandliya, S., Vishwakarma, S., Bora, M., Bag, S. K., Dalbhagat, C. G., Mishra, H. N. (2024). Ready-to-Eat Foods Development through Composite Extrusion Technological Development. In *Recent Advances in Ready-to-Eat Food Technology* (pp. 35–51). CRC Press.

Manoj Kumar, C. T., Sabikhi, L., Singh, A. K., Raju, P. N., Kumar, R., Sharma, R. (2019). Effect of incorporation of sodium caseinate, whey protein concentrate, and transglutaminase on the properties of depigmented pearl millet based gluten free pasta. *LWT-Food Science and Technology*, 103, 19–26. https://doi.org/10.1016/j.lwt.2018.12.071

Martins, Z. E., Pinho, O., Ferreira, I. M. P. L. V. O. (2017). Food industry by-products used as functional ingredients of bakery products.

Trends in Food Science and Technology, 67. https://doi.org/10.1016/j.tifs.2017.07.003

Mildner-Szkudlarz, S., Bajerska, J., Zawirska-wojtasiak, R., Gorecka, D. (2012). White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *Journal of Science of Food and Agriculture*, 93(2), 389-395. https://doi.org/10.1002/jsfa.5774

Nogales-Bueno, J., Baca-Bocanegra, B., Rooney, A., Hernández-hierro, J. M., José, F., Byrne, H. J. (2017). Talanta Linking ATR-FTIR and Raman features to phenolic extractability and other attributes in grape skin. *Talanta*, 167, 44–50. https://doi.org/10.1016/j.talanta.2017.02.008

Pandiselvam, R., Manikantan, M. R., Sunoj, S., Sreejith, S., Beegum, S. (2019). Modeling of coconut milk residue incorporated rice-corn extrudates properties using multiple linear regression and artificial neural network. *Journal of Food Process Engineering*, 42(2), e12981. https://doi.org/10.1111/jfpe.12981

Pérez-Jiménez, J., Serrano, J., Tabernero, M., Arranz, S., Díaz-Rubio, M. E., García-Diz, L., Goñi, I., Saura-Calixto, F. (2008). Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. *Nutrition*, 24(7–8), 646–653. https://doi.org/10.1016/j.nut.2008.03.012

Preethi, P., Mangalassery, S., Shradha, K., Pandiselvam, R., Manikantan, M. R., Reddy, S. V. R., Devi, S. R., Nayak, M. G. (2021). Cashew apple pomace powder enriched the proximate, mineral, functional and structural properties of cereal based extrudates. *LWT-Food Science and Technology*, 139, 110539. https://doi.org/10.1016/j.lwt.2020.110539

Radulescu, C., Buruleanu, L. C., Olteanu, R. L., Nicolescu, C. M., Bumbac, M., Gorghiu, L. M., Nechifor, M. D. (2023). *Grape by-Products: Potential Sources of Phenolic Compounds for Novel Functional Foods*, Intech Open: London, UK. Available online: https: //www.intechopen.com/online-first/88679 (accessed on 2 September 2024).

Rice-Evans, C. A., Miller, N. J., Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. In *Free Radical Biology and Medicine* 20(7), 933-956. https://doi.org/10.1016/0891-5849(95)02227-9

Różańska-Boczula, M., Wójtowicz, A., Piszcz, M., Soja, J., Lewko, P., Ignaciuk, S., Milanowski, M., Kupryaniuk, K., Kasprzak-Drozd, K. (2023). Corn-Based Gluten-Free Snacks Supplemented with Various Dried Fruits: Characteristics of Physical Properties and Effect of Variables. *Applied Sciences*, 13(19), 10678. https://doi.org/10.3390/app131910678

Sánchez-Moreno, C., A. Larrauri, J., Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Research International*, 32(6), 407–412. https://doi.org/https://doi.org/10.1016/S0963-9969(99)00097-6

Saura-Calixto, F. (1998). Antioxidant dietary fiber product: a new concept and a potential food ingredient. *Journal of Agricultural and Food Chemistry*, 46(10), 4303–4306.

Selani, M. M., Brazaca, S. G. C., Dos Santos Dias, C. T., Ratnayake, W. S., Flores, R. A., Bianchini, A. (2014). Characterisation and potential application of pineapple pomace in an extruded product for fibre enhancement. *Food Chemistry*, 163, 23–30. https://doi.org/10.1016/j.foodchem.2014.04.076

Sousa, E. C., Uchôa-Thomaz, A. M. A., Carioca, J. O. B., Morais, S. M. de, Lima, A. de, Martins, C. G., Alexandrino, C. D., Ferreira, P. A. T., Rodrigues, A. L. M., Rodrigues, S. P. (2014). Chemical composition and bioactive compounds of grape pomace (Vitis vinifera L.), Benitaka variety, grown

in the semiarid region of Northeast Brazil. *Food Science and Technology*, 34, 135–142.

Sun, L., Miao, M. (2020). Dietary polyphenols modulate starch digestion and glycaemic level: a review. In *Critical Reviews in Food Science and Nutrition*, 60(4), 541-555. https://doi.org/10.1080/10408398.2018.1544883

Tang, G.-Y., Zhao, C.-N., Liu, Q., Feng, X.-L., Xu, X.-Y., Cao, S.-Y., Meng, X., Li, S., Gan, R.-Y., Li, H.-B. (2018). Potential of grape wastes as a natural source of bioactive compounds. *Molecules*, 23(10), 2598.

Wang, S., Kowalski, R. J., Kang, Y., Kiszonas, A. M., Zhu, M.-J., Ganjyal, G. M. (2017). Impacts of the particle sizes and levels of inclusions of cherry pomace on the physical and structural properties of direct expanded corn starch. *Food and Bioprocess Technology*, 10, 394–406.

Zhu, F., Du, B., Zheng, L., Li, J. (2015). Advance on the bioactivity and potential applications of dietary fibre from grape pomace. *Food Chemistry*, 186, 207–212.

Acknowledgment

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS - UEFISCDI, project number PN-III-P4-PCE-2021-0718, within PNCDI III.

The results were first used in a patent application entitled “Process for obtaining an extruded non-gluten product, direct-expanded and product so obtained” (“Procedeu de obţinere a unui produs aglutenic extrudat, direct-expandat şi produs astfel obţinut”).

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

PHYSICOCHEMICAL CHARACTERISTICS AND ACCEPTABILITY OF COOKIES MADE WITH MOCAF AND MILLET *(Setaria italica* L*)* FLOUR

Eni Purwani[1]✉, Aan Sofyan[1], Fitriana Mustikaningrum[1], Sudrajahwarajati Kisnawaty[1], Hilda Alifia Risma Putri[1], Nikma Khoiriya[1]

[1]Department of Nutrition Science, Faculty of Health Sciences, Universitas Muhammadiyah Surakarta, A. Yani Street, Mendungan, Pabelan, Kartasura Subdistrict, Sukoharjo District, Central Java, Indonesia, 57169
✉eni.purwani@ums.ac.id

https://doi.org/10.34302/crpjfst/2024.16.4.2

Article history:
Received:
August 10th, 2024
Accepted:
October 9th, 2024

Keywords:
Cookies;
Mocaf Flour;
Millet Flour;
Physicochemical;
Acceptance.

ABSTRACT

Mocaf and millet flour are local ingredients suitable for functional cookies. Therefore, this research aimed to analyze the effect of mocaf and millet flour ratio on the physicochemical properties and acceptance of cookies. The experimental treatments included the ratio of mocaf : millet flour comprising (P1) 100% : 0%, (P2) 75% : 25%, (P3) 50% : 50%, (P4) 25% : 75%, and (P5) 0 % : 100%. Chemical characteristics such as water content, protein, fat, reducing sugar, and dietary fiber were analyzed using the AOAC method. The hardness, brittleness, and cohesiveness values were examined using a texture analyzer, while organoleptic tests were carried out on panelists. The results showed that the highest values for water content (6.25%) and fat content (29.65%) of cookies were found in P3 treatment. Protein and dietary fiber levels increased along with the percentage of millet flour. The highest value for reducing sugar content (16.88%) was discovered in treatment P3. The hardness (212.94N) and brittleness (5.19N) values were the highest in treatment P5, while cohesiveness (0.42) was in treatment P2. The preferred acceptance was cookies with a ratio of mocaf : millet flour of 50% : 50%. However, there was no effect of the ratio of mocaf : millet flour on water content (p= 0.331), fat (p= 0.174), reducing sugar (p= 0.056), and cohesiveness (p= 0.425). A significant correlation was observed between protein (p= 0.002), dietary fiber (p= 0.007), hardness (p= 0.005), brittleness (p= 0.016), and acceptance.

1. Introduction

Biscuits are crispy, thin, and flat cookies commonly made in small sizes (Ihromi et al., 2018). The stages of making cookies include the preparation, mixing ingredients, kneading, molding, baking, cooling, and packaging process (Pangestika et al., 2021). Based on the Indonesian National Standard (SNI) on the quality of biscuits (Badan Standarisasi Nasional, 2011), cookies are a kind of biscuits made from soft and crunchy dough, which have a less dense texture.

Despite the numerous benefits, cookies with functional components have not been widely developed. Functional food is processed food that contains one or more functional components based on scientific research with physiological benefits. These components are proven to be harmless and beneficial for health (Sidiq et al., 2022);(Philia et al., 2020). One of the ingredients that can be used as a base for cookies with functional components is fermented cassava (mocaf) and millet flour.

Cassava is a local food that has several advantages, including high micro and micronutrient levels, with a low glycemic index (cassava GI 46). Other advantages are soluble dietary fiber content, which can be used as a

probiotic in the intestine and stomach. Furthermore, cassava comprises glucoside compounds that are harmful (poisonous) and can produce cyanide acid (Rauf et al., 2018). The process to remove these glucoside compounds is by fermenting cassava into mocaf flour (Muhammad Iqbal Nusa, Budi Suarti, 2012).

Mocaf flour can replace wheat flour due to the presence of similar chemical and physical characteristics. Specifically, the starch content of mocaf flour is 78.27% - 85.63%, approximately similar to wheat of 78.36%, is white, neutral odor, and neutral flavor (Suarti et al., 2016). The fermentation process of cassava into mocaf flour uses Lactic Acid Bacteria (LAB) (Kristanti et al., 2020). This process creates changes in flour characteristics, including increased viscosity, rehydration power, easily dissolved, and neutral flavor to cover the taste by 70%. Therefore, mocaf flour has similar characteristics to wheat, showing potential as a substitute for various products such as noodles, bread, nuggets, and other snacks (Asmoro et al., 2017).

The dietary fiber content of mocaf flour (6%) is 19% higher than wheat (0.3%), while the protein (1.2%) is 650% lower compared to wheat (9%). Furthermore, the fat content at 0.6% is 67% lower compared to wheat flour (1%) (Ihromi et al., 2018). According to (R. M. Putri & Kurnia, 2017), mocaf flour contains energy (350 calories), which is 5% higher than wheat flour (333 calories), and carbohydrates (85%) are 10% greater compared to wheat flour (77.2%). The high fiber in mocaf can serve as a functional component in cookies, which are consumed by pregnant women to reduce the risk of lousy defecation habits on pregnant women (Mutalazimah & Cahyanti, 2019).

Millet is another cereal crop with high dietary fiber and carbohydrate content, although it is only used for bird food (R. A. N. Putri et al., 2020). Previous research has shown that millet flour (Suparti & Sholihah, 2021) contain GI of 54.5% with 12.1% protein, 1.68% fat, 81.52% carbohydrates, and 7.8% dietary fiber (Muhammad et al., 2020). It also contains a high fiber content, such as cellulose, hemicellulose, phenolic esters, and glycoproteins (Sulistyaningrum, A., Hayati, N.Q, Rahmawati, n.d.). Millet flour has the potential as a basic ingredient for processed food to strengthen food security. This serves as a method of diversifying food with local raw materials (Sulistyaningrum, A., Hayati, N.Q, Rahmawati, n.d.).

The quality of cookies from millet and mocaf flour is decided based on their physicochemical properties. These include water content, protein, fat, sugar, and dietary fiber, while physical properties are hardness, brittleness, and cohesiveness. Organoleptic properties are characteristics that evaluate the acceptance of a product (Yasinta, 2017).

2. Materials and methods

The sample of mocaf from *Ladang Lima* and millet from *Organis* were taken in September 2022. This experimental research was carried out to examine the physicochemical properties of cookies by comparing mocaf and millet flour. The design used a Completely Randomized Design (CRD) with four treatments. These comprise (P1) 100% mocaf and 0% millet flour, (P2) 75% mocaf and 25% millet flour, (P3) 50% mocaf and 50% millet flour, (P4) 25% mocaf and 75% millet flour, and (P5) 0% mocaf and 100% millet flour. Each treatment was repeated and analyzed in duplicate, leading to a total of 20 treatments. The acceptance research used a panelist test to allow the implementation of the code of ethics at the UMS Faculty of Medicine, **No.4246/B.1/KEPK-FKUMS/IV/2022**.

2.1.Procedure for Cookies Ratio of Mocaf with Millet Flour

The dough was a mixture of butter, powdered sugar, and skim milk using a mixer for approximately 3-7 minutes. Egg yolks were added to the mixture and mixed until expanded with a change of color (Yasinta, 2017). Subsequently, flour was poured according to the treatment and blended for 5 minutes. The dough obtained was molded and baked. The compound of the ingredients was adjusted to the percentage

of flour in the mixture used in cookies made of mocaf and millet flour.

2.2. Water content

Analysis of water content in mocaf flour cookies with millet was carried out using the Thermo Gravimetric method (Shariff et al., 2023). Initially, the crucible was weighed at a constant rate (a gram), and the sample was added (b grams). The crucible was baked in the oven for 6 hours at a temperature of 105^0C and weighed to achieve a constant weight (C grams).

2.3. Protein, Fat, Reducing Sugar, and Total Food Fiber Content

Analysis of protein levels in food ingredients applied the Micro Kjeldahl method (Chang & Zhang, 2017). Meanwhile, fat content analysis was performed using the Soxhlet method (Ellefson, 2017). Analysis of reducing sugar content applied the spectrophotometric method (Shariff et al., 2023) and total food fiber content used the Multienzyme method (Kristanti et al., 2020).

2.4. Physical Properties Testing

Hardness tests, brittleness, and cohesiveness of cookies were investigated by a Texture Analyzer tool which was commenced by cutting of sample to the size of (2x2x2) cm^3. The Texture Analyzer machine was turned on for a minimum of 30 minutes before use and programmed according to the determined parameters, namely hardness, brittleness, and cohesiveness. Subsequently, the sample was put under the pressure tool and operated. The magnitude of hardness, brittleness, and cohesiveness were observed on the monitor screen. The gel strength value was the force required to define the sample expressed in Newton units (N).

2.5. Acceptance Testing

Acceptance was carried out using organoleptic tests (Sofyan et al., 2022). The parameters tested were color, odor, flavor, texture, and overall, with 7 scales. These included like extremely, like, like moderately, neutral, dislike, dislike moderately, and dislike extremely with a total of 34 fairly trained panelists.

2.6. Data analysis

Data analysis was carried out using SPSS version 20. Specifically, chemical test results, physical properties, and acceptance were conducted using normality and homogeneity tests with the Kolmogorov-Smirnov. When the data were not normally distributed, the Kruskal-Wallis test was performed. Meanwhile, when there was an influence with a significance value of $p<0.05$, the Dunnet difference test was performed.

3. Results and discussions

3.1. Water content

Water content data were not normally distributed ($p= 0.028$), hence, the Kruskal Wallis test proceeded with the analysis results in Table 1.

Table 1. Moisture Content of Cookies Ratio of Mocaf and Millet Flour

Treatment	Water Content (%)
P1 (100% : 0%)	5.81 ± 0.59
P2 (75% : 25%)	5.68 ± 0.52
P3 (50% : 50%)	6.25 ± 0.07
P4 (25% : 75%)	5.45 ± 1.21
P5 (0% : 100%)	5.46 ± 0.52
p-value	**0.331**

The results of the Kruskal-Wallis statistical test on water content showed p value = 0.331. This showed that cookies with a ratio of mocaf flour : millet flour of 100% : 0%, 75% : 25%, 50% : 50%, 25% : 75%, and 0% : 100% did not have a significant difference. According to the average value of water content of mocaf with millet flour, the results did not meet the quality required for cookies. This was based on the 2011 Indonesian National Standard (SNI) (Badan Standarisasi Nasional, 2011), where cookies accommodated water content of more than 5%. Cookies with the lowest water content of 5.45% were from the ratio of 25% mocaf flour: 75%

millet flour. The highest results obtained were 6.25%, found in 50% : 50% cookies.

3.2. Protein Content

The protein content data were normally distributed (p= 0.297), but not homogeneous (p= 0.032). Therefore, the next analysis was carried out by the Kruskal Wallis Test, with the results shown in Table 2.

Table 2. Protein Content of Cookies Ratio of Mocaf and Millet Flour

Treatment	Protein Content (%)
P1 (100% : 0%)	3.19 ± 0.13[a]
P2 (75% : 25%)	4.27 ± 0.61[ab]
P3 (50% : 50%)	5.51 ± 0.71 [bc]
P4 (25% : 75%)	6.24 ± 0.46[c]
P5 (0% : 100%)	8.07 ± 0.58 [d]
p-value	**0.002**

Value with different letter notations shows significant differences (Duncan P<0.05).

Protein content of cookies with a ratio of mocaf : millet flour 100% : 0%, 75% : 25%, 50% : 50%, 25% : 75%, and 0% : 100% had a significant difference. In the average protein content of mocaf and millet, cookies that met the quality requirements per Indonesian National Standard (SNI) in 2011 (Badan Standarisasi Nasional, 2011) had a minimum protein of 5 grams in every 100 grams. These cookies were obtained in treatments P3, P4, and P5. Cookies with the lowest protein content of 3.19% were found in the ratio of 100% mocaf : 0% millet flour. Meanwhile, the highest protein content results obtained were 8.07% in 0% mocaf flour cookies: 100% millet flour ratio. This suggested that the use of higher millet in mocaf flour cookies led to increased protein content.

Research by (Izwardy, 2018) stated that protein content of mocaf flour was 1.2 grams, while (Muhammad et al., 2020) reported 12.1 grams. Based on the previous research, the higher the use of millet flour, the greater the protein content in cookies.

3.3. Fat content

Fat content data in the normality test were not normally distributed (p= 0.059). Therefore, analysis was carried out using the Kruskal-Wallis test, with the results shown in Table 3.

Table 3. Fat Content of Cookies Ratio of Mocaf and Millet Flour

Treatment	Fat Content (%)
P1 (100% : 0%)	30.28 ± 0.37
P2 (75% : 25%)	29.53 ± 1.37
P3 (50% : 50%)	29.91 ± 0.29
P4 (25% : 75%)	29.65 ± 0.79
P5 (0% : 100%)	28.78 ± 0.57
p-value	**0.174**

The results of the Kruskal Walls statistical test showed p= 0.174. This suggested that cookies with a ratio of mocaf : millet flour in all treatments did not have a significant effect. Based on the average fat content of cookies, the lowest value of 28.78% was found in the ratio of 0% mocaf : 100% millet flour. The highest value of 30.28% was obtained in 100% mocaf : 0% millet flour. The results showed that as the use of millet flour increased, fat content reduced. This could be attributed to the lower fat content of millet flour at 1.68%, while mocaf flour had 2.72% (Gusriani et al., 2021).

3.4. Reducing Sugar Levels

The results of the normality test data on reducing sugar levels were not normally distributed (p= 0.000). Consequently, further analysis was carried out using the Kruskal-Wallis test, with results presented in Table 4.

Reducing sugar content in this research, based on the results of the Kruskal-Walls statistical test, showed p= 0.056. This suggested that cookies with a ratio of mocaf : millet flour of 100% : 0%, 75% : 25%, 50% : 50%, 25% : 75%, and 0% : 100% did not have a significant difference. Based on the average reducing sugar content of cookies, the lowest value of 13.52% was obtained in the ratio of 25% mocaf : 75% millet flour.

Table 4. Reducing Sugar Content of Cookies Ratio of Mocaf and Millet Flour

Treatment	Reducing Sugar Content (N)
P1 (100% : 0%)	18.33 ± 2.22
P2 (75% : 25%)	16.11 ± 0.11
P3 (50% : 50%)	16.88 ± 0.70
P4 (25% : 75%)	13.52 ± 4.57
P5 (0% : 100%)	15.41 ± 0.98
p-value	**0.056**

3.5. Total Dietary Fiber Content

Data on total dietary fiber content were normally distributed (p= 0.386), but not homogeneous (p= 0.000). This led to further analysis using the Kruskal-Wallis test, with results shown in Table 5.

Table 5. Total Food Fiber Content of Cookies Ratio of Mocaf and Millet Flour

Treatment	Total Food Fiber Content (%)
P1 (100% : 0%)	2.42 ± 1.81[a]
P2 (75% : 25%)	4.10 ± 1.31[a]
P3 (50% : 50%)	4.69 ± 0.59[a]
P4 (25% : 75%)	5.65 ± 0.63[a]
P5 (0% : 100%)	8.41 ± 0.67[b]
p-value	**0.007**

Value with different letter notations shows significant differences

The results of the Kruskal-Wallis statistical test showed that the p-value = 0.007, indicating a significant influence on the total food fiber content of cookies. Further testing was conducted with the Dunnett T3 test, where the results showed significant differences ($p<0.05$) between treatments P1, P3, P4, and P5, as well as P5 and all treatments. This suggested that cookies with a ratio of mocaf : millet flour 100% : 0%, 75% : 25%, 50% : 50%, 25% : 75%, and 0% : 100% had a significant difference.

In average total dietary fiber content cookies, the lowest value of 2.42% was obtained in the ratio of 100% mocaf : 0% millet flour. The highest value of 8.41% was obtained at a ratio of 0% mocaf : 100% millet flour. The data showed that a high millet flour ratio in making mocaf flour cookies caused an increase in total food fiber content. According to (Izwardy, 2018), the fiber content of mocaf flour was 6 grams, while (Muhammad Iqbal Nusa, Budi Suarti, 2012) reported a 7.8 grams. Based on observation, the use of millet flour increased along with fiber content in cookies. Moreover, dietary fiber in food will affect the properties of the food, as higher content correlated with lower glycemic index (Wahyuningtias, 2010). The dietary fiber in millet flour was higher compared to mocaf. Due to the high glycemic index content, as millet flour increased, there would be a significant decrease in reducing sugar.

3.6. Hardness Value

Hardness data were not normally distributed, with the results of the Kruskal-Wallis test shown in Table 6. Based on the results, the p-value <0.05 showed that there was a significant effect of treatment (P1, P2, P3, P4, and P5) on the hardness (N) of cookies made of millet and mocaf flour.

The results of the hardness test on cookies show that P5 had the highest average value of 212.94N. This value was influenced by the fiber and protein content of the raw materials, which played a role in absorbing water causing weakly bound (Dias et al., 2010). Millet flour contained a protein of 12.1% and a fiber of 7.8% (Dias-Martins et al., 2018). Mocaf flour comprised a protein of 1% and a fiber of 1.9% - 3.4% (Widasari & Handayani, 2014).

3.7. Brittleness Value

Brittleness analysis was examined using the ANOVA, with the results presented in Table 6. This analysis was carried out to determine whether there was a significant influence of the ratio of millet and mocaf flour on the brittleness of cookies. According to the results of the Kruskal-Wallis statistical test with a value of $p<0.05$, there was a significant effect of treatment (P1, P2, P3, P4, and P5) on the brittleness value of millet and mocaf flour cookies.

The results of the brittleness test on cookies showed that mocaf and millet flour ratio of 0%: 100% had the highest average value of 5.19N. Meanwhile, mocaf and millet flour ratio of 100%: 0% had the lowest average value, namely 2 .89N.

3.8. Value of Cohesiveness

Cohesiveness is the pressure area from the second to the first compression and has no units. It is often measured as the degree to which the product is destroyed mechanically [20]. Therefore, analysis was carried out using the Kruskal-Wallis test to determine the significant effect of the ratio of millet and mocaf flour on the cohesiveness of cookies. The results of the cohesiveness test analysis on cookies are shown in Table 6.

The results of the cohesiveness test on cookies show that P2, the ratio of mocaf flour to millet flour 75%: 25% had the highest average value of 0.42. According to (Shaliha et al., 2017), a high cohesiveness material correlated with elevated integrity of materials.

Table 6. Hardness Value, Brittleness, and Cohesiveness of Cookies Ratio of Mocaf Flour and Millet Flour

Treatment	Means ± SD		
	Hardness (N)	**Brittleness (N)**	**Cohesiveness**
P1 (100% : 0%)	165,29±8,69^{b}	2,89 ± 0,63^{a}	0,27 ± 0,15
P2 (75% : 25%)	142,76±18,14^{ab}	3,77 ± 0,54^{a}	0,42 ± 0,03
P3 (50% : 50%)	129,57±27,18^{ab}	3,17 ± 1,12^{a}	0,40 ± 0,03
P4 (25% : 75%)	188,53±7,10^{a}	3,34 ± 0,72^{a}	0,40 ± 0,03
P5 (0% : 100%)	212,94±48,98^{ab}	5,19 ± 1,14^{b}	0,40 ± 0,05
p-value	**0,005**	**0,016**	**0,425**

Value with different letter notations shows significant differences

3.9. Acceptance

The acceptance of cookies with mocaf flour and millet flour ratio of 100% : 0%, 75% : 25%, 50% : 50%, 25% : 75%, and 0% : 100% includes color, odor, flavor, texture, and overall. The results of the analysis were completed using the Kruskal-Wallis test to find out the real effect of the ratio of millet flour and mocaf flour on the acceptance of cookies. The results of cookies acceptance analysis are shown in Table 7.

Table 7. Cookies Acceptance Test Results Ratio of Mocaf Flour and Millet Flour

Treatment	Nilai Mean ± SD				
	Color	**Odor**	**Flavor**	**Brittleness**	**Overall**
P1 (100%:0%)	4,59±1,86^{a}	5,38±0,98^{ab}	5,26±1,31^{ab}	5,29±1,54^{ab}	5,09±1,54^{ab}
P2 (75%:25%)	5,59±1,04^{abc}	5,94±0,88^{b}	5,06±1,20^{a}	4,97±1,38^{ab}	5,26±0,99^{ab}
P3 (50%:50%)	6,03±0,71^{b}	5,71±0,90^{ab}	5,79±0,77^{b}	5,59±1,13^{b}	5,82±0,71^{b}
P4 (25%:75%)	5,06±1,23^{ac}	5,44±1,21^{ab}	4,56±1,37^{ac}	4,62±1,57^{ac}	4,68±1,14^{ac}
P5 (0%:100%)	4,85±1,37^{ac}	5,00±1,39^{a}	4,38±1,47^{a}	4,47±1,46^{a}	4,62±1,39^{a}
p-value	**0,000**	**0,013**	**0,000**	**0,006**	**0,000**

Value with different letter notations shows significant differences

Based on Table 7, the results of the acceptance test with color parameters showed that the ratio of millet and mocaf flour 50%: 50% had the highest average of 6.03. This could be attributed to the presence of millet flour, which contributed color to cookies. Treatment P3 was preferable because the color was as bright and attractive as general cookies. Meanwhile, treatment P1 had a pale color or was less attractive than other treatments. Cookies made with P4 and P5 showed a darker color, which was less attractive. According to (Martins et al., 2000), proteins with reducing sugars during heating would generate browning which formed melanoidin compounds.

Figure 1. Cookies Acceptance Test Results Ratio of Mocaf and Millet Flour

The results of the odor acceptance test in Figure 1 showed that P2 had the highest average of 5.94. This was because cookies with treatment P2 had a similar odor to general products. The decrease in the level of odor preference in cookies was caused by the dominance of millet flour. According to (Pakhri et al., 2017), the unpleasant odor was due to the breaking down of protein into amino acids by heat. The reaction between amino acids and sugar would produce aroma, while the fat in the ingredients was oxidized and broken down by heat. Therefore, some of the active ingredients produced by the process would react with amino acids and peptides to produce odor.

The results of the flavor acceptance test showed that the ratio of millet and mocaf flour 50%: 50% had the highest average of 5.79, while P5 had the lowest value of 4.38. Generally, flavor plays a significant role in acceptance, particularly in new products, where consumers will greatly determine the quality (Verma et al., 2015). The flavor of cookies is influenced by several factors including temperature, chemical compounds, ingredient components, and interactions with other flavor components. The high level of preference for the flavor at P3 was because cookies had a savory flavor, while P5 was bitter (aftertaste). When making cookies with millet flour as a substitute, it leaves a bitter aftertaste due to the presence of the epidermis which emits tannin (Widyastuti et al., 2019).

Food texture is an important aspect of consumer acceptance, particularly in crunchy products such as cookies. Based on the results of the brittleness acceptance test on cookies, the treatment most preferred by the panelists was P3 with an average of 5.59, while P5 had the lowest value of 4.47. The low level of texture preference was because P5 had a slightly rough texture, while P3 was crunchy, soft, and smooth.

Based on Figure 1, the results of the overall acceptance test on all cookies showed that P3 had the highest average of 5.82, while P5 had the lowest of 4.62. As shown by the observed parameters, the best treatment was P3 at a ratio of millet and mocaf flour of 50%: 50%.

4. Conclusion

In conclusion, this research showed that there was no effect of the comparison of mocaf and millet flour on water content, fat, reducing sugar, and cohesiveness. Meanwhile, there was a significant effect on protein content, dietary fiber, hardness, brittleness, and acceptance. The overall acceptance test results showed that the most preferred cookies formulation with highest rating was obtained in P3 with 50%:50% ratio of mocaf and millet flour.

5. References

Asmoro, N. W., Hartati, S., & Handayani, C. B. (2017). Physical and organoleptic characteristics of mocatilla chips from mocaf and corn flour. *Journal of Food*

Science and Agricultural Products 1 (1):64–71, doi: 10.26877/jiphp.v1i1.1354.

Badan Standarisasi Nasional. (2011). Indonesian national standard for biscuits. National Standardization Agency [Internet]. 1–5. Available from: https://kupdf.net/download/sni-01-2973-1992_58e4a373dc0d60523cda9818_pdf #

Chang, S. K. C., & Zhang, Y. (2017). Protein analysis. Food Analysis:315-331, doi: 10.1007/978-3-319-45776-5_18.

Dias-Martins, A. M., Pessanha, K. L. F., Pacheco, S., Rodrigues, J. A. S., & Carvalho, C. W. P. (2018). Potential use of pearl millet (*Pennisetum glaucum* (L.) R. Br.) in Brazil. Food Security, Processing, Health Benefits and Nutritional Products. *Food Research International* [Internet]109(April):175–86, doi: 10.1016/j.foodres.2018.04.023

Dias, C. L., Ala-nissila, T., Wong-ekkabut, J., & Vattulainen, I. (2010). The hydrophobic effect and its role in cold denaturation. *Cryobiology* 60(1):91–9, doi: 10.1016/j.cryobiol.2009.07.005.

Ellefson, W. C. (2017). Fat analysis. Food Analysis:299–314, doi: http://dx.doi.org/10.1007/978-3-319-45776-5_17.

Gusriani, I., Koto, H., & Dany, Y. (2021). Application of mocaf flour (Modified Cassava Flour) utilization in several food products at Madrasah Aliyah Mambaul Ulum, Central Bengkulu Regency. *Journal of Education Community Service Innovation* 2(1):57–73, doi: 10.33369/jurnalinovasi.v2i1.19142.

Ihromi, S., Marianah, M., & Susandi, Y. A. (2018). Subsitution of wheat flour with mocaf flour in the preparation of pastries. *Journal of Agrotech UMMAT* 5(1):73, doi: 10.31764/agrotek.v5i1.271.

Izwardy, et al. (2018). Indonesian food composition table. In Indonesian Ministry of Health (Vol. 2, Issue 2). https://www.academia.edu/44325122/food_indonesia_2017_table_composition

Kristanti, D., Setiaboma, W., & Herminiati, A. (2020). Physicochemical and organoleptic characteristics of mocaf cookies with added tempe flour. *E-journal of Biopropal Industry* 11(1):1, doi: 10.36974/jbi.v11i1.5354.

Martins, S. I. F. ., Jongen, W. M. ., & van Boekel, M. A. J. . (2000). A review of maillard reaction in food and implications to kinetic modelling. *Trends Food Science Technology*. Sep;11(9–10):364–73, doi: 10.1016/S0924-2244(01)00022-X.

Muhammad, D. R. A., Sasti, T. G., Siswanti, S., & Anandito, R. B. K. (2020). Characteristics of steamed chocolate brownis made from arrowroot starch with partial substitution of barley flour. *Journal of Agricultural Product Technology* 12(2):87, doi: 10.20961/jthp.v12i1.36184.

Muhammad Iqbal Nusa, Budi Suarti, A. (2012). Preparation of mocaf flour through the addition of starter and fermentation duration (Modified Cassava Flour). *Agrium Journal of Agricultural Sciences* 17(3):210–217, doi: https://doi.org/10.30596/agrium.v17i3.322

Mutalazimah, M., & Cahyanti, N. (2019). Fiber intake, fluid intake and defecation habits of pregnant women in Indonesia. *International Journal of Pharmaceutical Research* [Internet] 11(1):740 – 745, https://doi.org/10.31838/ijpr/2019.11.01.125

Pakhri, A., Yani, N., Mas'ud, H., & Sirajuddin, S. (2017). Cookies with barley flour substitution. *Food Nutrition Media XXIV(edisi 2):*21–27 Available from: https://www.scribd.com/document/389807804/4-Cookies-Dengan-Subtitusi-Tepung-Jewawut

Pangestika, W., Putri, F. W., & Arumsari, K. (2021). Utilization of catfish bone meal and tuna bone meal for making cookies. *Journal of Food and Agroindustry* 9(1):44–55, https://doi.org/10.21776/ub.jpa.2021.009.01.5

Philia, J., Widayat, Hadiyanto, Suzery, M., & Budianto, I. A. (2020). Diversification of mocaf flour into healthy noodle products at PT. Mocaf Solusindo Flour. *Indonesian Journal of Halal* 2(2):40–5, doi: https://doi.org/10.14710/halal.v2i2.7341.

Putri, R. A. N., Rahmi, A., & Nugroho, A. (2020). Chemical, microbiological, sensory characteristics of cereal flakes based on nagara yam flour (*Ipomoea batatas* L.) and barley flour (*Setaria italica*). *Journal of Technology Agro-Industry* 7(1):1–11, doi: 10.34128/jtai.v7i1.106.

Putri, R. M., & Kurnia, P. (2017). Utilization of mocaf (Modified Cassava Flour) with sago (*Metroxylon sago* Rottb) on elongation properties and acceptability of wet noodles. *Proceeding 6th University Research Colloquium* [Internet] 241–8. Available from: https://journal.unimma.ac.id/index.php/urecol/article/view/1052/867

Rauf, R., Nurdiana, N., & Sarbini, D. (2018). Iron content, hardness and acceptability of biscuits from composite cassava and wheat flours substituted with green spinach flour. *Journal of Health* 11(1):1–8, doi: 10.23917/jk.v11i1.6998.

Shaliha, L. A., Abduh, S. B. M., & Hintono, A. (2017). Antioxidant activity, texture and brightness of purple sweet potato (*Ipomoea batatas*) steamed at various heating times. *Journal of Food Technology Applications* 6(4):141-144 Available from: https://jatp.ift.or.id/index.php/jatp/article/view/260

Shariff, A. H. M., Hainusa, N. A., Huda, N., Zakaria, M., Ullah, S., Huyop, F., & Wahab, R. A. (2023). Antioxidant activity, total phenolic content, and nutrient composition of chayote shoot (*Sechium edule* Jacq. Swartz) from Kundasang, Sabah. *Journal of Tropical Life Science*. 13(1):147–56, doi: 10.11594/jtls.13.01.15.

Sidiq, Y., Tamaoki, D., & Nishiuchi, T. (2022). Proteomic profiling of plant and pathogen interaction on the leaf epidermis. *International Journal of Molecular Sciences* [Internet] 23(20). https://doi.org/10.3390/ijms232012171

Sofyan, A., Ikhsani, A. Y., Purwani, E., Hasanah, L. E. N., & Febriyadin, F. (2022). The effect of suweg (*Amorphophallus paeoniifolius*) flour and incubation temperature on characteristics of yogurt with the addition of *Bifidobacterium bifidum* as probiotic. *In: Materials Today: Proceedings* [Internet] p. S507 – S512. https://doi.org/10.1016/j.matpr.2022.04.538

Suarti, B., Fuadi, M., & Budiono, E. (2016). Flour treatment of oyster mushroom (*Pleurotus ostreatus*) and old dried noodles drying on quality of wheat mocaf (modified cassava flour). *Agrium, Journal of Agricultural Sciences* 20(2):109–18, https://jurnal.umsu.ac.id/index.php/agrium/article/view/626/558

Sulistyaningrum, A., Hayati, N.Q, Rahmawati, D. (2020). Linearity analysis of barley flour proportion on physical and chemical characteristics of roasted brownies. *Agricultural Informatics* 29(1): 23-32, doi: http://dx.doi.org/10.21082/jpasca.v14n1.

Suparti, S., & Lailia, Z. (2021). Growth of F0 seedlings of oyster mushroom and merang mushroom on alternative media of barley seed flour with different concentrations. *Bioexperiment Journal Biological Research* 7(1):27–33, doi: https://doi.org/10.23917/bioeksperimen.v4i2.6887.

Verma, S., Srivastava, S., & Tiwari, N. (2015). Comparative study on nutritional and sensory quality of barnyard and foxtail millet food products with traditional rice products. *Journal Food Science Technology* 52(8):5147–55, doi: 10.1007/s13197-014-1617-y.

Wahyuningtias, D. (2010). Organoleptic test of finished cakes using non instant and instant ingredients. *Binus Business Review*. 1(1):116, doi: 10.21512/bbr.v1i1.1060.

Widasari, M., & Handayani, S. (2014). Effect of wheat - mocaf (Modified Cassava Flour) proportion and addition of tempe formula

flour on flake yields. *E-Journal Boga* 3(3):222–8, https://ejournal.unesa.ac.id/index.php/jurnal-tata-boga/article/view/9497/9390.

Widyastuti, R., Asmoro, N. W., & Aini, A. N. (2019). Characteristics of biscuits substituted with millet flour (*Setaria italika* L). *Agrisaintifika Journal of Agricultural Sciences* 3(2):98–103, doi: https://doi.org/10.32585/ags.v3i2.544.

Yasinta, U.N,A., Dwiloka, B., & Nurwantoro. (2017). Effect of substituting wheat flour with banana flour on physicochemical and organoleptic properties of cookies. *Journal of Food Technology Applications* 6(3):119–23, doi: http://dx.doi.org/10.17728/jatp.200.

Acknowledgment

This research was financially supported the UMS Research and Innovation Institute

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

THE IMPACT OF THE INFUSION METHOD OF CHOKEBERRY POWDER IN WHITE TEA

Mirabela Ioana Lupu[1], Cristina Maria Canja[1]✉, Alina Maier[1], Vasile Padureanu[1], Geronimo Raducu Branescu[1] and Ana-Maria Manolica [1,2]

[1]Faculty of Food and Tourism, Transilvania University of Brasov, Romania
[2] Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
✉*canja.c@unitbv.ro*

https://doi.org/10.34302/crpjfst/2024.16.4.3

Article history:
Received
August 7th , 2024
Accepted
October 10th, 2024

Keywords:
Chokeberry powder;
White tea;
Infusion method.

ABSTRACT

The objective of this study was to evaluate the impact of chokeberry powder on the properties of white tea, with the aim of developing a new product with high bioactive compounds. Initially, the influence of the infusion technique on the characteristics of white tea was investigated. A comparative analysis was conducted between hot and cold infusion methods for both plain white tea and white tea with chokeberry powder at varying concentrations (0.6%, 0.8%, 1%). In the course of the experimental research, the total polyphenol content, antioxidant activity, viscosity, and pH of the tea were evaluated. A sensory analysis was also conducted on all varieties of tea presented in this paper. The experimental research demonstrated that the incorporation of chokeberry powder has a beneficial impact on the properties of white tea, resulting in a notable increase in polyphenol content (1% chokeberry powder in a cold infusion resulted in a total phenol concentration of 12.7 ± 0.6 mg GAE/100 mL). This enhancement in polyphenol content was accompanied by an increase in the beneficial effects of tea on the human body. Additionally, the sensory analysis indicated that the 1% chokeberry powder cold infusion (TC3) was the most preferred tea among consumers. This suggests that the TC3 sample exhibited the optimal balance between bioactive properties and consumer acceptance.

1.Introduction

Tea is a beverage with a global popularity that transcends geographical boundaries. It is consumed in a variety of forms, including hot and cold. The most commonly consumed teas (green tea, black tea, oolong tea, white tea, and yellow tea) are derived from the leaves and buds of the *Camellia sinensis (L.)* plant, belonging to the *Theaceae* family. These teas are distinguished by variations in harvesting, processing, and the degree of oxidation of the polyphenols present in fresh tea leaves (Sharangi, 2009; Unachukwu et al., 2010). As stated by some authors (Damiani et al., 2014), the two most prevalent grades, Silver Needle and White Peony, are commercially accessible. However, there are numerous other varieties with diverse trade names.

The Silver Needle (Bai Hao Yinzhen in its traditional name) is manufactured exclusively from the unopened buds of the plant, without any leaves. As its name indicates, it is characterized by a silver-white hue and comprises long, thin needles. The buds are initially sun-dried on sieves or drying mats for a period of approximately 24 hours, representing the initial phase of the processing method. This is then followed by baking over a low fire until the buds are fully desiccated. The final product exhibits a subtle flavor profile and a pale-yellow

hue. The White Peony (traditional name Bai Mudan) is manufactured from the bud and one or two leaves derived from the plant's vegetative apex. The processing of the leaves involves two simple phases: withering (sun drying/airing/low temperature) and basket drying. The resulting tea exhibits a light golden-brown color and a pleasant roasted aroma. The flowers and leaves of the *Camellia sinensis* plant contain a variety of bioactive substances, including nutrients (carbohydrates, proteins, and minerals), alkaloids (methylxanthines), and phenolic compounds (phenolic acids, flavonoids, and tannins) (Sharma et al., 2021). White tea (WT) is described as "tea for one year, medicine for three years, and treasure for seven years" (Cheng et al., 2021), which indicates the increasing nutritional and functional values of aging WT. White tea (WT) has been demonstrated to exert beneficial effects on human health, including the prevention and treatment of diabetes, cancer, bacterial infections, and obesity (Olcha et al., 2022).

The chokeberry, also known as Aronia, is a *Rosaceae* shrub that is native to North America and was introduced to Europe approximately a century ago (Chrubasik et al., 2010; Sidor et al., 2019). Black chokeberries are a rich source of polyphenols, which contribute to their high biological activity. The polyphenols present in these berries include anthocyanins, flavonols, flavanols, proanthocyanidins, and phenolic acids (Tolić et al., 2017). A previous study conducted by Kokotkiewicz identified the phenolic chemicals present in chokeberry fruit, including procyanidins (0.7-5.2%) and anthocyanins (0.6-2.0%), as the primary classes with therapeutic characteristics (Kokotkiewicz et al., 2010). The high concentrations of anthocyanin and polyphenols may exert a protective effect against the development of cancer, diabetes, gastrointestinal disease, and cardiovascular disease (Burdejova et al., 2020). Given the popularity of chokeberry and its associated health benefits, a variety of preparations have been developed on an industrial scale, including concentrated extracts, juices, and Aronia food products. One particularly valuable product is fruit powder, which is obtained by drying and grinding the fruit. The primary advantage of fruit drying is the extension of the product's shelf life.

Given the numerous advantages offered by both white tea and chokeberry powder, our objective is to enhance the biological activity of white tea by developing a new product. This will be achieved by incorporating chokeberry powder into white tea, thereby investigating the impact of this addition on the tea's properties, including pH, acidity, and viscosity. Additionally, a sensory analysis of the teas was conducted.

2. Materials and methods

2.1. Materials

2.1.1. Raw materials

The white tea utilized in the experimental research is a dry white peony tea procured from the supermarket and produced by the Basilur company. The chokeberry powder is an organic powder from the Aronia Charlottenburg company, procured from a commercial establishment. The nutritional information, as indicated on the product label, is as follows: The energy value is 1013kJ/242kcal, with fat comprising 2.5g-6.0g, of which saturated fatty acids account for 0.4g. Carbohydrates constitute 40-8.0g, with sugars amounting to 1.8-2.8g. Fiber is present in quantities of 70.0-80.0g, while proteins are found in amounts of 5.0-10.0g. Salt is present in quantities of less than 0.01g.

2.1.2. Sample preparation

In the experimental research, two infusion methods were employed in accordance with the methodology illustrated in Figure 1: hot infusion and cold infusion. Hot tea infusions were prepared by adding 100 mL of water at 70° C to 2.5 g of white tea and allowing the infusion to proceed for seven minutes (Damiani et al., 2014). The cold infusion was prepared by adding 100 mL of water at room temperature to 2.5 g of white tea and allowing the infusion to stand at room temperature (20–25 °C) for two hours, agitating continuously using a magnetic stirrer (IKA, RET basic, Germany) (Damiani et

al., 2014). The same recipes were used for both types of infusion, with the same chokeberry powder concentrations. The concentrations of the chokeberry powder were 0%, 0.6%, 0.8%, and 1%. Prior to analysis, all samples were filtered through a 0.45 µm membrane filter.

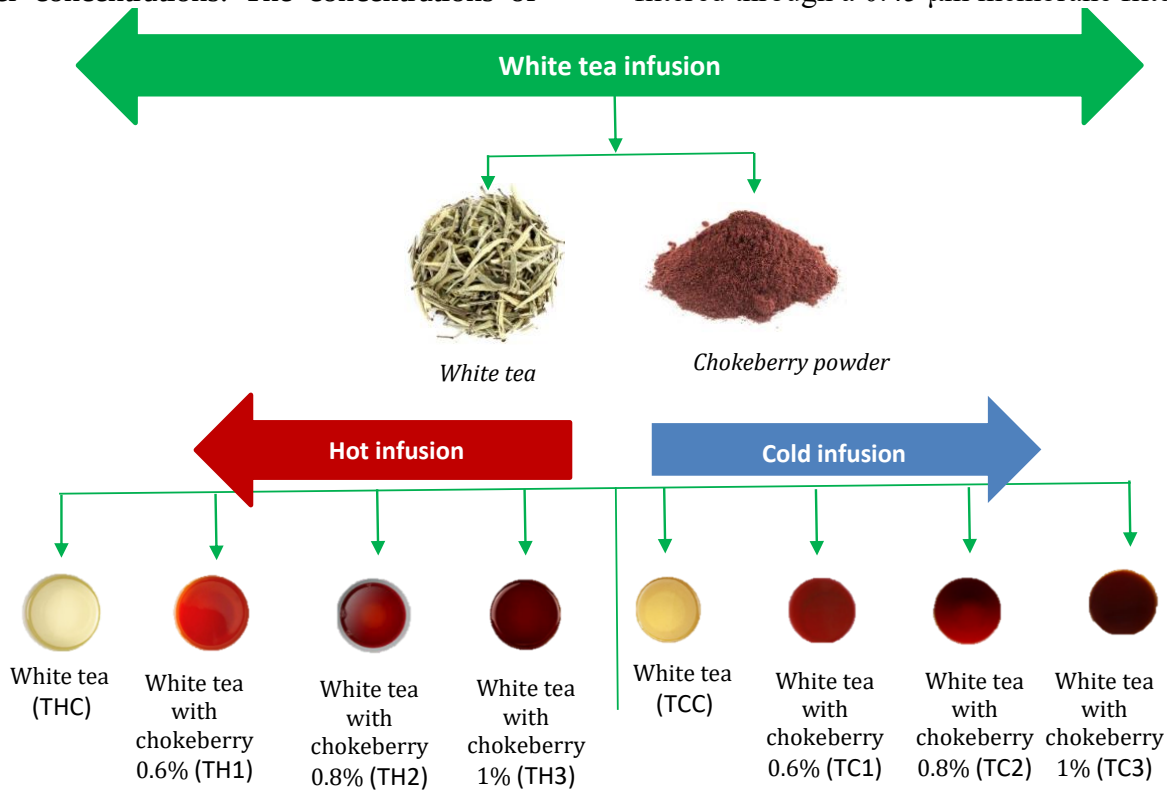


Figure 1. Diagram of the infusion process of white tea with chokeberry powder. THC -White tea hot infusion; TH1- White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3- White tea with chokeberry powder 1% hot infusion; TCC -White tea cold infusion; TC1 - White tea with chokeberry powder 0.6% cold infusion; TC2 - White tea with chokeberry powder 0.8% cold infusion; TC3 - White tea with chokeberry powder 1 % cold infusion.

2.2. Methods

2.2.1. Total polyphenolic content

Using gallic acid as a reference, the total polyphenolic content (TPC) was calculated using the Folin-Ciocalteu spectrophotometric method and represented as milligrams of gallic acid equivalents per milliliter (µg GAE/mL) (Singleton et al., 1999). Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), 1.25 mL, was applied to a 0.5 mL sample after being diluted 1:10 (v/v) with distilled water. 1 mL of Na_2CO_3 60 g/L was added after the mixture had been incubated for 5 minutes at room temperature. The sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany) after 30 min of incubation at 50 °C. Gallic acid was used as the standard, with concentrations ranging from 5 to 25 mg GAE/mL, to create the calibration curve.

2.2.2. Antioxidant activity (ABTS assay)

The ABTS assay measurement of the different teas was performed according to the method described by (Damiani et al., 2014; Re et al., 1999). A 7.0 mmol/L aqueous ABTS [2,20-azinobis-(3-ethylbenzothiazoline-6)-diammonium salt] solution was mixed in a 0.9:0.1 ratio with a 2.45 mmol/L aqueous solution of potassium persulfate as an oxidizing agent to quantify the radical cation (ABTS•+).

Prior to use, the combination was stored at room temperature in the dark for 12–16 hours. To get an absorbance at 734 nm ranging from 0.6 to 0.8, the ABTS•+ solution stock was 80-fold diluted with water before to use. 0.025 mL of previously diluted tea, adequately diluted Trolox (6-hydroxy-2,5,7,8-tetramethylchro- man-2-carboxylic acid) standard ethanolic solution, or water as a control were added to 2.475 mL of this ABTS•+ solution, and the mixture was then added. The samples were kept at room temperature in the dark for two hours, and the samples' absorbance was measured against water at 734 nm. The following equation was used to compute inhibition percentage values (Equation 1):

$$Inhibition\ of A_{734}(\%) = \left(1 - \frac{A_C}{A_0}\right) \cdot 100 \quad (1)$$

where Ac is an absorbance of the samples, A0 is an absorbance of the control. Antioxidant activity was expressed as mmol/L Trolox Equivalents (TE) using the linear regression value obtained from the Trolox calibration curve.

2.2.3. pH measurement

The pH was measured using the electrochemical method (Webster, 2003) with a potentiometer (Consort C1010, Consort, Turnhout, Belgium).

2.2.4. Viscosity

The viscosity (η [cP]) was determined using the Brookfield rheometer (Brookfield Engineering Laboratories, Inc., Middleborough, MA, USA). The torque required to drive a disk, immersed at a predetermined depth, in the liquid whose viscosity is to be determined, into a rotational movement with a certain speed, will be measured. It is established that a thin layer of liquid adheres to the surface of a body in contact with a liquid due to adhesion forces. This layer, known as the boundary layer, moves as a single entity with the surface to which it has adhered, thus exhibiting the same velocity. The molecular cohesion forces exerted by the molecules in the layer under consideration will cause the molecules in the neighboring layer to move at a slower speed due to the sliding between the layers of molecules.

The accuracy of the Brookfield viscometer was evaluated using a standard-viscosity liquid provided by the Cannon Instrument Company (State College, PA). The results demonstrated that the viscosity measurement data shear rate of 300 s^{-1} from the Brookfield viscometer was found to be within 5% of the standard viscosity value. The torque output became unstable at the shear rate of 300 s^{-1}, which equaled 40 rpm. This instability is likely due to secondary flow caused by centrifugal force in the gap between the cone and plate geometries (Lee et al., 2012).

2.2.5. Sensory analysis

A total of one hundred evaluators, comprising both male and female participants between the ages of 21 and 60, were invited to score the tea samples on a 5-point hedonic scale, ranging from least favored (1, "dislike very much") to most liked (5, "like very much"). This was done in order to ascertain consumer preference for the tested tea samples. Infusions were evaluated considering the criterion of color, brightness, clarity, astringency, aroma and bitterness. The panelists received eight distinct tea samples in 150-ml tea cups, each bearing a randomly assigned number. Tea samples were interspersed with opportunities for participants to rinse their palates with water. The sensory analysis was conducted at room temperature in a facility equipped with LED lighting. The evaluators were selected from the personnel and student body at Transilvania University of Brașov.

2.2.6. Statistical analysis

Each tea sample was tested in triplicate, and the results of the three separate tests were averaged to yield a single value for each sample. All data are presented as the mean of the three replicates, followed by the standard deviation (SD). The significance of mean differences was assessed by one-way ANOVA. Tukey's test ($p \leq 0.05$) was used to compare mean differences. The correlation analysis was employed to estimate the degree of correlation between the data sets, while the regression analysis was utilized to model the relationship between the

predictor variables and the investigated parameters (JASP Team, 2023).

3.Results and discussions

As illustrated in Figure 1, eight distinct tea varieties were obtained through both cold and hot infusion methods. These teas were then subjected to comprehensive analysis, evaluating their polyphenol content, antioxidant activity, viscosity, and pH. The findings from this experimental research were collated and presented in Table 1.

Table 1. The properties of white tea

Analysis	Hot infusion				Cold infusion			
	THC	**TH1**	**TH2**	**TH3**	**TCC**	**TC1**	**TC2**	**TC3**
TPC [mg/mL]	4.05± 0.02^{a}	8.28± 0.78^{b}	9.11± 0.56^{b}	11.13± 0.27^{bc}	8.25± 0.21^{b}	19.4± 0.09^{c}	21.9± 0.20^{c}	33.4± 0.71^{d}
Antioxidant activity [mmol/L]	14.98± 1.76^{a}	31.27± 1.23^{c}	33.03± 1.02^{c}	36.31± 1.76^{c}	26.3± 1.01^{b}	71.78± 1.98^{d}	74.32± 1.65^{d}	82.45± 1.89^{d}
pH	7.82± 0.76^{a}	7.78± 0.98^{ab}	7.75± 0.02^{b}	7.73± 0.98^{b}	8.08± 0.09^{c}	7.97± 0.97^{d}	7.95± 0.1^{d}	7.92± 0.12^{d}
η [cP]	1.11± 0.97^{a}	1.14± 0.21^{ab}	1.15± 0.05^{ab}	1.21± 0.87^{b}	1.23± 0.11^{b}	1.27± 0.78^{c}	1.31± 0.23^{d}	1.34± 0.55^{d}

THC-White tea hot infusion; TH1-White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3-White tea with chokeberry powder 1% hot infusion; TCC-White tea cold infusion; TC1-White tea with chokeberry powder 0.6% cold infusion; TC2-White tea with chokeberry powder 0.8% cold infusion; TC3-White tea with chokeberry powder 1 % cold infusion; TPC-Total polyphenolic content; η-Viscosity. The results are expressed as the mean value of the three replicates ± the standard deviation (SD). Data with different superscripts reported in the same row are significantly different (one-way ANOVA, $p < 0.05$). Data within a row with the same superscripts are not significantly different (one-way ANOVA, $p > 0.05$).

3.1. Total polyphenolic content

Table 1 presents a summary of the total phenol contents (TPC) of the tea infusions, as determined by Folin-Ciocalteu's reagent. A comparison of the total phenol contents (TPC) of hot and cold teas reveals that the latter consistently exhibits a significantly higher TPC, a phenomenon particularly pronounced in the case of the higher concentration of chokeberry powder TC3, with a TPC of 33.4 ± 0.71 mg GAE/mL. A comparison of the control samples (THC and TCC) revealed that the TPC of white tea obtained by cold extraction (8.25±0.21 mg GAE/mL) was significantly different ($p<0.05$) than that of white tea obtained by hot infusion (4.05±0.02 mg GAE/mL). This suggests that thermal treatment has a significant impact on total phenol content. These values are consistent with those reported in the literature (Damiani et al., 2014; Dasdemir et al., 2023; Perera et al., 2015; Ramalho et al., 2013). The infusion method and fruit concentration have a significant impact on the total phenol content (TPC). Furthermore, an examination of the samples with varying chokeberry powder content revealed a significant increase ($p<0.05$) in total polyphenol (TPC) content in those obtained through both hot and cold infusion methods.

Research on white and green tea infusions reveals that brewing conditions significantly impact the extraction of bioactive compounds and antioxidant capacity. Cold infusion (20-25°C) was found to be more efficient in extracting bioactive compounds compared to

hot infusion (80°C) (de Carvalho Rodrigues et al., 2015). However, brewing at 98°C for 7 minutes yielded optimal antioxidant polyphenols in white tea (Pérez-Burillo et al., 2018). White teas exhibited the highest concentrations of chlorophylls, carotenoids, and total phenolic compounds (Popoviciu & Mălureanu, 2022). Total catechin content varied widely among white and green teas, with some white teas containing comparable levels to green teas (Unachukwu et al., 2010). Particle size also influenced extraction, with milled leaves producing greater antioxidant activity than whole leaves (Castiglioni et al., 2015). Cold brewing for 120 minutes or hot brewing at 90°C resulted in maximum extraction efficiency, particularly for whole, large leaves (Castiglioni et al., 2015).

White teas exhibited the highest concentrations of chlorophylls, carotenoids, and total phenolic compounds (Popoviciu & Mălureanu, 2022).

3.2. Antioxidant activity (ABTS assay)

The antioxidant activity of the tea infusions was evaluated using the ABTS method. As evidenced in Table 1, the ABTS assay results demonstrate that all hot tea infusions exhibit significantly diminished antioxidant activity (14.98-36.31 mmol/L TE) in comparison to cold tea infusions (26.3-82.45 mmol/L TE). The highest antioxidant activity was observed in the case of the cold tea infusion with a 1% chokeberry powder concentration, which exhibited an antioxidant capacity of 82.45 ± 1.89 mmol/L TE. This evolution of antioxidant capacity in cold tea in comparison with hot tea was also observed by other authors (Damiani et al., 2014). Moreover, the antioxidant activity of the samples obtained through hot and cold infusion with an identical chokeberry powder content was found to be significantly different ($p<0.05$).

Research on white tea's antioxidant activity in cold and hot infusions reveals varying results across studies. White tea generally demonstrates high antioxidant capacity, with some studies finding prolonged hot steeping or cold extraction to be most effective (Castiglioni et al., 2015; Hajiaghaalipour et al., 2016). However, one study reported optimal antioxidant activity at 70°C for white tea, decreasing at higher temperatures (Chernousova et al., 2018). Cold extraction (20-25°C) was found to be more efficient in extracting bioactive compounds compared to hot extraction (80°C) in some cases (de Carvalho Rodrigues et al., 2015). Factors influencing antioxidant activity include steeping time, temperature, and particle size, with milled leaves generally yielding higher antioxidant activity than whole leaves (Castiglioni et al., 2015). White tea often exhibits greater antioxidant capacity than black tea and comparable or higher levels than green tea (Chernousova et al., 2018; Hajiaghaalipour et al., 2016). Additionally, some white tea extracts have shown bacteriostatic activity against S. aureus and E. coli (de Carvalho Rodrigues et al., 2015).

3.3. pH measurement

The pH values for both methods fell within the range of 7.73 to 8.08, exhibiting minimal discrepancy. A slight decrease in pH was observed in both types of infusion. The sample TCC exhibited the highest pH value. A reduction in pH was similarly documented by other authors (Lunkes & Hashizume, 2014; S. Zhang et al., 2023). The pH of the white tea hot infusion sample differs significantly from that of the white tea cold infusion sample ($p<0.05$). The hot and cold infusion samples with chokeberry powder do not exhibit a significant difference in pH ($p>0.05$), whereas a significant difference is observed between the hot and cold infusion samples with the same addition of chokeberry powder ($p<0.05$).

pH values of tea infusions were generally mildly acidic, ranging from 3.85 to 6.45 (Kaczmarek, 2004), with white teas showing similar pH levels to other teas, except for highly acidic hibiscus tea (Popoviciu & Mălureanu, 2022).

3.4. Viscosity

In regard to viscosity, it was determined that as the concentration of chokeberry powder increases, the viscosity of the tea for both infusion methods also increase. The lowest viscosity was observed in the case of the hot infusion, with a value of THC, 1.11 ± 0.97 cP. A comparison of the control samples from the hot and cold infusions reveals that the viscosity of the cold tea (TCC: 1.23 ± 0.11) is higher than that of the hot tea (THC: 1.11 ± 0.97 cP). Viscosity decreases as temperature increases. This is due to the fact that the powder particles increase the viscosity of the tea, thus demonstrating that the viscosity of the tea is directly proportional to the concentration of the powder (Pérez et al., 2022). Another explanation is that, as tea heats up, the molecules within the liquid move more quickly, reducing the friction between them, and thus decreasing viscosity. Cold tea, by contrast, will have a slightly higher viscosity than hot tea.

3.5. Correlation of infusion method and chokeberry powder concentration

The statistical analysis of the data indicates that the investigated parameters were affected by two factors: infusion method and chokeberry powder concentration, either independently or in combination. The analysis of variance conducted on the analytical parameters for different infusion methods and chokeberry powder concentrations showed significant differences in total polyphenolic content, antioxidant activity, pH, and viscosity.

It is noteworthy that the correlation coefficient (R) for TPC is 0.935, for pH it is 0.992, and for viscosity it is 0.995. Furthermore, the antioxidant activity also appears to exhibit variability, with a correlation coefficient of 0.958. The adjusted R^2 for the two predictors, infusion method and chokeberry powder concentration, indicates that they can predict 70.8% of the variation in TPC results, 81% of the variation in antioxidant activity results, 96.3% of the variation in pH results, and 97.8% of the variation in viscosity results.

The results of the ANOVA indicate that the model is statistically significant. The predictors introduced into the model, both individually and in combination, exerted a notable influence on the analyzed parameters, as detailed below: The total polyphenolic content was found to be significantly influenced ($p < 0.05$) only by the infusion method, while antioxidant activity, pH, and viscosity were significantly influenced ($p < 0.05$) by both the infusion method and the concentration of chokeberry powder.

3.6. Correlation of the analyzed parameters

Figure 2 illustrates a correlation heat map for Pearson r. Pearson's product-moment correlation coefficient is a measure of the linear relationship between two variables. The correlation analysis enables the estimation of the parameters of the correlation. As can be observed, the heatmap
is symmetric along the diagonal. Furthermore, the color blue represents positive correlation coefficients, while the color red represents negative correlation coefficients. The saturation of colors is indicative of the absolute value of the correlation coefficient. The significant correlations are marked with: *$p < 0.05$ if the correlation is significant at alpha=0.05 level; **$p < 0.01$ if the correlation is significant at alpha=0.01 level and ***$p < 0.001$ if the correlation is significant at alpha=0.001 level.

3.7. Sensory evaluation of tea samples

The success of a novel product or formula is primarily contingent upon consumer demand and acceptability based on sensory perception. To ascertain consumer approval and identify any shortcomings in sensory attributes, a sensory evaluation was conducted in conjunction with an assessment of the product's intrinsic qualities.

The outcomes of the hot and cold infusion processes for all eight samples are presented in Figure 3. The graph demonstrated a positive correlation between color, brightness, clarity, astringency, aroma, and bitterness. The tea with the highest level of appreciation was the one with a 1% concentration of chokeberry powder cold infusion (TC3), which received the

maximum score (5 points) for the majority of the analyzed properties, with the exception of clarity, which received 4 points.

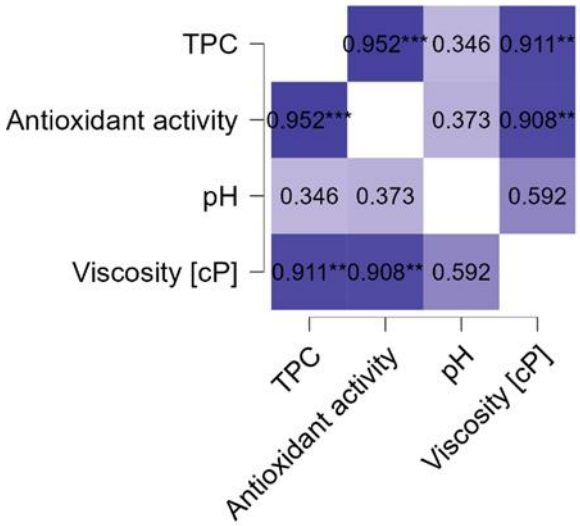


Figure 2. Heatmap for Pearson's r. TPC-Total polyphenolic content

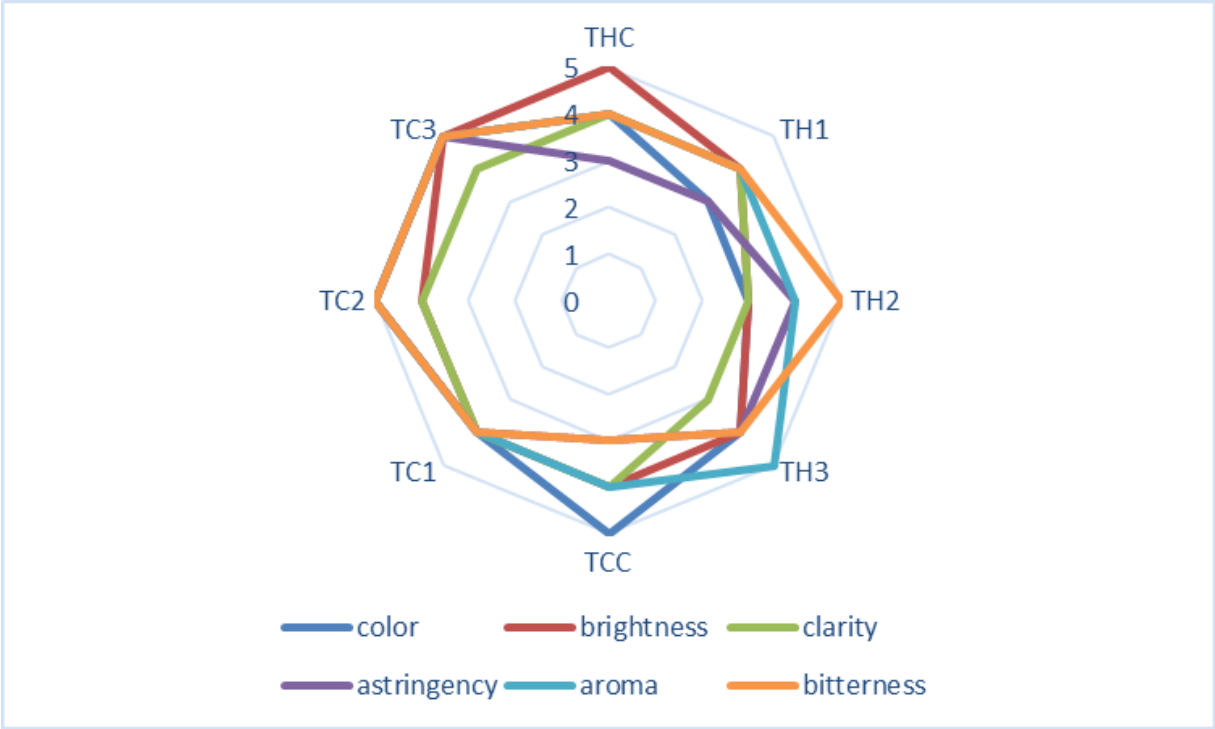


Figure 3. Sensory evaluation of tea samples. THC -White tea hot infusion; TH1- White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3- White tea with chokeberry powder 1% hot infusion; TCC -White tea cold infusion; TC1 - White tea with chokeberry powder 0.6% cold infusion; TC2 - White tea with chokeberry powder 0.8% cold infusion; TC3 - White tea with chokeberry powder 1 % cold infusion

White tea infusions have been studied for their sensory properties and antioxidant content under various brewing conditions. Cold brewing for 120 minutes or hot brewing at 90°C for 7 minutes yielded the highest antioxidant activity, with milled leaves providing greater extraction (Castiglioni et al., 2015). However, whole leaf infusions were preferred in sensory evaluations, particularly for cold-brewed white teas (Castiglioni et al., 2015). Optimal conditions for both antioxidant content and sensory properties were found to be 98°C for 7 minutes (Pérez-Burillo et al., 2018). For Fuding white tea, a 3-minute infusion at 100°C with a 1:50 tea-to-water ratio produced the highest sensory scores (H. Zhang et al., 2017). In cold infusions of Taiwanese teas, consumers could distinguish between unfermented/lightly fermented and heavily/fully fermented teas, with lightly fermented teas preferred for their balanced bitterness, astringency, fresh flavor, and late sweetness (Liu et al., 2021).

4. Conclusion

The findings of the experimental research indicated that cold tea infusion is an effective method for enhancing the active biological properties of tea. A comparison of the total phenol contents (TPC) of hot and cold teas indicates that cold teas consistently have a significantly elevated TPC, most notable in the greater concentration of chokeberry powder TC3, which has a TPC of 33.4 ± 0.71 mg GAE/mL.

It was shown that the application of heat treatment leads to a reduction of these compounds,obtaining a TPC value of 11.13±0.27 mg GAE/mL (TH3) for the highest concentration of aronia powder.

The ABTS assay results indicate that all hot tea infusions display markedly reduced antioxidant activity (14.98-36.31 mmol/L TE) relative to cold tea infusions (26.3-82.45 mmol/L TE). The pH values for both procedures ranged from 7.73 to 8.08, demonstrating negligible variation, with a small reduction in pH noted in both infusion modalities. A comparison of the control samples from the hot and cold infusions indicates that the viscosity of the cold tea (TCC: 1.23 ± 0.11 cP) surpasses that of the hot tea (THC: 1.11 ± 0.97 cP), demonstrating that viscosity diminishes with rising temperature. In the context of sensory analysis, the tea infused with a 1% concentration of chokeberry powder (TC3) achieved the highest score, attaining the maximum rating of 5 points for most of the evaluated attributes.

In conclusion, chokeberry fruits represent a valuable resource for the tea industry, offering multiple health benefits and a distinctive taste and aroma. The exploitation of these fruits can bring advantages from both an economic and a health and sustainability perspective, through the development of a prosperous chokeberry tea sector and the promotion of a healthy and sustainable lifestyle.

5.References

Burdejova, L., Tobolkova, B., & Polovka, M. (2020). Effects of Different Factors on Concentration of Functional Components of Aronia and Saskatoon Berries. *Plant Foods for Human Nutrition*, *75*(1), 83–88. https://doi.org/10.1007/s11130-019-00780-4

Castiglioni, S., Damiani, E., Astolfi, P., & Carloni, P. (2015). Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas. *International Journal of Food Sciences and Nutrition*, *66*(5), 491–497. https://doi.org/10.3109/09637486.2015.1042842

Cheng, L., Wang, Y., Zhang, J., Zhu, J., Liu, P., Xu, L., Wei, K., Zhou, H., Peng, L., Zhang, J., Wei, X., & Liu, Z. (2021). Dynamic changes of metabolic profile and taste quality during the long-term aging of Qingzhuan Tea: The impact of storage age. *Food Chemistry*, *359*, 129953. https://doi.org/10.1016/j.foodchem.2021.129953

Chernousova, O. V., Krivtsova, A. I., & Kuchmenko, T. A. (2018). The study of antioxidant activity of white tea.

Proceedings of the Voronezh State University of Engineering Technologies, *80*(1), 133–139. https://doi.org/10.20914/2310-1202-2018-1-133-139

Chrubasik, C., Li, G., & Chrubasik, S. (2010). The clinical effectiveness of chokeberry: a systematic review. *Phytotherapy Research*, *24*(8), 1107–1114. https://doi.org/10.1002/ptr.3226

Damiani, E., Bacchetti, T., Padella, L., Tiano, L., & Carloni, P. (2014). Antioxidant activity of different white teas: Comparison of hot and cold tea infusions. *Journal of Food Composition and Analysis*, *33*(1), 59–66. https://doi.org/10.1016/j.jfca.2013.09.010

Dasdemir, Y., Findik, B. T., Yildiz, H., & Birisci, E. (2023). Blueberry-added black tea: Effects of infusion temperature, drying method, fruit concentration on the iron-polyphenol complex formation, polyphenols profile, antioxidant activity, and sensory properties. *Food Chemistry*, *410*, 135463. https://doi.org/10.1016/j.foodchem.2023.135463

de Carvalho Rodrigues, V., da Silva, M. V., dos Santos, A. R., Zielinski, A. A. F., & Haminiuk, C. W. I. (2015). Evaluation of hot and cold extraction of bioactive compounds in teas. *International Journal of Food Science & Technology*, *50*(9), 2038–2045. https://doi.org/10.1111/ijfs.12858

Hajiaghaalipour, F., Sanusi, J., & Kanthimathi, M. S. (2016). Temperature and Time of Steeping Affect the Antioxidant Properties of White, Green, and Black Tea Infusions. *Journal of Food Science*, *81*(1). https://doi.org/10.1111/1750-3841.13149

Kaczmarek, U. (2004). [PH values and fluoride levels in some tea brands]. *Annales Academiae Medicae Stetinensis*, *50 Suppl 1*, 58–61.

Kokotkiewicz, A., Jaremicz, Z., & Luczkiewicz, M. (2010). A Review of Traditional Use, Biological Activities, and Perspectives for Modern Medicine. *Journal of Medicinal Food*, *13*(2), 255–269. https://doi.org/10.1089/jmf.2009.0062

Lee, D. H., Jung, J. M., Kim, S. Y., Kim, K. T., & Cho, Y. I. (2012). Comparison tests for plasma viscosity measurements. *International Communications in Heat and Mass Transfer*, *39*(10), 1474–1477. https://doi.org/10.1016/j.icheatmasstransfer.2012.10.018

Liu, S.-L., Jaw, Y.-M., Wang, L.-F., Chuang, G. C.-C., Zhuang, Z.-Y., Chen, Y.-S., & Liou, B.-K. (2021). Evaluation of Sensory Quality for Taiwanese Specialty Teas with Cold Infusion Using CATA and Temporal CATA by Taiwanese Consumers. *Foods*, *10*(10), 2344. https://doi.org/10.3390/foods10102344

Lunkes, L. B. F., & Hashizume, L. N. (2014). Evaluation of the pH and titratable acidity of teas commercially available in Brazilian market. *RGO, Rev. Gaúch. Odontol. (Online)*, *62*(no.1 Porto Alegre).

Olcha, P., Winiarska-Mieczan, A., Kwiecień, M., Nowakowski, Ł., Miturski, A., Semczuk, A., Kiczorowska, B., & Gałczyński, K. (2022). Antioxidative, Anti-Inflammatory, Anti-Obesogenic, and Antidiabetic Properties of Tea Polyphenols—The Positive Impact of Regular Tea Consumption as an Element of Prophylaxis and Pharmacotherapy Support in Endometrial Cancer. *International Journal of Molecular Sciences*, *23*(12), 6703. https://doi.org/10.3390/ijms23126703

Perera, G. A. A. R., Amarakoon, A. M. T., Illeperuma, D. C. K., & Muthukumarana, P. K. P. (2015). Effects of raw material on the chemical composition, organoleptic properties, antioxidant activity, physical properties and the yield of instant black tea. *LWT - Food Science and Technology*, *63*(1), 745–750. https://doi.org/10.1016/j.lwt.2015.03.060

Pérez, E., Alviso, D., Manrique, E., & Artana, G. (2022). Estimation of the rheological curve of HPAM solutions from measurements using the Brookfield viscometer. *Journal of Petroleum Science*

and Engineering, *216*, 110793. https://doi.org/10.1016/j.petrol.2022.110793

Pérez-Burillo, S., Giménez, R., Rufián-Henares, J. A., & Pastoriza, S. (2018). Effect of brewing time and temperature on antioxidant capacity and phenols of white tea: Relationship with sensory properties. *Food Chemistry*, *248*, 111–118. https://doi.org/10.1016/j.foodchem.2017.12.056

Popoviciu, D. R., & Mălureanu, R. A. (2022). Comparative quantitative analysis of some classes of bioactive compounds in some tea sortiments and tea like infusions available on the Romanian market. *Annals of The University of Craiova, Biology, Horticulture, Food Products Processing Technology, Environmental Engineering*, *27*(63). https://doi.org/10.52846/bihpt.v27i63.52

Ramalho, S. A., Nigam, N., Oliveira, G. B., de Oliveira, P. A., Silva, T. O. M., dos Santos, A. G. P., & Narain, N. (2013). Effect of infusion time on phenolic compounds and caffeine content in black tea. *Food Research International*, *51*(1), 155–161. https://doi.org/10.1016/j.foodres.2012.11.031

Re, R., Nicoletta, P., Anna, P., Ananth, P., Min, Y., & Catherine, R.-E. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*((9-10)), 1231–1237.

Sharangi, A. B. (2009). Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) – A review. *Food Research International*, *42*(5–6), 529–535. https://doi.org/10.1016/j.foodres.2009.01.007

Sharma, R., Verma, S., & Kumar, D. (2021). Polyphenolics and therapeutic insights in different tissues extract and fractions of Camellia sinensis (L.) Kuntze (Kangra Tea). *Food Bioscience*, *42*, 101164. https://doi.org/10.1016/j.fbio.2021.101164

Sidor, A., Drożdżyńska, A., & Gramza-Michałowska, A. (2019). Black chokeberry (Aronia melanocarpa) and its products as potential health-promoting factors - An overview. *Trends in Food Science & Technology*, *89*, 45–60. https://doi.org/10.1016/j.tifs.2019.05.006

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth Enzymol*, *299*, 152–178.

Tolić, M.-T., Krbavčić, I., Vujević, P., Milinović, B., Jurčević, I., & Vahčić, N. (2017). Effects of Weather Conditions on Phenolic Content and Antioxidant Capacity in Juice of Chokeberries (Aronia melanocarpa L.). *Polish Journal of Food and Nutrition Sciences*, *67*(1), 67–74. https://doi.org/10.1515/pjfns-2016-0009

Unachukwu, U. J., Ahmed, S., Kavalier, A., Lyles, J. T., & Kennelly, E. J. (2010). White and Green Teas (Camellia sinensis var. sinensis): Variation in Phenolic, Methylxanthine, and Antioxidant Profiles. *Journal of Food Science*, *75*(6). https://doi.org/10.1111/j.1750-3841.2010.01705.x

Webster, D. (2003). pH – Principles and measurement. In *Encyclopedia of Food Sciences and Nutrition* (pp. 4501–4507). Elsevier. https://doi.org/10.1016/B0-12-227055-X/00913-5

Zhang, H., Li, Y., Lv, Y., Jiang, Y., Pan, J., Duan, Y., Zhu, Y., & Zhang, S. (2017). Influence of brewing conditions on taste components in Fuding white tea infusions. *Journal of the Science of Food and Agriculture*, *97*(9), 2826–2833. https://doi.org/10.1002/jsfa.8111

Zhang, S., Li, Q., Wen, S., Sun, L., Chen, R., Zhang, Z., Cao, J., Lai, Z., Li, Z., Lai, X., Wu, P., Sun, S., & Chen, Z. (2023). Metabolomics reveals the effects of different storage times on the acidity quality and metabolites of large-leaf black tea. *Food Chemistry*, *426*, 136601.

https://doi.org/10.1016/j.foodchem.2023.136601

Acknowledgments:

The research was performed in the Food and Tourism Faculty, Transilvania University of Brasov.

Conflicts of Interest:

The authors have no conflict of interest regarding the content of this paper.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

POTENCY OF INDONESIA NATIVE SPICES AS UNPLEASANT SENSORY REMOVER IN HIGH PROTEIN AND FIBER OKARA-BASED SNACK BAR

Aminullah[1]✉, Assyifa Moulidia[1], Muhammad Fakih Kurniawan[1]

[1]Department of Food Technology and Nutrition, Faculty of Halal Food Science, Universitas Djuanda, Bogor, Indonesia
✉aminullah@unida.ac.id

https://doi.org/10.34302/crpjfst/2024.16.4.4

Article history:
Received
August 19th, 2024
Accepted
October 15th, 2024

Keywords:
Cinnamon;
Ginger;
Local Bambara nut;
Pandan leaf;
Rate-All-That-Apply (RATA);
Tofu dregs flour.

ABSTRACT

A snack bar of tofu dregs or okara flour and local Bambara groundnut was the protein and fiber-rich food product; however, it had unpleasant odors. This research aimed to utilize cinnamon, ginger, and pandan leaves to improve snack bar products' sensory profile based on okara and local Bambara groundnut. The method used was Rate-All-That Apply, which included determining sensory attributes through Focus Group Discussion and panelist sensory testing. There were six treatments: the ratio of cinnamon, ginger, and pandan leaf powder of 1% and 2%, respectively, to the formulation. The data analysis used was the Friedman and Nemenyi Test, Principal Component Analysis (PCA), and Preferences Mapping in XLSTAT 2019 software. The results showed that snack bars have 17 sensory attributes, where the attributes of cinnamon taste and aroma, ginger taste and aroma, pandan taste and aroma, and bitter aftertaste have significant differences. In addition, all panelists gave the highest preference for the snack bar by adding 1% pandan leaf powder, which could eliminate unpleasant odors in the snack bar. Its dominant sensory attributes were sweetness, salty taste, nutty flavor, pandan leaf taste and aroma, baked product aroma, and fudgy, crumbly, and starchy textures.

1.Introduction

Snack bars can be categorized as healthy snacks because they have balanced nutrition that can be adjusted according to needs. One of the ingredients that have the potential to be developed into a snack bar product is tofu dregs, soy pulp, or okara. Okara flour contains 45.38% and 21.94% of total dietary fiber and protein, respectively (Rachmayani et al., 2017). When making snack bars, ingredients such as nuts, which are high in nutrients, are often used. Bambara nuts are a type of legume that has the potential to be developed as a high-value local commodity because they are rich in nutrients, and their processing is still limited. Bambara groundnut contains an average of 63% carbohydrates, 19% protein, 6.5% fat, 5% crude fiber, calcium, potassium, iron, nitrogen, and vitamins E, C, and A (Temegne et al., 2018; Anhwange & Atoo, 2015).

The manufacture of okara snack bars have been carried out, including snack bars with tofu pulp and purple sweet potato flour (Rachmayani et al., 2017), tofu and pumpkin flour snack bars (Rohmawati et al., 2018), and snacks bar with the addition of Ambon sale bananas (Yudarsi et al., 2017). These snack bar made of okara flour has a good chemical content. However, it tends to have poor distinctive aroma of okara, which is unpleasant. Subsequent research by Purnama et al. (2019) regarding the okara and local Bambara nut snack bars was done by reformulating the ingredients using wheat flour to reduce the unpleasant aroma of okara flour. In addition, Ahaotu et al. (2021) added that snack bars from Bambara nut and maize had a protein

content of 6.32–15.00% and fiber content of 2.60-3.10%; however, sensory studies in this research also produced sensory values that tended to decrease when the proportion of Bambara nut increased. Based on the research of Purnama et al. (2019), the okara flour and local Bambara nut snack bar has pretty good chemical characteristics that meet the 2019 USDA snack bar standards, which contained 57.18% carbohydrates, 14.40% protein, 15.69% fat, 10.34% dietary fiber, and 427 Kcal/100 grams. However, based on the organoleptic test, the snack bar still had an unpleasant aroma and was not liked by the panelists. This problem can be corrected by adding special flavored spices consumers like, such as cinnamon, ginger, and fragrant pandan leaves.

Several studies regarding the potential of Indonesia native spices, such as cinnamon, ginger, and pandan leaves, as ingredients to increase sensory value in food products. Cardoso-Ugarte et al. (2016) reported that cinnamon usually was used as a flavoring and aroma enhancer in the food industry. Shobur et al. (2021) explained that increasing cinnamon extract in soy milk ice cream could cover the unpleasant aroma of soy milk. Pramitasari et al. (2011) also reported that adding ginger extract to instant powdered soy milk could reduce the unpleasant odor of soy milk. This report was supported by Karseno et al. (2021) and Safitri et al. (2019), who claimed that panelists like adding ginger extract into syrup coconut sap, and mayonnaise products, respectively. Roihanah & Ismawati (2014) also explained that adding pandan leaf extract could increase the preference value of Moringa leaf jelly drink, where it could reduce the unpleasant aroma and bitter taste. Laohakunjit & Kerdchoechuen (2007) also successfully enriched the aroma of non-aromatic rice using natural pandan extract. Utilize cinnamon, ginger, and pandan leaves to improve the sensory profile of an okara flour-based snack bar, which has yet to be previously studied.

In this study, sensory profile analysis was performed using the Rate-All-That-Apply (RATA) method. Ares et al. (2014) stated that the RATA method is a consumer-based sensory evaluation method that provides an intensity rating on sensory attributes that describe the product than CATA (Check-All-That-Apply). Adawiyah et al. (2020) used the RATA method to obtain the sensory profile of table-top sweeteners specifically. While Nurazizah et al. (2021) used the CATA for obtaining sensory profile of black pepper coffee. The research objective was to improve sensory profile of okara based snack bar using cinnamon, ginger, and pandan leaves.

2. Materials and methods

2.1. Sample preparation

Research on making snack bars consisted of three main stages, namely the manufacture of okara flour refers to Yustina & Farid (2012), the manufacture of chopped local Bambara groundnut, and the manufacture of snack bars based on Purnama et al. (2019).

The okara flour was started with a draining or pressing process by manually squeezing it using a filter cloth to separate the water content from speeding up the drying process—furthermore, the steaming okara for 15 minutes at 100 °C. They were then dried in the oven at 60-70 °C for 5 hours. The dried okara was ground using a blender and sifted through 100 mesh to make a uniform size. In addition, the local Bambara groundnut was sorted, washed using clean water, and boiled at 100 °C for 30 minutes. After that, the Bambara groundnut was drained, and the peel was separated. The local Bambara groundnut was then chopped.

Making snack bars starts with weighing the ingredients according to the treatment. The first mixing process was started by mixing the dry ingredients according to the formulation, which consisted of wheat flour, okara flour, chopped Bambara groundnut, cinnamon powder, ginger powder, and pandan leaves powder. The second mixing process was the manufacture of the binder. Caster sugar was added to the margarine and then stirred using a mixer. Then, eggs were mixed at high speed for 5 minutes. Then, the dry ingredients from the first mixing were added and stirred until evenly distributed, and the dough

was molded. Snack bar dough was printed with a size of 7 cm × 2 cm × 1 cm, put in a baking dish, and baked in an oven at 150 °C for 20 minutes. The snack bar formulation can be seen in Table 1.

Table 1. Snack bar formulation (flour base 100 g)*

Ingridients (g)	Formulation					
	A	B	C	D	E	F
Wheat flour	80	80	80	80	80	80
Okara flour	20	20	20	20	20	20
Local Bambara groundnut	20	20	20	20	20	20
Cinnamon powder	1	2	-	-	-	-
Ginger powder	-	-	1	2	-	-
Pandan leaf powder	-	-	-	-	1	2
Caster sugar	45	45	45	45	45	45
Margarine	30	30	30	30	30	30
Egg	30	30	30	30	30	30
Salt	0.5	0.5	0.5	0.5	0.5	0.5

*Purnama et al. (2019)
A: snack bar with addition of 1% cinnamon powder
B: snack bar with addition of 2% cinnamon powder added
C: snack bar with addition of 1% ginger powder
D: snack bar with addition of 2% ginger powder
E: snack bar with addition of 1% pandan leaf powder
F: snack bar with addition of 2% pandan leaf powder

2.2. Determination of sensory attributes

In this study, the determination of sensory attributes was carried out by Focus Group Discussion (FGD) (Dooley et al., 2010). FGDs were conducted to determine snack bar products' sensory attributes and the appropriate lay equivalent in describing sensory attributes to untrained panelists (consumers). The FGD was conducted with six participants who were trained panelists at snack companies and researchers who became moderators. The FGD was conducted online through the Google Meet application to adjust to the Covid-19 pandemic. The FGD activity began with panelists describing each sensory attribute: taste, aftertaste, aroma, and texture of snack bar products. All panelists discussed the overall sensory attributes of the product. After obtaining the results of the sensory attributes, the panelists then discussed the appropriate equivalents that could explain the sensory attributes to consumers as untrained panelists.

2.3. Data retrieval

The consumer panelist selection stage was conducted to get panelists according to the snack bar consumer target. The panelist categories were adolescent panelists with an age range of 13-19 years and adult panelists with an age range of 20-49 years. Locations for data collection were carried out around Bogor, with a total of 50 panelists. Panelists involved in the test must not have a history of allergy to nuts and have previously tried consuming snack bar products. Panelists were selected by filling out questionnaires, which were distributed directly. The questionnaire was designed to determine the background of the panelists to obtain the desired panelist criteria consisting of gender, age, and occupation.

Snack bar products were served in ziplock plastic coded with three-digit random numbers and presented in random order to avoid bias. Panelists tasted each product without comparing it with other products. The serving was equipped with a glass of mineral water to neutralize the mouth during product change. The first test carried out by the panelists was hedonic testing. Panelists tasted the product and carried out hedonic testing with a scale of six levels of preference, namely 1 (disliked very much), 2 (disliked), 3 (did not like it a little), 4 (somewhat liked), 5 (liked), and 6 (liked very much). The next test was the RATA test. Panelists tasted the product again and answered the RATA question, which contained the sensory attributes resulting from the FGD discussion. Panelists evaluated and determined the sensory attributes contained in the product by giving a tick on the intensity level. The intensity level of the sensory attribute used six levels, namely 1 (very weak), 2 (somewhat weak), 3 (moderate), 4 (somewhat strong), 5 (slightly strong), and 6 (very strong). If the attribute was not perceived, then the attribute was left blank and given a score of 0.

2.3. Data analysis

The data was analyzed using the XLSTAT 2019 program, which included several analyses: the Friedman Test, Principal Component Analysis (PCA), and Preference Mapping. The Friedman Test was carried out to identify differences between products on each sensory attribute; if the p-value was less than 5% or there were significantly different attributes in each treatment, it was continued with Nemenyi's post hoc test. PCA analysis was conducted to explain the relationship and correlation between one variable and another observed variable based on several dimensions. Preference Mapping analysis was conducted to understand the direction of the sensory attributes favored by consumers and to obtain product characteristics following consumer preferences in the form of a 2-dimensional contour plot.

3.Results and discussions

3.1. Sensory attributes of snack bar

The description of sensory attributes using an effective qualitative method can be done using the focus group discussion (FGD) method, a systematic and focused group discussion system (Bisjoe, 2018). This discussion focuses on describing the product's sensory attributes, and if there is a different language, then an agreement is made using the same language (Setyaningsih et al., 2010). The results of the discussion on the sensory attributes of snack bar products can be seen in Table 2.

Table 2. Sensory attributes of okara flour snack bar products

Attribute type	Attribute sensory	Note
Taste	Sweet taste	-
	Salt taste	-
	Nutty taste	Peanut taste from local Bambara nut
	Cinnamon taste	typical Cinnamon taste
	Ginger taste	typical Ginger taste
	Pandan leaf taste	typical Pandan leaf taste
	Unpleasant taste	There is an impression of a distinctive taste of okara
Aftertaste	Bitter aftertaste	Bitter taste that lingers in the mouth after being swallowed
Aroma	Baked product aroma	Typical aroma of bakery products
	Unpleleaasant aroma	Typical unpleasant smell of okara
	Cinnamon aroma	Typical Cinnamon spice aroma
	Ginger aroma	Typical ginger aroma
	Pandan leaf aroma	Typical Pandan leaf aroma
Texture	Hard texture	Hard texture when chewed
	Fudgy texture	Wet and soft texture on the inside of the snack bar
	Starchy texture	Texture of full of floury taste in every bite
	Crumbly texture	Crumb texture in the mouth when chewed

3.2. Panelist profile

The consumer panelists involved in this study were 50 people, with a ratio of 70% women and 30% men living in the Bogor area. Agriculture and Agri-Food Canada (2014) stated that women under 35 living in large households and those with children recorded the highest cereal or snack bars consumption. In addition, the distribution of the panelists' ages and professions can be seen in Figure 1.

Figure 1 shows the age distribution of panelists, consisting of 98% adults (20-49 years) and 2% adolescents (13-19 years), and the distribution of panelists' professions consists of 48% students or students, 38% employees, 8% self-employed and 6% housewife. The selection of panelists is targeted at snack bar consumers with busy activities, such as students and employee workers. This selection follows Agriculture and Agri-Food Canada (2014),

which reported that the age range of 16-24 years and 35-44 years was the age range that consumes the most snack bars in the UK. People with busy activities more often consume snack bars classified as healthy snacks. These healthy snack products were more nutritious and practical, so they were suitable for consumption on the sidelines of busy daily activities (Taulabi et al., 2021).

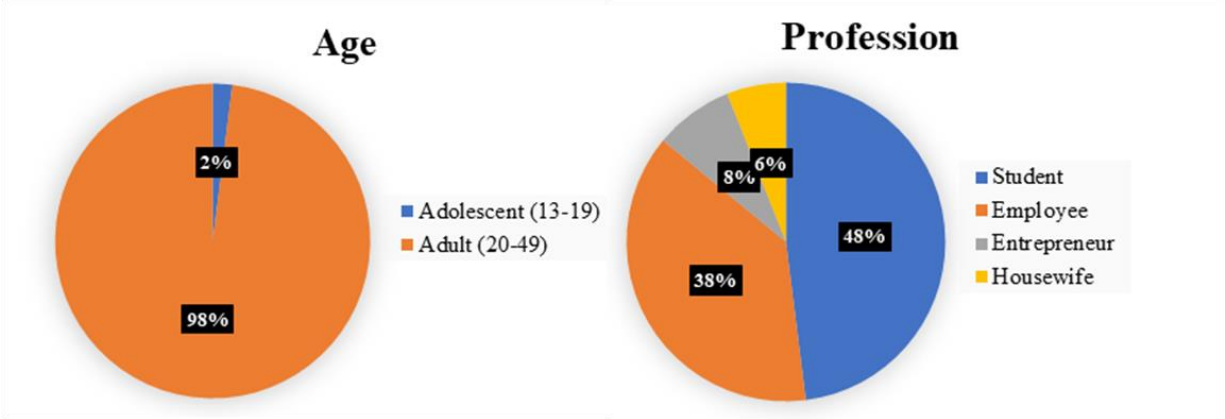


Figure 1. Distribution of panelists' ages and professions

3.3. Sensory profile of snack bar

Sensory profiles and attribute correlations on snack bar products evaluated by panelists were presented with a Principal Component Analysis (PCA) biplot graph and analyzed using the Friedman test at a significant level of 5%. The PCA biplot graph groups the tested products based on their sensory attributes, and the Friedman test shows that the comparison of each attribute for each product is significantly different. Friedman test results can be seen in Table 3.

Table 3. Friedman test of snack bar sensory attribute

Sensory attribute	***p-value***
Sweet taste	0.316
Salt taste	0.405
Nutty taste	0.619
Cinnamon taste	**< 0.0001**
Ginger taste	**<0.0001**
Pandan leaf taste	**< 0.0001**
Unpleasant taste	0.851
Bitter aftertaste	**0.045**
Baked product aroma	0.260
Unpleasant aroma	0.634
Cinnamon aroma	**< 0.0001**
Ginger aroma	**< 0.0001**
Pandan leaf aroma	**< 0.0001**
Hard texture	0.155
Fudgy texture	0.465
Starchy texture	0.771
Crumbly texture	0.287

Note: Numbers in bold print indicate a significant difference between snack bar products on each attribute at a significant level of 5%

Table 3 shows that the attributes of cinnamon taste, ginger taste, pandan taste, bitter aftertaste, cinnamon aroma, ginger aroma, and pandan aroma have significant differences. The comparative treatment of adding cinnamon, ginger, and pandan leaves causes the taste and aroma to be significantly different in snack bar products. The difference in the intensity of adding these spices can affect the bitter aftertaste attributes so that the bitter aftertaste attributes are significantly different for each product.

Meanwhile, the sensory attributes of sweet taste, salty taste, nutty taste, unpleasant taste, the aroma of baked products, unpleasant aroma, hard texture, fudgy texture, starchy texture, and crumbly texture are not significantly different for each product. The attributes are then further tested by Nemenyi post hoc. The results of Nemenyi post hoc test analysis can be seen in Table 4.

Table 4. The intensity value of the sensory attributes of snack bar

Attribute	Product sample					
	A	B	C	D	E	F
Sweet taste	2.78[a]	2.82[a]	2.72[a]	2.66[a]	2.90[a]	2.74[a]
Salt taste	2.40[a]	2.32[a]	2.42[a]	2.20[a]	2.36[a]	2.34[a]
Nutty taste	3.00[a]	2.94[a]	3.00[a]	3.14[a]	3.14[a]	3.24[a]
Cinnamon taste	**3.16[b]**	**3.78[b]**	**0.92[a]**	**1.06[a]**	**0.60[a]**	**0.74[a]**
Ginger taste	**0.94[a]**	**0.82[a]**	**3.14[b]**	**3.74[b]**	**0.50[a]**	**0.72[a]**
Pandan leaf taste	**0.60[a]**	**0.60[a]**	**0.60[a]**	**0.56[a]**	**2.88[b]**	**3.76[b]**
Unpleasant taste	1.66[a]	1.66[a]	1.66[a]	1.64[a]	1.50[a]	1.66[a]
Bitter aftertaste	**1.12[a]**	**1.18[b]**	**0.90[a]**	**1.16[b]**	**0.88[a]**	**1.00[a]**
Baked product aroma	2.76[a]	2.68[a]	2.74[a]	2.62[a]	2.86[a]	2.78[a]
Unpleasant aroma	1.54[a]	1.58[a]	1.72[a]	1.64[a]	1.54[a]	1.48[a]
Cinnamon aroma	**3.10[b]**	**3.38[b]**	**0.92[a]**	**1.08[a]**	**0.76[a]**	**0.80[a]**
Ginger aroma	**0.82[a]**	**0.84[a]**	**2.82[b]**	**3.34[b]**	**0.62[a]**	**0.64[a]**
Pandan leaf aroma	**0.56[a]**	**0.60[a]**	**0.68[a]**	**0.56[a]**	**2.84[b]**	**3.62[b]**
Hard texture	2.52[a]	2.64[a]	2.40[a]	2.38[a]	2.62[a]	2.42[a]
Fudgy texture	2.30[a]	2.40[a]	2.48[a]	2.28[a]	2.36[a]	2.46[a]
Starchy texture	2.86[a]	2.82[a]	2.84[a]	2.76[a]	2.86[a]	2.84[a]
Crumbly texture	2.62[a]	2.68[a]	2.54[a]	2.60[a]	2.86[a]	2.64[a]

Note: different superscript letters in the same line show a significant difference at the 5% level
A: snack bar with addition of 1% cinnamon powder
B: snack bar with addition of 2% cinnamon powder
C: snack bar with addition of 1% ginger powder
D: snack bar with addition of 2% ginger powder
E: snack bar with addition of 1% pandan leaf powder
F: snack bar with addition of 2% pandan leaf powder

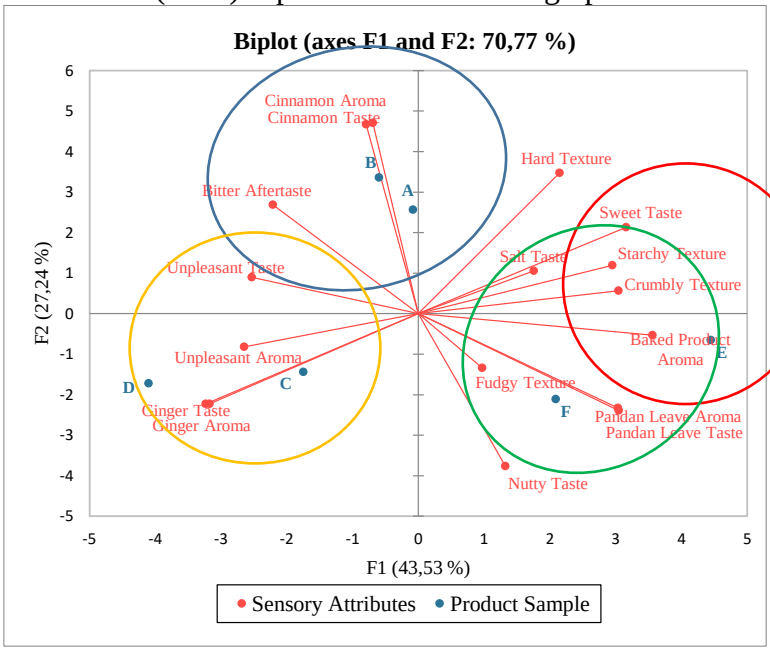
Table 4 shows a tendency for snack bar products added with spices with a higher concentration (2% concentration) to have a higher intensity of bitter aftertaste. Drewnowski & Gomez-Carneros (2000) explained that phenolic compounds are responsible for the bitterness and astringency of many foods and beverages. Muchuweti et al. (2007) and Aravind et al. (2012) reported that cinnamon has a relatively high phenolic component. Prasad & Tyagi (2015) reported that ginger has large amounts of phenolic compounds such as gingerols, shogaols, paradols, and flavonoids. Quyen et al. (2020) also reported that pandan leaves contain many phenolic compounds, such as alkaloids, flavonoids, and terpenoids. However, according to Yan & Asmah (2010), the content of phenolic compounds in pandan leaves is lower than in ginger.

Table 4 also shows that the sensory attributes of taste and aroma of snack bars are significantly different. However, it was also seen that the concentrations of 1% and 2% did not differ significantly based on the panelists' assessment of the aroma and taste parameters of both spices on the snack bar, with cinnamon, ginger, and pandan leaves added. The distinctive aroma and taste of cinnamon come from the components of essential oils and oleoresin, which can give cinnamon a distinctive aroma and taste. The cinnamaldehyde compound gives a slightly spicy-sweet taste and aroma, typical of cinnamon (Prasetyaningrum et al., 2012). The aroma of ginger rhizome comes from the content of essential oils, while the spicy taste of ginger

is caused by gingerol compounds (Setyaningrum & Saparinto, 2013). Meanwhile, the distinctive aroma of pandan leaves is caused by the volatile oil content derived from the chemical compound 2-Acetyl-1-Pyrroline (ACPY) (Murtini et al., 2020).

The PCA biplot can describe the interaction on each sensory attribute more clearly, which shows the close relationship between each sensory attribute assessed. The PCA biplot of the cinnamon, ginger, and pandan leaf spice snack bars can be seen in Figure 2.

Figure 2 shows the total diversity of 70.77%. McPherson (2011) explained that the PCA graph with a diversity of 70% was considered good enough and valid to explain the data variables well. In addition, there are four groups (quadrants) for attributes, namely 1) Quadrant I: attributes of hard texture, sweet taste, salty taste, starchy texture, and crumbly texture; 2) Quadrant II: attributes of cinnamon taste, unpleasant taste, bitter aftertaste, and cinnamon aroma; 3) Quadrant III: attributes of ginger taste, ginger aroma and unpleasant aroma; and 4) Quadrant IV: attributes of pandan leaf taste, peanut flavor, baked product aroma, pandan leaf aroma, and fudgy texture.



A: snack bar with addition of 1% cinnamon powder
B: snack bar with addition of 2% cinnamon powder
C: snack bar with addition of 1% ginger powder
D: snack bar with addition of 2% ginger powder
E: snack bar with addition of 1% pandan leaf powder
F: snack bar with addition of 2% pandan leaf powder

Figure 2. PCA biplot graph of sensory attributes and snack bar sample

Gower et al. (2011) explained that a slight angle (less than 90°) indicates the variables were positively correlated. So, suppose attributes are positively correlated with other attributes when these attributes experience an increase in intensity. In that case, it can be perceived that one of the other attributes will experience an increase in intensity. On the other hand, points not in a different quadrant or far from each other and do not form an angle of less than 90º from the center point indicate that the attribute has a negative correlation or does not have a correlation. Variable points that form small vector angles show a positive correlation, and product points in a close position or the same quadrant have the same characteristics (Apandi et al., 2016).

Cinnamon, ginger, and pandan leaf taste attributes increase when cinnamon, ginger, and pandan leaf aroma attributes increase, respectively. The bitter aftertaste and the unpleasant taste attributes positively correlate with the cinnamon and ginger taste and aroma attributes. It can be perceived that the bitter aftertaste and the unpleasant taste attributes will increase if the intensity of the cinnamon and the ginger taste and aroma attributes increase. This is related to cinnamon and ginger, which have

cinnamaldehyde and shogaol compounds that could cause off-flavors such as a bitter aftertaste (Dwijatmoko et al., 2016; Widiantoko & Yunianta, 2014).

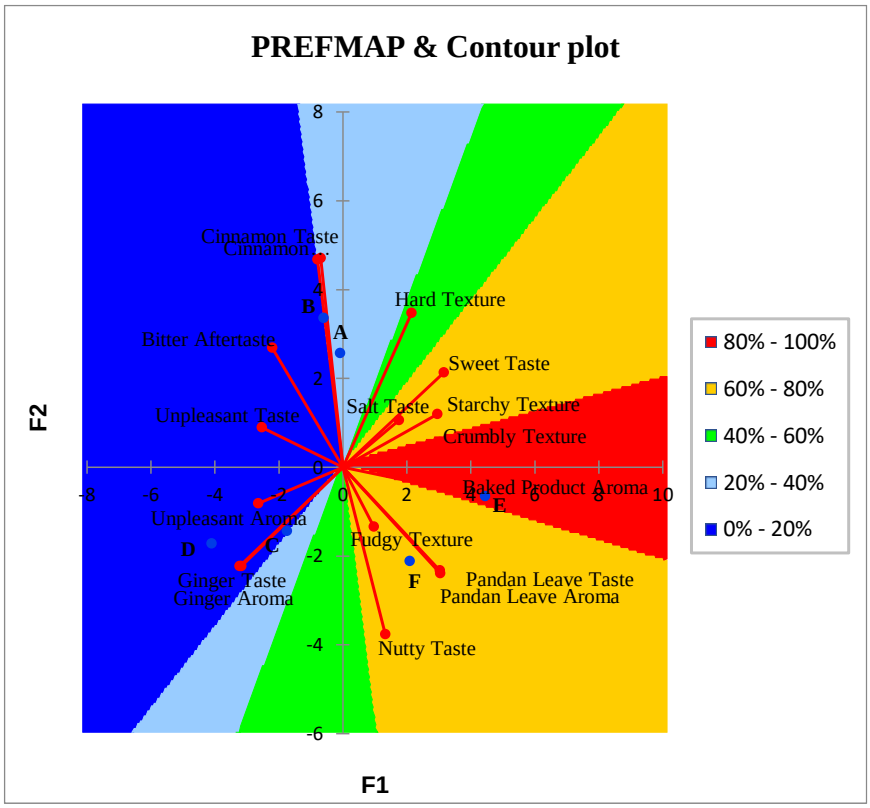
There is a positive correlation between the unpleasant taste attributes and the taste and aroma attributes of cinnamon and ginger. Adding cinnamon powder and ginger powder from okara flour to the snack bar still does not entirely cover the unpleasant taste of okara. The unpleasant aroma attribute is also related to ginger taste and aroma attributes, which are positively correlated. This relation implies that adding ginger powder to the okara snack bar has not been effective in reducing the unpleasant aroma of okara. However, the unpleasant aroma attribute with the cinnamon taste and aroma attributes are negatively correlated, so it can be perceived that adding cinnamon powder to the okara snack bar effectively reduces the unpleasant aroma of okara flour. This correlation also applies to the taste and aroma of pandan leaves, where Figure 2 shows that pandan leaf taste and aroma have negatively correlated to the unpleasant taste and aroma.

In addition, Figure 2 shows that sweetness positively correlates with the aroma and taste of pandan leaves and cinnamon aroma and taste. They can support the sweet taste of the snack bar. USDA (2019) reported that cinnamon had a sugar content of 2.17 g/100 g ingredients, so adding this spice can increase the sweetness level of snack bar products. In addition, Cheetangdee & Chaiseri (2006) reported that fresh pandan leaves contained 2.38 mg/g fructose and 1.77 mg/g glucose. While the attributes of ginger aroma and taste have a negative correlation with the sweet taste attribute, it can be perceived that the higher the intensity of the ginger taste and aroma attributes, the lower the intensity of the sweet taste attribute. The spicy taste created by the phenolic compounds in ginger will reduce the sweet taste. This reduction is also confirmed by Karseno et al. (2021), who stated that adding ginger extract could reduce the product's sweetness.

In addition to the relationship between one attribute and another, Figure 2 shows that the snack bar adding 1% and 2% cinnamon has dominant sensory attributes of cinnamon aroma and taste, bitter aftertaste, and unpleasant taste. At the same time, the sensory attributes of ginger aroma and taste, as well as unpleasant aroma and taste, are the dominant attributes of the snack bar, with the addition of 1% and 2% ginger. Figure 2 also shows that snack bars with 1% pandan leaf powder have sensory attributes of pandan leaf aroma and taste, baked product aroma, starchy texture, crumbly texture, sweet taste, and salty taste. The snack bar with 2% pandan leaf powder added has pandan leaf aroma and taste, fudgy texture, baked product aroma, nutty flavor, crumbly texture, and salty taste.

The panelist's preference map is obtained from the preference mapping analysis. The preference mapping analysis results are described in the form of a contour plot. The contour plot positions the product based on the panelist's preference value above the average. The contour plot is divided into five color areas, each giving a different interpretation of the above-average preference value. The results of the preference mapping analysis are shown in Figure 3.

Based on Figure 3, the panelist's preference map can be seen as the attributes that affect the panelist's preference for the product. The attribute highly favored by panelists with a preference value percentage above the average of 80-100% was in the red area, namely the aroma attribute of the baked product. Preferred attributes with a preference value percentage above an average of 60-80% are in the yellow area, namely the attributes of sweet taste, salty taste, nutty taste, pandan taste, pandan aroma, starchy texture, crumbly texture, and fudgy texture.



A: snack bar with addition of 1% cinnamon powder
B: snack bar with addition of 2% cinnamon powder
C: snack bar with addition of 1% ginger powder
D: snack bar with addition of 2% ginger powder
E: snack bar with addition of 1% pandan leaf powder
F: snack bar with addition of 2% pandan leaf powder

Figure 3. Panelists' preferences map of snack bars

Attributes with a preference value percentage above the average of 40-60% are in the green area, namely the hard texture attribute. Attributes not favored by panelists with a percentage of preference values above an average of 0-20% are in the dark blue area, namely the attributes of cinnamon taste, ginger taste, unpleasant taste, cinnamon aroma, ginger aroma, unpleasant aroma, and bitter aftertaste.

In addition, Figure 3 shows that snack bar products with the addition of 1% and 2% pandan leaf powder are located in red and yellow areas, respectively. This result shows that adding pandan leaf powder can increase consumer acceptance of snack bars based on okara flour and local Bambara nuts. These data are also supported by PCA analysis data that snack bars of 1% and 2% pandan leaf powder can remove the unpleasant taste and aroma, which, in the research of Purnama et al. (2019), became a problem for snack bar products from okara flour. In addition, this snack bar, with pandan leaf powder, tends not to leave a bitter aftertaste when consumed. The snack bar, with the addition of 1% cinnamon powder, is in the light blue area, which means that 40% of the panelists gave a preference value above the average. The snack bar, with the addition of 2% cinnamon powder, 1% ginger powder, and 2% ginger powder, is in the dark blue area, which means that as many as 20% of the panelists gave a preference value above the average. Based on the percentage of panelists' acceptance that gives a preference value above the average, it can be perceived that the higher the intensity of adding spices, the lower the preference level of the panelists. Also, the panelists' acceptance value of snack bar products, with the addition of 1%

pandan leaf powder, is the highest percentage. It has been selected based on the panelists' preference values. This snack bar has a sensory profile of sweet taste, salty taste, pandan leaf, peanut flavor, baked aroma product, pandan leaf aroma, fudgy texture, crumbly texture, and starchy texture.

4. Conclusions

Based on the results of research that has been carried out, it was known that from the Friedman test at the 5% test level, the effect of adding cinnamon, ginger, and pandan leaves powder was significantly different on the sensory characteristics of cinnamon taste and aroma, ginger taste and aroma, pandan leaf taste and aroma, and bitter aftertaste. However, the addition was not significantly different in the attributes of sweet taste, salt taste, nutty taste, unpleasant taste, aroma of baked products, unpleasant aroma, hard texture, fudgy texture, starchy texture, and crumbly texture. PCA analysis and preference mapping showed that adding pandan leaf powder to the okara flour snack bar was better than adding cinnamon and ginger. As many as 80-100% of panelists gave a preference value above the average on the snack bar, adding 1% pandan leaf powder, which has dominant sensory attributes of sweet taste, salty taste, peanut taste, pandan leaf taste, pandan leaf aroma, baked product aroma, fudgy texture, crumbly texture, and starchy texture. Attributes that affected panelists' preferences were the aroma of baked products, sweetness, salty taste, peanut flavor, pandan taste, pandan aroma, starchy texture, crumbly texture, and fudgy texture.

5. References

Adawiyah, D.R., Tjiptoputri, O.M., Lince, L. (2020). Sensory profile of table-top sweeteners by Rate-All-That-Apply (RATA) Method. Jurnal Mutu Pangan: Indonesian Journal of Food Quality, 7(1), 2020, pp. 38-45. In Indonesian.

Agriculture and Agri-Food Canada. (2014). Consumer trends snack bar in the United Kingdom. Global Analysis Report. Ministry of Agriculture and Agri-Food, Canada.

Ahaotu, I., Ichendu, M.O., Maduka, N. (2021). Microbiological, nutritional and sensory evaluation of snack bars developed using Bambara groundnut (Vigna subterranean L.) and maize (Zea mays). *African Journal of Microbiology Research*, 16(1), 8-23.

Apandi, I., Fajar, R., Yusmarini. (2016). Consumers preference mapping analysis for the sensory attribute of soyghurt product within the student Agricultural Faculty of University of Riau. *Jurnal Online Mahasiswa Fakultas Pertanian Universitas Riau*, 3(1), 1-16. In Indonesian.

Anhwange, B.A., Atoo, G.H. (2015). Proximate composition of indigenous bambara nuts (Vigna subterranean (L.) Verdc). *SCSR Journal of Pure and Applied Sciences*, 2(1), 11-16.

Aravind, R., Aneesh, T., Bindu, A., Bindu, K. (2012). Estimation of phenolics and evaluation of antioxidant activity of Cinnamomum malabatrum (Burm. F). Blume. *Asian Journal of Research in Chemistry*, 5(5), 628–632.

Ares, G., Bruzzone, F., Vidal, L., Cadena, R.S., Gimenez, A., Pineau, B., Hunter, D.C., Paisley, A.G., Jaeger, S.R. (2014). Evaluation of a rating-based variant Check-All-That-Apply questions: Rate-All-That-Apply (RATA). *Journal Food Quality and Preferences*, 36, 87-95.

Bisjoe, A.R.H. (2018). Menjaring data dan informasi penelitian melalui FGD (Focus Group Discussion): Belajar dari praktik lapang. *Buletin Eboni*, 15(1), 17-27. In Indonesian.

Cardoso-Ugarte, G. A., López-Malo, A., Sosa-Morales, M. E. (2016). Cinnamon (Cinnamomum zeylanicum) essential oils. *Essential Oils in Food Preservation, Flavor and Safety*, 339–347.

Cheetangdee, V., Chaiseri, S. (2006). Free amino acid and reducing sugar composition of pandan (Pandanus amaryllifolius) leaves. *Kasetsart Journal - Natural Science*, 40, 67-74.

Dooley, L., Lee, Y.S,, Meullenet, J. F. (2010). The application of check-all-that-apply (CATA) consumer profiling to preference mapping of vanilla ice cream and its comparison to classical external preference mapping. *Food Quality and Preference*, 21(4), 394–401.

Drewnowski, A., Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: A review. *The American Journal of Clinical Nutrition*, 72(6), 1424–1435.

Dwijatmoko, M.I., Danar, P., Dimas, R.M. (2016). Effect of cinnamon essential oils addition in the sensory attributes of dark chocolate. *Journal Nusantara Bioscience*, 8(2), 301-305. In Indonesian.

Gower, J. C., Lubbe, S. G., Le Roux, N. J. (2011). Understanding Biplots. London: John Wiley & Sons.

Karseno, Setyawati, R., Haryanti, P. Tri Yanto. (2021). Addition of selected ginger extract on total phenolic, antioxidant and sensory properties of the syrup coconut sap (Ginger – SCS). *Food Research*, 5(6), 283 – 289.

Laohakunjit, N., Kerdchoechuen, O. (2007). Aroma enrichment and the change during storage of non-aromatic milled rice coated with extracted natural flavor. *Food Chemistry*, 101, 339-344.

McPherson, G. (2011). Applying and Interpreting Statistics: A Comprehensive Guide. New York (US): Springer-Verlag.

Muchuweti, M., Kativu, E., Mupure, C.H., Chidewe, C., Ndhlala A.R., Benhura, M.A.N. (2007). Phenolic composition and antioxidant properties of some spices. *American Journal of Food Technology*, 2, 414-420.

Murtini, E.S., Yuwono, S.S., Setyawan, H.Y., Nadzifah, N. (2020). Pandan leaf powder: Characteristics and its application in pandan sponge cake making. *IOP Conference Series: Earth and Environmental Science*, 475, 012041.

Nurazizah, I., Nur'utami, D.A., Aminullah. (2021). Application of check-all-that-apply (CATA) in sensory profile assessment of arabica dark roast and black pepper mixed coffee. *Future of Food: Journal on Food, Agriculture and Society*, 9(4), 1-12.

Pramitasari, D., Anandhito, R.B.K., Fauza, G. (2011). The addition of ginger extract in making soymilk powder by spray drying method: Chemical constituents, sensory characteristic and antioxidant activity. *Biofarmasi*, 9(1), 17-25. In Indonesian.

Prasad, S., Tyagi, A.K. (2015). Ginger and its constituents: Role in prevention and treatment of gastrointestinal cancer. *Gastroenterology Research and Practice* 2015, 1-11.

Prasetyaningrum, Utami, R., Anandito, R.B.K. (2012). Antioxidant activity, total phenolic content, and antibacterial activity of cinnamon bark oil and oleoresin (Cinnamomum burmanii). *Jurnal Teknosains Pangan*, 1(1), 24-31. In Indonesian.

Purnama, H., Hutami, R., Novidahlia, N. (2019). Karakteristik fisikokimia dan sensori snack bar ampas tahu dengan penambahan kacang bogor. *Jurnal Ilmu Pangan Halal*, 1(2), 75-82. In Indonesian.

Quyen, N.T.C, Quyen, N.T.N, Nhan, L.T.H., Toan, T.Q. (2020). Antioxidant activity, total phenolics and flavonoids contents of Pandanus amaryllifolius (Roxb.). *IOP Conference Series: Materials Science and Engineering*, 991, 012019.

Rachmayani, N., Rahayu, W.P., Faridah, D.N., Syamsir, E. (2017). Snack bar tinggi serat berbasis tepung ampas tahu (okara) dan tepung ubi ungu. *Jurnal Teknologi dan Industri Pangan*, 28(2), 139-149. In Indonesian.

Rohmawati, M.G., Yannie, A.W., Akhmad, M. (2018). Utilization of tofu by products for snack bars with the addition of pumpkin flour (Cucurbita moschata Durch) and various types of sugar. *Jurnal Teknologi dan Industri Pangan*, 3(1), 1–9. In Indonesian.

Roihanah, M., Ismawati, R. (2014). The effect of amount carrageenan and pandan leafextract (Pandanus amaryllifolius) on the organoleptic characteristic of moringa leaf

jelly drink (Moringa oleifera). *E-Journal Boga*, 3(3), 96-105. In Indonesian.

Safitri, A.R., Evanuarini, H., Thohari, I. (2019). The effect of ginger extract on emulsion stability, total acidity and sensory evaluation of full fat mayonnaise. *International Research Journal of Advanced Engineering and Science*, 4(4), 284-289.

Setyaningrum, H.D., Saparinto, C. (2013). Jahe. Depok: Penebar Swadaya. In Indonesian.

Setyaningsih, D., Anton, A., Maya, P.S. (2010). Analisis Sensori untuk Industri Pangan dan Argo. Bogor: IPB Press. In Indonesian.

Shobur, F., Wikanastri, H., Yunan, K.S. (2021). Physical, chemical and sensory properties of soy milk ice cream with the addition of cinnamon extract. *Jurnal Pangan dan Gizi*, 7(1), 73-87. In Indonesian.

Taulabi, M.S.D., Oessoe, Y.E., Sumual, M.F. (2021). Study of the chemical composition of snack bars from various local raw materials: Systematic review. *Agrio-Sosio Ekonomi*, 5(17), 15-20. In Indonesian.

Temegne, N. C., Gouertoumbo, W. F., Wakem, G. A., Nkou, F. T. D., Youmbi, E., Ntsomboh-Ntesefong, G. (2018). Origin and ecology of bambara groundnut (Vigna Subterranea (L.) Verdc: A review. *Journal of Ecology and Natural Resources,* 2(4), 1-10.

USDA United States Department of Agriculture (2019). Spices, cinnamon, ground. https://fdc.nal.usda.gov/fdc-app.html#/food-details/171320/nutrients

Widiantoko, R.K., Yunianta. (2014). The making of ice cream from tempe and ginger (study of raw materials proportion and stabilizers proportions on the physical, chemical and organoleptic properties). *Jurnal Pangan dan Agroindustri*, 2(1), 54-66. In Indonesian.

Yan, S.W., Asmah, R. (2010). Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *International Food Research Journal*, 17, 417-423.

Yudarsi, D., Ali, A., Ayu, D.F. (2017). Utilization of okara flour with addition of ambon banana sale in making snack bars. *Jurnal Teknologi Pertanian*, 4(2), 2-15. In Indonesian.

Yustina, I., Farid, R.A. (2012). Potensi tepung dari ampas industri pengolahan kedelai sebagai bahan pangan. In: Prosiding Seminar Nasional Kedaulatan Pangan dan Energi, Fakultas Pertanian, Universitas Trunojoyo Madura; Juni 2012. In Indonesian.

Acknowledgement

We would like to thank the Faculty of Halal Food Science and the Department of Food Technology and Nutrition for completing this research.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

INSTANT GERMINATED VD20 RICE CULTIVATED IN VIETNAM: EFFECT OF COOKING CONDITIONS

Le Thi Kim Loan[1,✉], Truong Quoc Tat[2], Pham Do Trang Minh[1], Vo Thi Thu Thao[1], Pham Thi Minh Hoang[1], Tran Thi Yen Nhi[3], Bach Long Giang[3], Dao Tan Phat[3], Ngo Van Tai[4,✉]

[1]Faculty of Agriculture and Food Technology, Tien Giang University, My Tho city, Tien Giang, Vietnam
[2]Faculty of Technology and Engineering, Dong Thap University, Pham Huu Lau Street, Cao Lanh City, Dong Thap, Vietnam
[3]Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam
[4]School of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
✉ngovantai1509@gmail.com & ✉lethikimloan@tgu.edu.vn

https://doi.org/10.34302/crpjfst/2024.16.4.5

Article history:
Received
August 27th, 2024
Accepted
October 7th, 2024

Keywords:
Instant rice;
Germination;
Cooking;
Physical properties;
Sensory evaluation.

ABSTRACT

Instant rice is ideal for convenient use as a low-moisture, lightweight ration and emergency food in our fast-paced lifestyle. It has a longer shelf life and can be quickly rehydrated. This study examined the impact of various cooking circumstances, such as the ratio of rice to water and the kind and concentration of food additives, on the physical and sensory characteristics of quick germinated VD20 rice. Using the correct ratio of rice to water could decrease the percentage of broken cooked grains, increase porosity and volume expansion, and improve customer satisfaction. The study revealed that germinated VD20 rice cooked with a water-to-rice ratio of 1:2 had superior sensory qualities, as well as an appealing appearance when compared to both cooked and quick rice. Furthermore, the notable impact of food additives, specifically sodium bicarbonate and sodium chloride, on the characteristics of instant rice was noticed. Sodium bicarbonate adversely affects the organoleptic characteristics and causes structural damage and dark yellowing in instant rice. In a theoretical sense, the addition of 0.5% NaCl as a supplement can improve the physical characteristics of rice, resulting in instant rice with a well-formed structure and a higher rate of acceptance compared to the control sample which does not contain any food additives. This study is the first to explore the manufacturing of instant rice from germinated products. It aims to provide fundamental knowledge for future research on utilizing the high nutritional value of these sources and optimizing the production process.

1. Introduction

Germinated rice is a type of rice created from brown rice raw materials. Due to the germination process, rice is enhanced nutritional profiles, including vitamins E, PP, B1, B6, magnesium... especially gamma amino butyric acid (GABA). GABA is a free amino acid neurotransmitter, which have potential to regulates blood pressure, accelerates metabolism in the brain, prevents loss of control of some hormones during aging and perimenopause and beneficial for Alzheimer's patients. In addition to GABA compound, in germinated rice also contains γ-oryzanol, a ferulic acid with antioxidant effects that prevents skin aging and regulates blood cholesterol levels (Hepsomali et al., 2020). GABA is a water-soluble non-protein amino acid, it exists in small amounts in some plants used as food such as vegetables (spinach, potatoes, cabbage, asparagus, cauliflower and eggplant), sour fruits (apples and grapes), some

grains (oats and corn) and in beans (Maqbool et al., 2017). GABA has been shown to have many benefits for animal bodies such as reducing blood pressure, reducing blood cholesterol (Inoue et al., 2003). Furthermore, it also has the effect of inhibiting neurotransmitter impulses in the central nervous system, effectively preventing pain, reducing stress and anxiety states as well as inhibiting the development of tumors of cancer cells (Huang et al., 2022). Therefore, research and development of GABA-rich food products has become a potential trend and is increasingly expanding.

Along with the continuous development of today's society, fast food products are becoming more and more popular and increasingly have a place in the hearts of consumers because of the convenience they bring. Rice is the main ingredient that needs to be supplied to the body every day. One of the rice varieties that has been successfully restored in Tien Giang is the VD20 rice variety, a rice variety with good quality thanks to its high quality, fragrant and sticky rice, suitable for consumer tastes. As mentioned earlier, germinated VD20 rice also has the potential to development into products, and one of them is instant rice. Instant rice is becoming increasingly popular as a result of people's hectic and fast-paced lifestyles (Loan et al., 2023; Yadav et al., 2023). Yadav et al. (2023) reported that the manufacturing techniques employed to produce instant rice are as follows: The three methods mentioned in the literature are as follows: (a) precooking the rice grain followed by sterilization; (b) precooking the rice grain followed by drying; (c) extruding rice flour followed by drying. The most easy method is the precooking and then drying (Lin & Li, 2023). When the rice kernel is boiled in extra water to attain full translucence, its swelling ratio is approximately 3–3.5, signifying that the rice kernel can absorb up to 2–2.5 times its weight in water. In a constrained water system (water-to-rice ratio of 1.4), all water is absorbed by the rice kernel. While comparable water absorption occurs for rice grains cooked for 12 minutes and 35 minutes, the distribution of water is more uniform with extended cooking duration (Hsu et al., 2015; Kasai et al., 2005). Consequently, the cooking temperature enhances the rate of water diffusion within the rice kernel. Moreover, the water absorbed by the starch granules leads to the substitution of hydrogen bonds between starch molecules with hydrogen bonds between starch and water molecules during gelatinization. Hot-air drying, a traditional and cost-effective method for dehydrating rice kernels, influences the quality and starch digestibility of rehydrated instant rice (Lin & Li, 2023). Limited research has systematically explored the effects of rice variety, cooking parameters, retrogradation, and annealing on the starch digestibility of instant rice. Especially, there was no study on production instant rice from the germinated grain. Therefore, the aim of this study was to determine the effect of cooking process (usage of water and effect of some food additives) on physical characteristics and sensory properties of instant germinated rice. Research on the process of producing instant rice from VD20 germinated rice is carried out with the aim of both meeting consumer needs, diversifying products and generating income for farmers from rich nutritional value material in Tien Giang province (Vietnam).

2. Materials and methods

2.1. Materials

A sample of VD20 rice varieties was obtained from a local farmer in Tien Giang province, Vietnam. The paddy sample underwent a comprehensive cleaning process to eliminate dust particles and was then de-husked using a Dehusker machine for subsequent analysis. The germination process followed the previous established method of our group (Loan & Thuy, 2019).

2.2. Experimental design

Weigh 300 g of germinated rice (for each treatment) and wash it with clean water. Then, add water at the rate according to the experimental setup (1:1, 1:1.25, 1:1.5, 1:1.75, 1:2 for ratio of rice and water), and cook with a rice cooker with a capacity of 1.2 liters (Sunhouse SHD8217W, Vietnam). Cooking

time was determined when the pot turned on the "cook" button and the cooked rice was left to stabilize for another 5 minutes. After the rice was cooked, let it cool and then put it in the oven dryer at 60^{o}C until the humidity was reached ≤13% (Loan et al., 2023). Sample was put in PA packaging to keep it stable for at least 3 days, then take 20 g for reconstituting with 45 ml of boiling water for a certain time and then determine the physical and sensory characteristics.

After selecting the appropriate conditions, the study of effect of sodium bicarbonate ($NaHCO_3$)/sodium clorua (NaCl) on the physical and sensory characteristics was conducted. 300 g germinated rice also was used for this study. The ratio of rice and water was chosen from previous experiment. In this study, different concentrations of $NaHCO_3$ (0.1, 0.2, 0.3%) and NaCl (0.5, 1.0, 1.5%) were added in the cooking water, sample without added additive consider as control sample. The cooking procedure also was same as earlier mentioned.

2.3. Analysis of nutritional profile of germinated VD20 rice

The proximate composition (moisture, protein, fat, ash and fiber) of the rice samples were estimated by standard AOAC protocols (AOAC, 2005). The carbohydrate content was estimated by difference method. Total starch content and amylose contents were estimated by Anthrone reagent and colorimetric methods, respectively (Sharma et al., 2024). The total polyphenol content of rice was analysis by Folin-Ciocalteu 10%, follow the procedure described by Loan and Thuy (2019).

2.4. Determination of physical properties

Volume expansion and hardness of instant rice were determined follow the method of Loan et al. (2023) and Chavan et al. (2018) to analysis the increasing volume of instant rice compare to the initial volume of rice and the change of structure of rice after processed.

2.5 Sensory evaluation

A sensory analysis of sample was conducted by a panel of 20 semi-trained members using a five-point Hedonic scale (Sivaranjani et al., 2024). The selection of panelist was based on their pre-existing expertise and enthusiasm for the sensory evaluation of instant rice. At first, the members received training on the quality characteristics of instant rice, the evaluation form, and the process of assigning scores. The samples were evaluated using a rating scale that ranged from "1 - Dislike extremely" to "5 - Like extremely" on 3 mainly attributes, including color, aroma and taste, and appearance and structure. The sample was provided in a randomized sequence at a temperature of 25–27°C, and the average mean score was utilized for comparison analysis.

2.6. Data analysis

The average statistics from three replications for each character were conducted. The repeated data underwent statistical analysis using the Analysis of Variance (ANOVA) method, utilizing the Statistical Package for Social Science (SPSS) software.

3. Results and discussions

3.1 Nutritional profile of germinated VD20 rice

Raw materials are an important factor that determines product quality. Depending on the origin and type of rice, the chemical composition may vary. Therefore, it is necessary to analyze the chemical composition of raw materials to determine the nutritional value of raw materials and find a reasonable processing process. The proximate composition of germinated VD20 rice was comparable with the reported of Bolarinwa et al. (2019). It also could be beneficial to produce the gluten-free product. The polyphenol content was observed at value of 32.3 mgGAE/100 g, which also could be potential to nutraceutical food as well as low glycemic index food by different mechanism (Ngo et al., 2023).

Table 1. Physicochemical properties of germinated VD20 rice

Parameters	Value
Moisture content	11.52%
Protein content	7.99%
Lipid content	2.29%
Carbohydrate content	69.47%
Ash content	4.33%
Polyphenol content	32.3 mgGAE/100 g

3.2 Effect of ratio of rice and water on the physical and sensory properties of instant rice

Adding water during cooking is very important. The water ratio during cooking has significant different effects on the structure of rice after gelatinization at the same cooking temperature conditions. After cooking, starch gelatinization occurs, causing rice grains to swell. The swelling phenomenon occurs first in the spore of starch granules, then spreads over the surface, causing their volume to increase many times until the starch granule tears and becomes an irregular shape, and then stop increasing volume (Briffaz et al., 2014). According to Reed et al. (2013), rice varieties will often differ in the thickness of the silk layer, structure, composition as well as gelatinization temperature and gelatinization degree. To completely gelatinize starch, it is necessary to use the appropriate amount of water. That directly affects the swelling and structure of reconstituted dried rice products (Bui et al., 2018).

Through the process of performing the experiment under the same conditions, the collected results were shown in Table 2 and Table 3. Specifically, for a rice: water ratio of 1:1, the cooking time was about 15 minutes, the rice is hard after cooking, with little loose grain expansion, the rice expansion after cooking is low at 21.56 cm^3 due to the supplemented water not being enough to gelatinize the rice. After cooking, the rice grains are quite dry and the rice hardness was measured at 1051 grams of force, which is mainly because the amount of water is small, so the grains do not absorb enough water to expand well. Therefore, after cooking, the rice is still hard and quite dry, but the grains still retain their original shape and are less likely to break (Figure 1). Amornsin (2003) reported that rice grains are not adequately moistened in the interior, the starch in the interior of the grain may not be fully gelatinized by heating. The color of the rice after cooking is still light yellow, with a light aroma (Figure 1). Therefore, the acceptable aroma and taste were low (3.53). Because the amount of water is low when cooking, the water evaporates faster during the drying process, and the drying time at 60^oC was short (about 220 minutes).

However, for a rice: water ratio of 1:1.25, the cooking time was about 20 minutes, which was longer than the sample is from ratio of 1:1. After being cooked, rice grains are still quite hard and swell less because the amount of water also was not enough to gelatinize the rice starch. Therefore, the expansion is relatively low at 22.37 cm^3, but still higher than that of ratio of 1:1. The grain structure recorded 1027 grams of force, showing that the rice was still quite hard which would affect the sensory properties and expansion when reconstituted. The acceptable value on color, aroma and taste, appearance and structure of instant rice were 4.54, 3.43, 4.15, respectively. After cooking, the rice still retains its good color, light aroma, and the grains are intact. Due to the same reason that the amount of water retain in rice is small, the water evaporates relatively quickly during the drying process, and the drying time was quite short (240 minutes). However, because the rice grains have not been completely gelatinized, after reconstitution the rice grains bloom less and are still hard

At a rice: water ratio of 1:1.5, the cooking time for rice increased to about 25 minutes. After cooking, the rice grains are cooked evenly

and swell completely to an extent of 24.70 cm^3. The amount of water added is just enough, which led after cooking the rice was soft, the grains sticked together but not significantly and still retain their intact shape. The hardness of instant rice was about 997 grams of force. It has a decrease in hardness, comparing to rice: water ratios of 1:1 and 1:1.25. However, the appearance and structure acceptance were highest, reaching value of 4.98 out of 5. The drying time was about 260 minutes. After cooking, instant rice still retains its color, light aroma, and acceptable taste, which made the acceptance of color, and aroma reached value of 4.47 and 4.06, respectively.

Table 2. Effect of ratio of rice and water on hardness and volume expansion index of instant rice

Ratio of rice and water	Volume expansion (cm^3)	Hardness (g-force)	Color	Aroma and taste	Appearance and structure
1:1.0	21.56^{e}	1051^{a}	4.69^{a}	3.51^{b}	3.85^{e}
1:1.25	22.37^{d}	1027^{ab}	4.54^{b}	3.43^{c}	4.15^{c}
1:1.5	24.70^{c}	997^{b}	4.47^{c}	4.06^{a}	4.89^{a}
1:1.75	25.39^{b}	923^{c}	3.85^{d}	3.31^{d}	4.34^{b}
1:2.0	28.18^{a}	848^{d}	3.54^{e}	3.21^{e}	4.05^{d}

Note: Different superscript capital letters indicate significant difference within column ($p < 0.05$).

Figure 1. Appearance of cooked and dehydrated VD20 instant germinated rice under different ratio of rice and water: a. 1:1, b. 1:1.25, c. 1:1.5, d. 1:1.75, e. 1:2

It could be seen that with a rice: water ratio of 1:1.75 the cooking time increased due to large volume. By this ratio, the rice after cooking has a large expansion of 25.39 cm^3, which described as evenly cooked and soft. The expansion of the rice was quite large compared to the 3 ratios previously done. The structure of rice was recorded as 923 grams of force. Due to the amount of water was relatively high, led to undesirable properties as known as begin to appear mushy and grains stick together, some grains were broken. After cooking, the rice's color gradually fades due to the increased amount of water, the aroma is light, and the taste is quite delicious as can see from the value of acceptance in Table 3. When reconstituted, the rice grains swell evenly and quite sticky together. Moreover, the supplementary water ratio was high (2 times than weight of rice), led the rice after cooking was very mushy and too soft. The hardness recorded as 848 grams of force, showing that the rice is very soft

compared to previous samples. The rice was evaluated as stick together, many broken grains and unable to retain its original shape. The drying process also took long time. The color of rice after cooking is no longer typical of VD20 rice due to too much water, the smell is very light, and the taste is quite bland. When reconstituted, the rice grains are too soft, many grains stick together, and the shape is not beautiful.

Different water ratios create rice with different blooms and textures. At a higher water ratio, the rice grains swell evenly and become soft, but the rice grains become mushy and stick together, making the drying process difficult (Wang et al., 2020). On the contrary, at a lower water ratio, the rice will not be cooked, the rice's bloom will be low, the rice grain structure will be hard due to not enough water to gelatinize the starch, the rice after reconstitution will be very hard and have low bloom or no bloom at all affects the swelling of rice after reconstitution (Loan et al., 2023). Besides, the color and flavor gradually decrease because the more water there is, the lighter the flavor and color become. From the research results, it shows that the sample with a rice: water ratio of 1:1.5 cooked rice quickly and the rice has a moderate expansion and structure that is neither too puffy nor too hard, most suitable compared to other rice varieties. The color and flavor of instant rice still retain quite well after cooking and reconstitution. The drying time was relatively short, the rice after reconstitution blooms quite well, was relatively uniform, and soft. This sample had a higher sensory evaluation than the remaining samples.

3.3. Effect of type and concentration of food additive on the physical and sensory properties of instant rice

The purpose of adding additives during the cooking process is to improve the structure, swelling and help the rice grains separate and not too much sticky after cooking without affecting the drying process (Sharma et al., 2024). Therefore, it is necessary to choose the most suitable additive type and additive ratio, thus that the product after cooking and reconstitution has the best sensory properties. Two types of additives used for the additive survey experiment were $NaHCO_3$ and NaCl.

3.3.1. Effect of sodium bicarbonate concentration on the physical and sensory properties of instant rice

The addition of sodium bicarbonate ($NaHCO_3$) during the cooking process significantly affects the structure, color and taste of rice after cooking and reconstitution, when cooked under the same conditions with the optimal rice: water ratio of 1:1.5. Table 4 and 5 showed that rice cooked using $NaHCO_3$ at different additive ratios, which led to the rice after cooking has different colors, flavors and structures as well as physical properties. Because of the amount of water and just enough time for the starch to gelatinize, the control sample has an expansion value of 25.60 cm^3 and hardness of 933 grams force. Due to rice grains were completely gelatinized, thus after reconstitution, the rice grains swell evenly, are soft and separate from each other. However, when cooking water added $NaHCO_3$ led to the volume expansion was increase, while the reduction in hardness was found. The cooked rice became to mushy and softer (Figure 2). The panelists evaluated that even though the percentage of $NaHCO_3$ added was small, the rice was still a bit mushy and became quite dark yellow brown and cannot retain its original shape. The textural qualities of cooked rice are frequently determined by measuring the hardness and stickiness of rice grains (Jung et al., 2016). As a results, the acceptance rate on color, aroma and taste, appearance and structure also reduced when the cooking water was supplemented with sodium bicarbonate. As increasing concentration of $NaHCO_3$, the acceptance value was remarkable decreased. Xu et al. (2022) also reported that sodium carbonated could significantly decrease the color of instant noodle. Although in Cai Lay black rice, using $NaHCO_3$ in making reconstituted dried rice is effective in improving the structure (Loan et al., 2023). However, when applying in production of instant germinated

VD20 rice, $NaHCO_3$ was not effective in improving the structure and sensory value of rice after reconstitution. As above explanation, the buffering and dispersing properties of sodium carbonate could potentially influence the solubility and stability of such products (Đorđević et al., 2022). However, the fully pregelatinization during cooking and drying process, it make the product not significant changed structure of product.

Table 3. Effect of sodium bicarbonate concentration on sensory properties of instant rice

Concentration of $NaHCO_3$ (%)	Volume expansion (cm^3)	Hardness (g-force)	Color	Aroma and taste	Appearance and structure
0	25.60^{d}	933^{a}	4.47^{a}	4.07^{a}	4.84^{a}
0.1	26.77^{c}	857^{b}	3.96^{b}	3.84^{ab}	4.30^{ab}
0.2	28.46^{b}	797^{c}	3.42^{c}	3.37^{bc}	3.80^{bc}
0.3	29.83^{a}	597^{d}	2.92^{d}	3.00^{c}	3.36^{c}

Note: Different superscript capital letters indicate significant difference within column ($p < 0.05$).

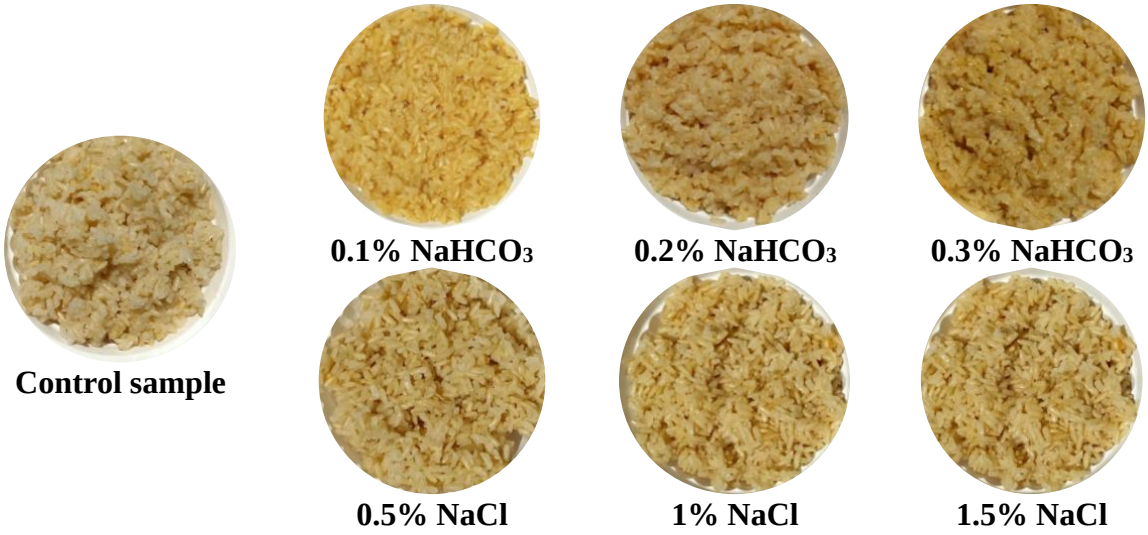


Figure 2. The appearance of cooked rice when supplementing different food additives with various concentration

3.3.2. Effect of sodium chloride concentration of food additive on the physical and sensory properties of instant rice

Sodium chloride (NaCl) is not only a familiar ingredient in cuisine but also greatly affects food from many different aspects. Salt is one of the basic tastes that humans are capable of sensing, the use of salt can enhance the flavor of food and make it more appealing. According to research by Sharma et al. (2024), have demonstrated the effects of salt treatments at different concentrations during the cooking process on the physicochemical, cooking and rehydration kinetics of instant rice. The application of salt pretreatment reduces bulk density and fraction of broken particles, while enhancing porosity, volumetric expansion rate, weight gain rate and rehydration properties.

Table 4 showed that different salt ratios resulted in instant rice with different flavors and structures. With the appropriate salt ratio, the rice grains swell well and rarely stick together. However, with a higher salt ratio, the rice bloom was quite poor, and the rice structure gradually increased in hardness. It was found that the highest hardness and lowest acceptance in appearance and structure from the sample was added 1.5% of NaCl (1049 g-forces and 3.31, respectively). The color and aroma of the rice are not much different, but the taste of the rice

has changed. When adding salt to the cooking process of rice, the dried rice noodle could prevent the hardness and increase the elasticity of the cooked rice (Sangpring et al., 2015). At a low salt ratio, the rice swells well after cooking, is soft, the rice flavor is not too different in this study.

For a salt ratio of 0.5%, the rice is evenly cooked and soft, do not break after cooking, separate from each other. The swelling and structure of the rice are not too different from the control sample because the added salt ratio is low, so the aroma and sweetness are still retained during the cooking process. Therefore, the combination of salty and sweet tastes when eaten will stimulate taste receptors on the tongue. When both salty and sweet are combined, they create a unique taste. When reconstituted, the rice will still be golden brown in color, the grains will expand evenly, be soft, and the grains will less stick to each other.

Table 4. Effect of sodium chloride concentration on physical properties of instant rice

Concentration of NaCl (%)	Volume expansion (cm^3)	Hardness (g-force)	Color	Aroma and taste	Appearance and structure
0	25.60^{d}	933^{c}	4.65^{a}	4.22^{a}	4.54^{a}
0.5	26.36^{c}	961^{c}	4.26^{b}	4.13^{a}	4.52^{a}
1	27.76^{b}	1012^{ab}	3.85^{c}	3.42^{b}	3.85^{b}
1.5	28.80^{a}	1049^{a}	3.36^{d}	2.91^{c}	3.31^{c}
CV (%)	4.85	5.80	12.31	18.02	12.61

Note: CV is coefficient of variation; Different superscript capital letters indicate significant difference within column ($p < 0.05$).

For a salt ratio of 1%, the rice grains are separated from each other and are drier than the 0.5% ratio, so the rice grain is dry. Similarly for the salt ratio of 1.5%, the rice is evenly cooked, soft and the grains are not broken or separated. The expansion of rice is 28.80 cm^3 and the structure of rice is 1049 grams. The rice still retains its aroma, but the salt ratio is quite high, so the rice tastes salty after cooking. Rice after cooking still retains its golden-brown color (Figure 2). When reconstituted, the rice still has a golden-brown color, the grains swell evenly, are less soft, and the grains rarely stick together, but the salty taste greatly affects the sensory value.

Because the nature of $NaHCO_3$ is to create swelling and porosity, it is not suitable for selection as an additive to improve the structure of products (Kabiri et al., 2003). When using additional salt during the cooking process, the rice after cooking has a stable structure, the rice grains are neither too soft nor too hard, and the color and flavor are completely acceptable. Salt, in addition to improving the structure of the product, also reduces water activity, thereby helping to inhibit the growth of bacteria and fungi (Albarracín et al., 2011). Therefore, it prevents the growth of harmful agents and reduces product failure. Research results showed that salt at a rate of 0.5% shows that rice, after cooking and reconstitution, has good elasticity, is soft, has a beautiful golden-brown color, has a light aroma, and tastes good.

4. Conclusions

The current study examines the impact of the ratio of rice to water, as well as the types and quantities of food additives, such as sodium bicarbonate and sodium chloride, on the quality of instant rice when prepared for cooking. Significant changes in the cooking quality features of rice were reported in all tests. A ratio of 1 part rice to 2 parts waters can result in a sample that has the desired level of hardness and a high rate of acceptance. The use of food additives had a substantial impact on the physical, rehydration, and sensory properties of instant rice. Sodium bicarbonate has the potential to enhance the expansion of volume, decrease hardness, and alter sensory

characteristics. Sodium chloride decreased the extent of damaged grains and improved the flavor of instant rice. Moreover, the method used to prepare instant rice is convenient for users, economical, does not require any specialized equipment, and can therefore be simply implemented on an industrial level. Thus, it is advisable for food enterprises that handle convenience and ready-to-eat food products to consider using a 0.5% sodium chloride treatment during the manufacture of instant rice.

5. References

Albarracín, W., Sánchez, I. C., Grau, R., & Barat, J. M. (2011). Salt in food processing; usage and reduction: a review. *International Journal of Food Science & Technology*, *46*(7),1329-1336. https://doi.org/10.1111/j.1365-2621.2010.02492.x

Amornsin, A. (2003). *Properties of waxy rice starch and rice grain: process development for an instant waxy rice product* University of Georgia.

AOAC. (2005). *Official methods of analysis of the Association of Official Analytical Chemists* (Vol. 2). Association of Official Analytical Chemists.

Bolarinwa, I., Lim, P., & Muhammad, K. (2019). Quality of gluten-free cookies from germinated brown rice flour. *Food Research*, *3*(3), 199-207. https://doi.org/10.26656/fr.2017.3(3).228

Briffaz, A., Bohuon, P., Méot, J.-M., Dornier, M., & Mestres, C. (2014). Modelling of water transport and swelling associated with starch gelatinization during rice cooking. *Journal of Food Engineering*, *121*, 143-151. https://doi.org/10.1016/j.jfoodeng.2013.06.013

Bui, L. T., Coad, R. A., & Stanley, R. A. (2018). Properties of rehydrated freeze dried rice as a function of processing treatments. *LWT*, *91*, 143-150. https://doi.org/10.1016/j.lwt.2018.01.039

Chavan, P., Sharma, S. R., Mittal, T. C., Mahajan, G., & Gupta, S. K. (2018). Effect of parboiling technique on physico-chemical and nutritional characteristics of basmati rice. *Agricultural Research Journal*, *55*(3). https://doi.org/10.5958/2395-146X.2018.00089.3

Đorđević, N., Mihajlović, S., Vlahović, M., Šajić, J. L., & Martinović, S. (2022). Thermal analysis and phase changes of mechanochemically activated sodium carbonate [Article]. *Thermochimica Acta*, *708*,Article,179139. 10.1016/j.tca.2021.179139

Hepsomali, P., Groeger, J. A., Nishihira, J., & Scholey, A. (2020). Effects of Oral Gamma-Aminobutyric Acid (GABA) Administration on Stress and Sleep in Humans: A Systematic Review. *Front Neurosci*, *14*, 923. https://doi.org/10.3389/fnins.2020.00923

Hsu, R. J.-c., Chen, H.-J., Lu, S., & Chiang, W. (2015). Effects of cooking, retrogradation and drying on starch digestibility in instant rice making. *Journal of Cereal Science*, *65*, 154-161. https://doi.org/10.1016/j.jcs.2015.05.015

Huang, D., Wang, Y., Thompson, J. W., Yin, T., Alexander, P. B., Qin, D., Mudgal, P., Wu, H., Liang, Y., & Tan, L. (2022). Cancer-cell-derived GABA promotes β-catenin-mediated tumour growth and immunosuppression. *Nature cell biology*, *24*(2),230-241. https://doi.org/10.1038/s41556-021-00820-9

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M., & Sansawa, H. (2003). Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. *European journal of clinical nutrition*,*57*(3),490-495. https://doi.org/10.1038/sj.ejcn.1601555

Jung, K.-j., Lee, H., Lee, S. H., & Kim, J. C. (2016). Retrogradation of heat-gelatinized rice grain in sealed packaging: investigation of moisture relocation. *Food Science and Technology*,*37*,97-102. https://doi.org/10.1590/1678-457x.07816

Kabiri, K., Omidian, H., & Zohuriaan-Mehr, M. (2003). Novel approach to highly porous superabsorbent hydrogels: synergistic effect of porogens on porosity and swelling rate. *Polymer International*, *52*(7), 1158-1164. https://doi.org/10.1002/pi.1218

Kasai, M., Lewis, A., Marica, F., Ayabe, S., Hatae, K., & Fyfe, C. A. (2005). NMR imaging investigation of rice cooking. *Food Research International*, *38*(4), 403-410. https://doi.org/10.1016/j.foodres.2004.10.012

Lin, J., & Li, C. (2023). Influence of instant rice characteristics and processing conditions on starch digestibility—A review. *Journal of Food Science*, *88*(8), 3143-3154. https://doi.org/10.1111/1750-3841.16627

Loan, L. T. K., & Thuy, N. M. (2019). Optimization of germination process of "Cam" brown rice by response surface methodology and evaluation of germinated rice quality. *Food Research*, *4*(2), 459-467. https://doi.org/10.26656/fr.2017.4(2).307.1

Loan, L. T. K., Thuy, N. M., & Van Tai, N. (2023). Mathematical and artificial neural network modeling of hot air drying kinetics of instant "Cẩm" brown rice. *Food Science and Technology*, *43*, e27623. https://doi.org/10.5327/fst.27623

Maqbool, M. A., Aslam, M., Akbar, W., & Iqbal, Z. (2017). Biological importance of vitamins for human health: A review. *J. Agric. Basic Sci*, *2*(3), 50-58.

Ngo, T. V., Kunyanee, K., & Luangsakul, N. (2023). Insights into Recent Updates on Factors and Technologies That Modulate the Glycemic Index of Rice and Its Products. *Foods*,*12*(19),3659. https://doi.org/10.3390/foods12193659

Sangpring, Y., Fukuoka, M., & Ratanasumawong, S. (2015). The effect of sodium chloride on microstructure, water migration, and texture of rice noodle. *LWT-Food Science and Technology*, *64*(2), 1107-1113. https://doi.org/10.1016/j.lwt.2015.07.035

Sharma, S., Semwal, A. D., Srihari, S. P., Govind Raj, T., & Wadikar, D. (2024). Effect of salt pretreatments on physico-chemical, cooking and rehydration kinetics of instant rice. *Journal of Food Science and Technology*, *61*(4), 770-781. https://doi.org/10.1007/s13197-023-05877-y

Sivaranjani, S., Singh, S. M., & P, S. R. (2024). Quality attributes of traditional food from blends of rice flour and jaggery syrup under different frying conditions. *Journal of Agriculture and Food Research*, *16*, 101074. https://doi.org/10.1016/j.jafr.2024.101074

Wang, L., Zhang, L., Wang, H., Ai, L., & Xiong, W. (2020). Insight into protein-starch ratio on the gelatinization and retrogradation characteristics of reconstituted rice flour. *International Journal of Biological Macromolecules*, *146*, 524-529. https://doi.org/10.1016/j.ijbiomac.2020.01.048

Xu, F., Chen, J., Ren, J. Y., Liu, S. H., Wang, L., & Wang, Y. h. (2022). Effect of sodium carbonate on rheological, structural, and sensory properties of wheat dough and noodle. *Journal of Food Processing and Preservation*, *46*(1), e16148. https://doi.org/10.1111/jfpp.16148

Yadav, G. P., Kumar, D., Dalbhagat, C. G., & Mishra, H. N. (2023). A Comprehensive Review on Instant rice: Preparation Methodology, Characterization, and Quality Attributes. *Food Chemistry Advances*, 100581. https://doi.org/10.1016/j.focha.2023.100581

Acknowledgment

The author would like to thank for Tien Giang University for facilitating this work.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECT OF INCORPORATION OF BIOPROCESSED LENTILS ON NUTRITIONAL AND TECHNOFUNCTIONAL PROPERTIES OF FLAT BREAD

Barinderjeet Singh Toor[1], Harsha Gandhi[2], Hardeep Singh[1], Amarjeet Kaur[2], Ankita Kataria[1,✉]

[1]Maharaja Ranjit Singh Punjab Technical University, Bathinda, Punjab-151001, India
[2]Punjab Agricultural University, Ludhiana, Punjab-141004, India
✉ankitakataria92@gmail.com

https://doi.org/10.34302/crpjfst/2024.16.4.6

Article history:
Received
August 29th, 2024
Accepted
October 10th, 2024

Keywords:
Lentil;
Processing;
Flat bread;
Dough rheology;
Texture.

ABSTRACT

The present study was carried out to observe the effect of incorporation of raw and processed lentils on nutritional and technofunctional characteristics of flat bread. Lentils were processed by soaking (25 ºC for 12 hours), atmospheric boiling (25 minutes), and germination (25 ºC for 48 hours) methods. Raw and processed lentil flours were used in production of flat bread which was evaluated for rheological, compositional and color characteristics. Shelf stability studies (textural properties, water activity and free fatty acid) of flat bread were carried out by storing in food-grade LDPE bags under refrigeration (4±1 ºC) for up to 7 days. Significant ($p<0.05$) variations were observed in rheological properties which were found to be optimum upon incorporation of raw and processed lentil flour at level of 10 % for flat bread dough. Processing treatments considerably enhanced the nutritional value, with germination and boiling leading to increased protein and fiber content and decreased fat content. Color analysis also exhibited substantial changes upon incorporation of raw and processed lentil flours. There was rise in hardness, gumminess and chewiness in control and lentil incorporated flat breads upon storage. Water activity ranged between 0.85-0.92 from 0th to 7th day. Elevation in free fatty acids was observed in all the products wherein rate of increase was highest in the control sample (133 %) during storage. Based on nutrition development, functional characteristics and organoleptic acceptability of flat breads, lentil flour, raw and processed, is a potential ingredient which can be utilized in development of functional foods.

1. Introduction

Since prehistoric times, legumes have played a significant role in human diets and agricultural systems. In recent years, they have made a major contribution to solving the pressing problems of food and nutritional security. This is ascribed to legumes' high nutritional content, which includes substantial amounts of high-quality proteins, complex carbohydrates, dietary fiber, minerals and vitamins. Legumes also contain a variety of bioactive components that protect against diabetes, colon cancer, and coronary heart disease (Dhillon *and al.*, 2022; Gandhi *and al.*, 2022).

Lentil, a commonly consumed legume, is an excellent source of protein (23-31 %), dietary fiber (7-23 %) and minerals; phosphorus (57-407 mg/100g), magnesium (99-726 mg/100g), calcium (59-463 mg/100g) and potassium (285-943 mg/100g). Furthermore, it contains significant amounts of essential amino acids except methionine, which can be complemented by consuming it with cereals. However, it possesses certain antinutritional compounds (phytates, saponin, tannins) which have the

ability to lower the bioavailability of nutrients like proteins and minerals. Processing techniques like soaking, boiling, autoclaving, roasting, germination, and fermentation have been reported to eliminate such compounds and thereby enhance the nutritional potential (Gandhi *and al*., 2022).

Recently, investigators have reported the utilization of raw and/or processed legume flours in the development of various food products. The investigations carried out by Alomari and Abdul-Hussain (2013), Atudorei *and al*. (2022), Bojňanská *and al*. (2021), Dhinda *and al*. (2012), Mohammed *and al*. (2012), Oskaybaş-Emlek *and al*. (2021), Sopiwnyk *and al*. (2020) and Turfani *and al*. (2017) reported the effect of utilization of flours of lentil, common bean, chickpea, red lentil and broad bean on the physicochemical characteristics of various bakery products. This could be attributed to the significant functionality of legumes which enhance the overall quality of final product. Modern research is primarily focused on enhancing bread by adding nutrient-rich and health-promoting ingredients (Olagunju *and al*., 2021). However, there is dearth of literature on the incorporation of raw and/or processed lentil flour in flat bread. Therefore, the present study was carried out to observe the effect of incorporation of raw and processed lentil on the nutritional and techno-functional characteristics of flat bread.

2. Materials and methods

2.1. Raw materials

Lentil was procured from the Punjab Agricultural University, India. Ingredients used for the preparation of flat bread were purchased from the local market of Ludhiana, India.

2.2. Processing of lentil grains

After sorting and cleaning, lentil seeds were processed using three techniques; a) soaking in distilled water for 12 hours at 25 ℃ (Rehinan *and al*., 2004); b) boiling under atmospheric conditions for 25 minutes at 100 ℃ (Yeo and Shahidi, 2017); c) germination of soaked (12 hours, 25 ℃) seeds for 48 hours at 25 ℃ (Vidal-Valverde *and al*., 2002). Following processing, seeds were tray dried (6 hours at 50 ℃) and milled to flour using domestic mill.

2.3. Dough rheology

Refined wheat flour was substituted with raw and processed lentil flour at different levels (0 – control, 5, 10, 15 and 20 %) to analyse the effect of incorporation on the rheological attributes. Perten doughLAB instrument (300 g capacity, Australia) working on the analytical conditions mentioned in the AACC International Method 54-21.02, was utilized to record following parameters – water absorption (%), dough development time (minutes), stability (FU) and peak energy (Wh/kg).

2.4. Preparation of flat bread

Flat bread was prepared using the procedure reported by Sharma *and al*. (1995). Refined wheat flour was substituted with raw and processed lentil flour at 0, 5, 10, 15 and 20 % levels in the flat bread. Other ingredients used were sugar (2.5 g), salt (1 g), compressed yeast (3 g), shortening (10 g), water (as per requirement) for 100 g of flour. All the ingredients were mixed to dough followed by fermentation for 60 minutes at 30 ºC and 75 % relative humidity (RH). Thereafter, dough was remixed, left for recovery time, sheeted, molded, proofed (30 minutes, 30 ºC, 75 % RH), and baked (4-5 minutes, 300 ºC). Flat bread prepared without incorporation of lentil flour was served as control sample.

2.5. Sensory evaluation

A nine-point hedonic scale ranging from 9 (Like extremely) to 1 (Dislike extremely) was adopted to evaluate prepared food products by a semi-trained panel of 25 members on the basis of appearance, texture, flavor, taste, mouthfeel, and overall acceptability. The optimum samples were selected on the basis of sensory evaluation and subjected to various physico-chemical analyses.

2.6. Proximate analysis

Standard procedures of AACC (2000) were adopted to analyse the proximate composition of flat bread samples. Moisture content was determined using hot air oven method at 130 ºC. Kjeldahl method was used for protein content estimation where in nitrogen conversion factor of 6.25 was applied. Fat content was evaluated with Soxhlet apparatus using petroleum ether (40-60 °C) as the extractant. For ash content, the samples were charred and kept in the muffle furnace at 550 ºC for 5 hours. Difference method was employed to calculate carbohydrate content by subtracting the contents of moisture, protein, fat and ash from 100. All the results were expressed as gram per 100 gram of sample (g/100 g).

2.7. Colour analysis

Hunter lab colorimeter (CR-300 Minolta Camera, Japan) was utilized to analyse the color properties. Results were expressed as L*, a* and b* values representing lightness, greenness/ redness and blueness/ yellowness, respectively.

2.8. Shelf stability

Flat bread was packed in low density polyethylene (LDPE) bags and stored for 7 days at refrigerated conditions. Samples were analysed on 0th, 3rd and 7th day for following parameters.

2.8.1. Texture analysis

TMS PRO was used for the analysis of texture profile of flat bread. Square shaped pieces of 1X1 cm^2 were placed on the platform of texture analyzer with the load cell of 100 N and probe with the diameter of 75 mm. The samples were compressed with 50 % compression. The hardness, springiness, cohesiveness, gumminess, chewiness and resilience were noted down for all the samples.

2.8.2. Water activity

The water activity was determined using a digital water activity meter (Pawkit, Decagon Devices, Inc., Pullman, Washington, USA). Water activity meter was calibrated before measuring the water activity.

2.8.3. Free fatty acid content

2 g sample and 20 mL benzene were mixed in a flask and kept for 30 minutes on shaker. Thereafter, 5 mL extract was poured into another flask and mixed with 5 mL ethanol and 5 mL benzene. 2-3 drops of phenolphthalein were added as indicator and contents were titrated against 0.02 N KOH until a light pink color appeared and persisted for 15 seconds (AACC, 2000). Titre value was noted down and results were calculated as percent (%) oleic acid.

2.9. Statistical analysis

Experiments were performed in duplicates/triplicates and final values were expressed as mean value ± standard deviation. Data sets were subjected to analysis of variance (ANOVA) at $p<0.05$ on IBM SPSS 22.0 software (SPSS Inc. Chicago II, USA).

3.Results and discussions

3.1. Effect of lentil incorporation on the rheological properties of flat bread dough

Dough characteristics exhibited considerable ($p<0.05$) variation upon incorporation of raw and processed lentil flours (Table 1). As the concentration of the lentil increased from 5 to 20 %, the values for water absorption percentage (%), peak energy (Wh/kg), and dough development time (minutes) showed a significant rise wherein highest values were observed in dough samples containing 20 % boiled lentil flour (6.7, 93.04 and 18.9 % respectively) followed by 20 % germinated lentil flour (4.5, 77.04 and 12.03 % respectively). However, a considerable decline of 72.34 % was observed in stability (minutes) upon incorporation of 20 % soaked lentil flour.

Table 1. Effect of lentil incorporation on the rheological properties of flat bread dough[+]

Treatment	Control	Raw				Soaked				Boiled				Germinated			
Level of Substitution (%)	**100%**	**5%**	**10%**	**15%**	**20%**	**5%**	**10%**	**15%**	**20%**	**5%**	**10%**	**15%**	**20%**	**5%**	**10%**	**15%**	**20%**
Water absorption (%)	60.15± 0.02^{J}	62.04± 0.02^{HIc}	63.26± 0.15^{EFb}	64.57± 0.06^{Ca}	64.84± 0.30^{Ba}	61.83± 0.14^{Id}	62.64± 0.17^{Gc}	63.52± 0.02^{Eb}	64.21± 0.36^{Da}	62.77± 0.22^{Gd}	63.06± 0.11^{Fc}	64.54± 0.13^{Cb}	65.95± 0.22^{Aa}	61.93± 0.03^{HId}	62.14± 0.08^{Hc}	64.74± 0.06^{BCb}	65.87± 0.07^{Aa}
Dough development time (minutes)	3.05± 0.03^{I}	1.50± 0.08^{Ld}	2.4± 0.07^{Jc}	4.80± 0.06^{Gb}	5.10± 0.05^{EFa}	1.50± 0.02^{Ld}	5.00± 0.04^{Fc}	5.20± 0.06^{DEb}	5.80± 0.02^{Aa}	1.70± 0.06^{Kd}	4.50± 0.08^{Hc}	5.60± 0.05^{Bb}	5.90± 0.04^{Aa}	1.50± 0.04^{Lc}	4.40± 0.12^{Hb}	5.30± 0.14^{CDa}	5.40± 0.03^{Ca}
Stability (minutes)	4.70± 0.07^{D}	6.80± 0.15^{Aa}	5.60± 0.04^{Cb}	3.60± 0.02^{Ec}	3.10± 0.07^{Fd}	6.20± 0.16^{Ba}	3.70± 0.07^{Eb}	3.20± 0.03^{Fc}	1.30± 0.14^{Id}	3.80± 0.07^{Ea}	3.30± 0.19^{Fb}	2.80± 0.04^{Gc}	2.10± 0.05^{Hd}	4.70± 0.20^{Da}	3.60± 0.02^{Eb}	3.10± 0.25^{Fc}	1.50± 0.13^{Id}
Peak energy (Wh/kg)	11.30± 0.13^{B}	8.34± 0.06^{Kd}	8.98±0. 03^{Ic}	9.44± 0.02^{GHb}	9.82± 0.04^{Fa}	8.91± 0.12^{Id}	9.34± 0.15^{Hc}	9.88± 0.17^{Fb}	10.27± 0.08^{Ea}	10.43± 0.06^{Dd}	10.87± 0.02^{Cc}	11.21± 0.01^{Bb}	13.44± 0.08^{Aa}	8.56± 0.12^{Jd}	8.98± 0.06^{Ic}	9.57± 0.14^{Gb}	9.94± 0.08^{Fa}

[+]Data is presented as mean± standard deviation.

Different letters from A, B, C to L indicate statistical differences by Tukey test ($p<0.05$) among different treatments.

Different letters from a, b to d indicate statistical differences by Tukey test ($p<0.05$) among different replacement levels for a single treatment.

According to Bojňanská *and al.* (2021), incorporating bean, lentils, and chickpea flours in bread led to an increase in water absorption capacity and development time, but a decline in dough stability. The higher water-holding capacity of legume flour can be attributed to the higher protein and pentosan (ribose and deoxyribose) contents which could change the water dynamics and distribution of the dough. The increased dough development time may be attributed to protein interactions causing a reduction in dough hydration and therefore dough development (Dhinda *and al.*, 2012). The variations observed upon the addition of boiled lentil flour can be attributed to the denaturation and unfolding of proteins which exposed the hydrophilic regions, leading to increased water absorption. Gelatinization of starch after boiling also aided in increasing the water absorption (Aguilera *and al.*, 2009). Shahzadi *and al.* (2005b) also noted a comparable decrease in dough stability attributing it to decrease in the content of wheat gluten. Similar observations have been reported by Turfani *and al.* (2017) who also found that lentil flour notably reduced stability and raised dough development time in bread due to the weakening of the protein (gluten) network. Additionally, germination of lentil flour can lead to proteolysis causing increase in low molecular weight proteins and exposure of the hydrophilic regions of the protein chains thereby increasing its water absorption capacity (Gandhi *and al.*, 2022). However, proteolysis also impacted the strength of gluten and decreased the stability of the dough. Similar results were observed for flat bread incorporated with sprouted oats which was attributed to the action on the wheat proteins upon sprouting (Sergiacomo *and al.*, 2024).

3.2. Effect of lentil incorporation on the sensory attributes of flat bread

The overall acceptability scores were highest for all the flat breads incorporated with 10 % processed lentil flours (Figure 1). Incorporation of lentil flour improved mouthfeel and aroma of the flat breads, however score for appearance declined. At higher substitution levels, an adverse impact on the final product's hardness, shape, color, and crumb elasticity was observed. Kohajdová *and al.* (2013) reported similar observations that baked rolls prepared with 10 % legume flour were most acceptable. Based upon the sensory evaluation, all the flat breads incorporated with 10 % raw and processed lentil flours were further subjected to physical-chemical analysis and shelf stability studies.

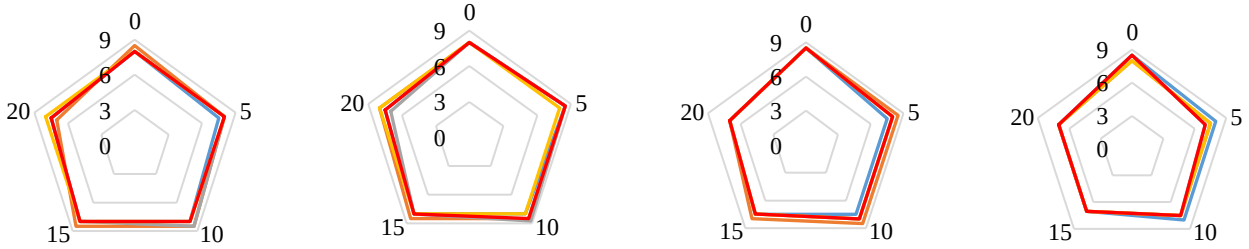


Figure 1. Effect on the sensory attributes (— appearance, — mouthfeel, — taste, — aroma, — overall acceptability) of flat bread upon substitution with lentil flours – a) raw, b) soaked, c) boiled and d) germinated – at 0 (control), 5, 10, 15 and 20 % replacement levels

3.3. Effect of lentil incorporation on the proximate composition of flat bread

Results obtained for proximate composition are summarized in Table 2. Incorporation of boiled lentil flour significantly ($p<0.05$) enhanced the moisture content of flat breads, that was in agreement with the findings of Dostalova *and al.* (1998) attributing it to the

increment in the water holding capacity of legume flour after boiling. A significantly ($p<0.05$) higher protein content was recorded in all the lentil flour (both raw and processed) incorporated flat breads owing to the higher protein content of legume (Kohajdová *and al*., 2013). Furthermore, germinated lentil flour resulted in highest increment in the protein content of the flat bread which might be due to the substantial reduction in the other constituents of the flour during the germination process leading to relative compositional variations (Xu *and al*., 2019). Conversely, flat bread prepared with soaked lentil flour exhibited lower ($p<0.05$) protein content. As explained by Agume *and al*. (2017), the breakdown as well as solubilization of proteins during soaking resulted in lower values. In context to ash content, flat bread prepared with lentil flours exhibited significantly ($p<0.05$) elevated values attributing to the higher content of minerals in legumes, as reported by Gandhi *and al*. (2022). Amongst lentil flours, highest increment in ash content was observed with raw lentil flour followed by germinated, soaked and boiled. It was believed that, during germination, the enzymatic activity caused relative compositional changes whereas leaching of minerals during boiling and soaking resulted in lower ash content (Gandhi *and al*., 2022). Fat content was found to reduce significantly ($p<0.05$) upon lentil incorporation which can be attributed to its low fat content as compared to wheat flour (Shahzadi *and al*., 2005a). Moreover, Gandhi *and al*. (2022) showed that soaking and boiling of lentils led to reduced fat content attributing this to the diffusion of the lipid bodies into processing water. Germination was demonstrated to decrease the fat content due to the utilization of lipid bodies as energy source for sprouting. Carbohydrate content of the flat bread substantially ($p<0.05$) reduced with addition of lentil flours owing to the lower carbohydrate content of the legumes (Kohajdová *and al*., 2013). Moreover, the incorporation of processed lentil flours in the flat bread led to reduction in carbohydrate content wherein lowest values were observed for boiled lentil flour followed by germinated and soaked. Dhull *and al*. (2023) observed leaching of sugars during hydrothermal treatments resulted in lower carbohydrates values. In context to germination, Fouad and Rehab (2015) highlighted that, the decline in carbohydrate content upon processing was due to the conversion of complex carbohydrates into simple sugars by α-amylase.

3.4. Effect of lentil incorporation on the color properties of flat bread

Color values (L*, a* and b*) of the flat bread exhibited substantial variations with the incorporation of processed lentil flour (Table 2). Significant ($p<0.05$) decrease of 4.77-9.45 % in L* values were recorded in raw and processed lentil flour incorporated flat breads implying decrease in lightness. Huang *and al*. (2017) reported similar observations upon addition of whole green lentils to bread which led to reduction in the L* value (darker coloration) by 10.31 % attributing it to the high phenolic content of lentils. However, a* and b* values were found to increase upon incorporation of raw and processed lentil flours. This color change can be explained by the Maillard reaction which caused the natural dark pigmentation of bread (Alomari and Abdul-Hussain, 2013). Flat bread incorporated with boiled lentil flour exhibited reduction in L*, a* and b* values which may be due to degradation of color pigment and water-soluble phenolic compounds during boiling (Monalisa *and al*., 2020).

3.5. Effect of lentil incorporation on the shelf stability of flat bread

3.5.1. Texture profile analysis

Texture profile analysis was used to evaluate hardness, cohesiveness, chewiness, springiness, gumminess, and resilience of the prepared flat breads (Table 3). During storage studies, the hardness, gumminess, and chewiness of the flat breads were significantly ($p<0.05$) increased till the 7th day of storage to 9.19 N (in germinated), 9.47 N (in soaked), and 62.4 J (in boiled) respectively. However, the values of

springiness, cohesiveness, and resilience showed a considerable ($p<0.05$) decline to 6.26 (in germinated), 0.24 (in raw), and 0.14 (in germinated) respectively on the 7th day. The values of hardness showed highest elevation in raw lentil flour flat breads during storage. Atudorei *and al*. (2022) also found similar trends in the texture profile attributes during the preparation of bread from lentil flour due to higher fiber content and a more compact bread structure. It was also explained that a decrease in the strength of the gluten network may be caused due to the higher water-binding capacity of dietary fibers. The dilution of the gluten resulting from the flour addition caused by a reduction in the strength of the gluten network and the result of a change in the nature of starch may be responsible for the reduction in the values of springiness, cohesiveness, and resilience. The increase in hardness upon storage in all the samples was attributed to moisture loss and starch retrogradation (Lin *and al*., 2013).

Table 2 Effect of lentil incorporation on the physico-chemical properties of flat bread[+]

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)	Color analysis		
						L*	a*	b*
Control	32.69± 0.13^{C}	12.46± 0.01^{D}	10.26± 0.02^{A}	0.5± 0.01^{E}	74.85± 0.31^{D}	85.56± 0.58^{A}	3.63± 0.16^{D}	16.47± 0.20^{C}
Raw	31.75± 0.08^{E}	15.00± 0.07^{B}	10.23± 0.04^{AB}	0.88± 0.03^{A}	80.36± 0.02^{B}	80.92± 0.30^{C}	4.81± 0.07^{A}	17.53± 0.05^{B}
Soaked	32.85± 0.09^{B}	14.71± 0.12^{C}	10.09± 0.04^{C}	0.75± 0.02^{C}	80.78± 0.03^{A}	79.76± 0.33^{D}	4.30± 0.06^{C}	18.40± 0.23^{A}
Boiled	34.78± 0.06^{A}	14.99± 0.23^{B}	10.03± 0.04^{C}	0.67± 0.02^{D}	80.68± 0.04^{A}	77.47± 0.11^{E}	3.16± 0.22^{E}	11.66± 0.07^{D}
Germinated	32.38± 0.04^{D}	15.46± 0.05^{A}	10.18± 0.05^{B}	0.83± 0.03^{B}	79.86± 0.12^{C}	81.48± 0.17^{B}	4.51± 0.05^{B}	17.34± 0.13^{B}

[+]Data is presented as mean± standard deviation.
Different letters from A, B to E indicate statistical differences by Tukey test ($p < 0.05$) among different treatments.

3.5.2. Water activity

The water activity for the flat breads made from different processed lentil flours showed significant ($p<0.05$) variation upon storage (Table 3). However, a slight rise in water activity was seen from the 0th to the 3rd and 3rd to the 7th day under refrigeration conditions. Increase in water activity upon storage can lead to quality degradation in relation to hydrolytic rancidity and organoleptic attributes like texture. Tiwari *and al*. (2011) also reported that the packaging material and storage conditions may be the cause of moisture absorption by the product since it acts as medium for microbial growth and can reduce the shelf by causing development of off-flavour thereby affecting its shelf life.

3.5.3. Free-fatty acid content

The values of free fatty acid content on the 0th day varied from 0.24-0.36 % oleic acid for the flat breads prepared from raw and processed flours. The free fatty acid content showed a significant ($p<0.05$) increase from day 0 to day 7 (Table 3). Due to enzymatic (lipase action) or non-enzymatic activities at high temperatures, triglycerides are hydrolyzed to produce free fatty acids (Tiwari *and al*., 2011). Surekha *and al*. (2013) reported the same results related to free fatty acid levels after a storage examination of cookies produced from barnyard millet. The considerable ($p<0.05$) rise in free fatty acids can be related to the presence of moisture in flat breads, as well as the use of low-density polyethylene material for packaging, which shows a higher water vapor transmission rate. However, it was also observed in the current study that the rate of increase of free fatty acid was higher in the control sample as compared to the flat breads prepared with raw and processed lentils which can be attributed to their antioxidant activity.

Table 3 Effect of lentil incorporation on the storage stability of flat bread[+]

Treatment	Days	Texture analysis						Water activity	Free fatty acid (% oleic acid)
		Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (J)	Resilience		
Control	**0**	5.43±0.14^{Oc}	12.27±0.02^{Ba}	0.70±0.02^{Aa}	1.86±0.03^{Kc}	12.40±0.03^{Nc}	0.67±0.01^{Ba}	0.85±0.01^{Eb}	0.24±0.02^{Fc}
	3	7.18±0.03^{Hb}	10.53±0.05^{Cb}	0.64±0.07^{Ba}	2.48±0.02^{Jb}	14.29±0.04^{Mb}	0.53±0.03^{Cb}	0.85±0.01^{Eb}	0.36±0.02^{Eb}
	7	10.27±0.03^{Aa}	7.86±0.04^{Jc}	0.47±0.02^{Db}	4.65±0.03^{Fa}	17.80±0.08^{La}	0.28±0.02^{Fc}	0.92±0.01^{ABa}	0.56±0.01^{BCa}
Raw	**0**	6.28±0.02^{Lc}	10.32±0.03^{Da}	0.56±0.02^{Ca}	3.45±0.03^{Hc}	35.85±0.02^{Fc}	0.68±0.02^{Ba}	0.90±0.01^{CDb}	0.26±0.01^{Fc}
	3	6.76±0.03^{Jb}	9.04±0.02^{Hb}	0.41±0.02^{EFb}	4.61±0.07^{FGb}	42.43±0.05^{Eb}	0.51±0.02^{CDb}	0.91±0.01^{BCb}	0.45±0.04^{Db}
	7	8.2±0.04^{Ea}	7.63±0.04^{Kc}	0.24±0.04^{Hc}	6.35±0.02^{Ca}	61.59±0.39^{Ba}	0.26±0.03^{Fc}	0.92±0.01^{ABa}	0.58±0.01^{Ba}
Soaked	**0**	5.68±0.03^{Nc}	12.69±0.04^{Aa}	0.66±0.01^{ABa}	4.55±0.05^{Gc}	28.72±0.08^{Ic}	0.74±0.01^{Aa}	0.91±0.01^{BCa}	0.35±0.01^{Ec}
	3	6.86±0.02^{Ib}	9.31±0.07^{Gb}	0.44±0.02^{DEb}	6.24±0.03^{Db}	32.34±0.09^{Hb}	0.47±0.05^{Db}	0.91±0.01 ^{BCa}	0.44±0.03^{Db}
	7	8.43±0.02^{Da}	6.92±0.07^{Mc}	0.33±0.04^{Gc}	9.47±0.06^{Aa}	51.58±0.04^{Da}	0.36±0.02^{Ec}	0.91±0.01 ^{BCa}	0.53±0.03^{Ca}
Boiled	**0**	6.06±0.01^{Mc}	10.19±0.06^{Ea}	0.57±0.02^{Ca}	3.28±0.02^{Ic}	21.00±0.23^{Kc}	0.48±0.01^{Da}	0.91±0.01 ^{BCa}	0.36±0.03^{Ec}
	3	7.57±0.03^{Gb}	8.64±0.02^{Ib}	0.37±0.01^{FGb}	5.86±0.02^{Eb}	32.44±0.02^{Hb}	0.34±0.03^{Eb}	0.91±0.01 ^{BCa}	0.47±0.01^{Db}
	7	8.87±0.03^{Ca}	6.91±0.09^{Mc}	0.28±0.03^{Hc}	6.87±0.06^{Ba}	62.40±0.16^{Aa}	0.24±0.05^{Fc}	0.93±0.01^{Aa}	0.63±0.02^{Aa}
Germinated	**0**	6.66±0.01^{Kc}	9.48±0.04^{Fa}	0.53±0.01^{Ca}	3.49±0.04^{Hc}	23.19±0.03^{Jc}	0.38±0.03^{Ea}	0.89±0.01^{Dc}	0.35±0.03^{Ec}
	3	7.94±0.05^{Fb}	7.44±0.03^{Lb}	0.36±0.02^{Gb}	4.58±0.09^{FGb}	34.67±0.05^{Gb}	0.25±0.02^{Fb}	0.91±0.01^{BCb}	0.46±0.03^{Db}
	7	9.19±0.12^{Ba}	6.26±0.12^{Nc}	0.25±0.01^{Hc}	6.26±0.03^{Da}	56.40±0.07^{Ca}	0.14±0.03^{Gc}	0.92±0.01^{Aba}	0.54±0.02^{Ca}

[+]Data is presented as mean± standard deviation.
Different letters from A, B, C to O indicate statistical differences by Tukey test ($p<0.05$) among different treatments.
Different letters a, b and c indicate statistical differences by Tukey test ($p<0.05$) among different days for a single treatment.

Saeed *and al*. (2020) also observed that the formation of free fatty acids content in black gram incorporated biscuits was low as compared to control samples and indicated this to the capability of the antioxidants present in the former samples.

4. Conclusion

Incorporation of raw and processed lentil flour in flat bread dough at 10 % level led to optimum rheological properties. The overall palatability of the flat bread containing 10 % processed lentil flour was observed to be enhanced in comparison to the control. Processing techniques significantly improved the nutritional value; boiling and germination resulted in higher protein and lower fat levels; potentially making it a healthier choice. Significant variations in color and textural characteristics (hardness, gumminess, and chewiness) were also observed after adding both processed and raw lentil flours. The water activity and free fatty acid values were in the optimum ranges for lentil incorporated flat bread. This comprehensive study on the application of processed lentil flour in the making of flat bread resulted in significant knowledge regarding the physical, chemical, sensory, and rheological characteristics.

5. References

AACC International Method 54-21.02, Rheological behaviour of flour by farinograph: Constant Flour Weight. Approved Methods of Analysis, 11th ed. Published online at http://methods.aaccnet.org. AACC International, St. Paul, MN.

Aguilera, Y., Esteban, R. M., Benítez, V., Molla, E., & Martin-Cabrejas, M. A. (2009). Starch, functional properties, and microstructural characteristics in chickpea and lentil as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, 57(22), 10682-10688. https://doi.org/10.1021/jf902042r

Agume, A. S. N., Njintang, N. Y., & Mbofung, C. M. F. (2017). Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour. *Foods*, 6(2), 12. https://doi.org/10.3390/foods6020012

Alomari, D. Z., & Abdul-Hussain, S. S. (2013). Effect of lupin flour supplementation on chemical, physical and sensory properties of Mediterranean flat bread. *International Journal of Food Science and Nutrition Engineering*, 3(4), 49-54. doi:10.5923/j.food.20130304.01

American Association of Cereal Chemists. Approved Methods Committee. (2000). Approved methods of the American Association of Cereal Chemists. AACC.

Atudorei, D., Mironeasa, S., & Codină, G. G. (2022). Effects of germinated lentil flour on dough rheological behavior and bread quality. *Foods*, 11(19), 2982. https://doi.org/10.3390/foods11192982

Bojňanská, T., Musilová, J., & Vollmannová, A. (2021). Effects of adding legume flours on the rheological and bread making properties of dough. *Foods*, 10(5), 1087. https://doi.org/10.3390/foods10051087

Dhillon, N. K., Toor, B. S., Kaur, A., & Kaur, J. (2022). Characterization and evaluation of yellow pea flour for use in 'Missi Roti'a traditional Indian flat bread in comparison with Desi chickpea flour. *The Pharma Innovation*, 11, 58-64. https://www.thepharmajournal.com/archives/2022/vol11issue5/PartA/11-5-220-942.pdf

Dhinda, F., Prakash, J., & Dasappa, I. (2012). Effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fibre and low carbohydrate bread. *Food and Bioprocess Technology*, 5, 2998-3006. https://doi.org/10.1007/s11947-011-0752-y

Dhull, S. B., Kinabo, J., & Uebersax, M. A. (2023). Nutrient profile and effect of processing methods on the composition and functional properties of lentils (*Lens culinaris* Medik): A review. *Legume Science*, 5(1), e156. https://doi.org/10.1002/leg3.156

Dostalova, J., Divisova, J., & Pokorny, J. (1998). Effect of soaking and cooking on water holding and sensory characteristics of cooked lentils. *Polish Journal of Food and Nutrition Sciences*, 3(07). https://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-article-553301f4-c657-4fad-8e63-3c354766b9e6

Fouad, A. A., & Rehab, F. M. (2015). Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris* Medik.) sprouts. *Acta Scientiarum Polonorum Technologia Alimentaria*, 14(3), 233-246. https://doi.org/10.17306/j.afs.2015.3.25

Gandhi, H., Toor, B. S., Kaur, A., & Kaur, J. (2022). Effect of processing treatments on physicochemical, functional and thermal characteristics of lentils (*Lens culinaris*). *Journal of Food Measurement and Characterization*, 16(6), 4603-4614. https://doi.org/10.1007/s11694-022-01549-1

Huang, S., Liu, Y., Zhang, W., Dale, K. J., Liu, S., Zhu, J., & Serventi, L. (2018). Composition of legume soaking water and emulsifying properties in gluten-free bread. *Food Science and Technology International*, 24(3), 232-241. https://doi.org/10.1177/1082013217744903

Kohajdová, Z., Karovičová, J., & Magala, M. (2013). Effect of lentil and bean flours on rheological and baking properties of wheat dough. *Chemical Papers*, 67, 398-407. https://doi.org/10.2478/s11696-012-0295-3

Lin, L. Y., Wang, H. E., Lin, S. D., Liu, H. M., & Mau, J. L. (2013). Changes in buckwheat bread during storage. *Journal of Food Processing and Preservation*, 37(4), 285-290. https://doi.org/10.1111/j.1745-4549.2011.00647.x

Mohammed, I., Ahmed, A. R., & Senge, B. (2012). Dough rheology and bread quality of wheat–chickpea flour blends. *Industrial Crops and Products*, 36(1), 196-202. https://doi.org/10.1016/j.indcrop.2011.09.006

Monalisa, K., Bhuiyan, J. A., Islam, M. Z., & Sayem, A. S. M. (2020). Boiling-induced changes on physicochemical, bioactive compounds, color, and texture properties of pumpkin (Cucurbita maxima). *Food Science and Technology International*, 26(4), 333-343. https://doi.org/10.1177/1082013219894402

Olagunju, A. I., Oluwajuyitan, T. D., & Oyeleye, S. I. (2021). Multigrain bread: dough rheology, quality characteristics, in vitro antioxidant and antidiabetic properties. *Journal of Food Measurement and Characterization*, 15, 1851-1864. https://doi.org/10.1007/s11694-020-00670-3

Oskaybaş-Emlek, B., Özbey, A., & Kahraman, K. (2021). Effects of germination on the physicochemical and nutritional characteristics of lentil and its utilization potential in cookie-making. *Journal of Food Measurement and Characterization*, 15(5), 4245-4255. https://doi.org/10.1007/s11694-021-00958-y

Rehinan, Z. U., Rashid, M., & Shah, W. H. (2004). Insoluble dietary fibre components of food legumes as affected by soaking and cooking processes. *Food Chemistry*, 85(2), 245-249. https://doi.org/10.1016/j.foodchem.2003.07.005

Saeed, S. M. G., Ali, S. A., Ali, R., Naz, S., Sayeed, S. A., Mobin, L., & Ahmed, R. (2020). Utilization of Vigna mungo flour as fat mimetic in biscuits: Its impact on antioxidant profile, polyphenolic content, storage stability, and quality attributes. *Legume Science*, 2(4), e58. https://doi.org/10.1002/leg3.58

Sergiacomo, A., Bresciani, A., Gallio, F., Varetto, P., & Marti, A. (2024). Sprouted Oats (*Avena sativa* L.) in baked goods: From the rheological properties of dough to the physical properties of biscuits. *Food and Bioprocess Technology*, 1-12. https://doi.org/10.1007/s11947-024-03362-8

Shahzadi, N., Butt, M. S., Rehman, S. U., & Sharif, K. (2005a). Chemical characteristics of various composite flours. *International Journal of Agriculture and Biology*, *7*(1), 105-108.

Shahzadi, N., Butt, M. S., Rehman, S. U., & Sharif, K. (2005b). Rheological and baking performance of composite flours. *International Journal of Agriculture and Biology*, 7(1), 100-104.

Sharma, S., Sekhon, K. S., & Nagi, H. P. S. (1995). Legume supplemented flat bread: Nutritive value, textural and organoleptic changes during storage. *Journal of Food Processing and Preservation*, *19*(3), 207-222. https://doi.org/10.1111/j.1745-4549.1995.tb00289.x

Sopiwnyk, E., Young, G., Frohlich, P., Borsuk, Y., Lagassé, S., Boyd, L., ... & Malcolmson, L. (2020). Effect of pulse flour storage on flour and bread baking properties. *LWT-Food Science and Technology*, 121, 108971. https://doi.org/10.1016/j.lwt.2019.108971

Surekha, N., Naik, R. S., Mythri, S., & Devi, R. (2013). Barnyard Millet (*Echinochloa Frumentacea* Link) cookies: development, value addition, consumer acceptability, nutritional and shelf-life evaluation. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 7(3), 1-10. https://doi.org/10.9790/2402-0730110

Tiwari, U., Gunasekaran, M., Jaganmohan, R., Alagusundaram, K., & Tiwari, B. K. (2011). Quality characteristic and shelf life studies of deep-fried snack prepared from rice brokens and legumes by-product. *Food and Bioprocess Technology*, 4, 1172-1178. https://doi.org/10.1007/s11947-09-0219-6

Turfani, V., Narducci, V., Durazzo, A., Galli, V., & Carcea, M. (2017). Technological, nutritional and functional properties of wheat bread enriched with lentil or carob flours. *LWT-Food Science and Technology*, 78, 361-366. https://doi.org/10.1016/j.lwt.2016.12.030

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., & Kuo, Y. H. (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology*, 215, 472-477. https://doi.org/10.1007/s00217-002-0602-2

Xu, M., Jin, Z., Simsek, S., Hall, C., Rao, J., & Chen, B. (2019). Effect of germination on the chemical composition, thermal, pasting, and moisture sorption properties of flours from chickpea, lentil, and yellow pea. *Food Chemistry*, 295, 579-587. https://doi.org/10.1016/j.foodchem.2019.05.167

Yeo, J., & Shahidi, F. (2017). Effect of hydrothermal processing on changes of insoluble-bound phenolics of lentils. *Journal of Functional Foods*, 38, 716-722. https://doi.org/10.1016/j.jff.2016.12.010

Acknowledgement

We are grateful to the Department of Food Science and Technology, Punjab Agricultural University, Ludhiana for providing the required facilities for conducting this study.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CURRENT DEVELOPMENTS IN THE VALORIZATION OF APPLE PROCESSING WASTE IN TO VALUE ADDED FUNCTIONAL BIOACTIVE COMPOUNDS: A COMPREHENSIVE OVERVIEW

Pranay Kr Baishya[1], Hemanta Deka[1], Dr. Subhajit Ray[1]✉

[1]Department of Food Engineering and Technology, Central Institute of Technology Kokrajhar, Kokrajhar, BTAD, Assam:783370 India

✉subhajit@cit.ac.in

https://doi.org/10.34302/crpjfst/2024.16.4.7

Article history:
Received
May 15th, 2024
Accepted
November 10th , 2024

Keywords:
Apple pomace;
Valorization;
Conventional extraction;
Non-conventional extraction;
Bioactive constituents.

ABSTRACT

Apple is a potential fruit and consumed throughout the globe. Processing apple results three fourth fraction of apple juice as a major product and one fourth fraction of apple pomace as by product or waste. Direct disposal of this waste creates environmental problem. However, apple pomace is considered to be a significant source of pectin, carbohydrates, amino acids, protein, essential fatty acids, and phenolic compounds. Effective utilization of apple pomace into food and nutraceutical industries could be a suitable waste management strategy. In order to extract these bioactive constituents, various conventional e.g. Soxhlet extraction (SE), maceration, and hydro-distillation (HD) and novel processing techniques e.g. ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized liquid extraction (PLE), pulse electric field extraction (PEF), enzyme assisted extraction (EAE), and liquid-liquid extraction techniques (LLE) are considered. Separation, purification, identification and quantification followed by high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy are important in order to characterize the bioactive constituents available in apple pomace. This paper reviews the valorisation of apple pomace into high valued bioactive constituents by different extraction strategies as a sustainable waste management approach.

1. Introduction

Apples (*Malus Domestica*), celebrated for their delicious taste and health benefits, stand as a formidable force in global agriculture (Perussello et al. 2017). India produces only 2.05 percent of the world's total apple production. Every time apple juice is produced, leftover apple pomace is generated. Constituting approximately 25% of the weight of fresh fruit, this byproduct poses a substantial challenge for waste management (Waldbauer et al. 2017). Apple pomace is a significant contributor to fruit waste, with up to 12 million tons generated globally each year. It is made up of apple peel, pulp, seeds, cores, stems, and a mixture of these components that are left over after the juicing process. The production involves several steps such as milling, liquefaction, and juice extraction (Hobbi et al. 2023). Addressing this waste has become imperative for environmental and economic considerations. Beyond being more pulp, apple pomace is a valuable repository of nutrients and bioactive compounds. Although it is naturally biodegradable, its sheer volume creates logistical challenges for producers, despite its traditional use as animal feed (Grigoras et al. 2013). Despite this, millions of tons of apple pomace go unused, representing an untapped resource. Apple pomace consists of generally

peel, fleshy part along with a insignificant fraction of seeds and stems. However, several factors e.g. variety, origin and processing techniques influences variation of nutritional ingredients of apple pomace (O'Shea et al. 2015). Typically, it features a nutrient-rich profile, including fiber, carbohydrates, sugars, and essential minerals like calcium, potassium, and magnesium (O'Shea et al. 2015). Realizing the potential of apple pomace involves exploring two main pathways: fermentable and non-fermentable applications. In the fermentable approach, pomace becomes a natural food source for microbes, leading to the development of various high quality products. On the other hand, non-fermentable methods focus on extracting inherent biologically active constituents for implementation in food processing, nutraceuticals, and other industries. Due to its perishability and high biodegradability, the large-scale apple processing industry produces a significant amount of nutrient-rich pomace which can harm the environment. Therefore, it is crucial to find ways to utilize and reduce the amount of pomace produced (Perussello et al. 2017). Repurposing apple pomace goes beyond waste management, it's a strategy to maximize the potential of an abundant resource. By harnessing its natural richness, we can elevate a byproduct into a valuable contributor to a more sustainable and circular economy.

2.Apple pomace constituents

Apples (*Malus domestica*) are a commonly consumed food in many cultures. Therefore, it is important to manage the by-product of apples, called apple pomace, efficiently. Several methods for utilizing apple pomace more effectively have been proposed. These include using it as fodder, producing organic acids, enzymes, bioethanol, and biogas through fermentation by the influence of microorganisms microbiological fermentation, and creating novel materials such as bio composites (Makris and Şahin . 2019). Apple pomace waste refers to the by-products of apple processing, such as peel, core, stem, and seeds. Disposing of this waste is a major environmental issue, as millions of tons are produced every year and often end up in landfills or are burned. This not only leads to wastage but also contributes to discharge of greenhouse gas and air pollution, worsening environmental problems (Perussello et al. 2017).

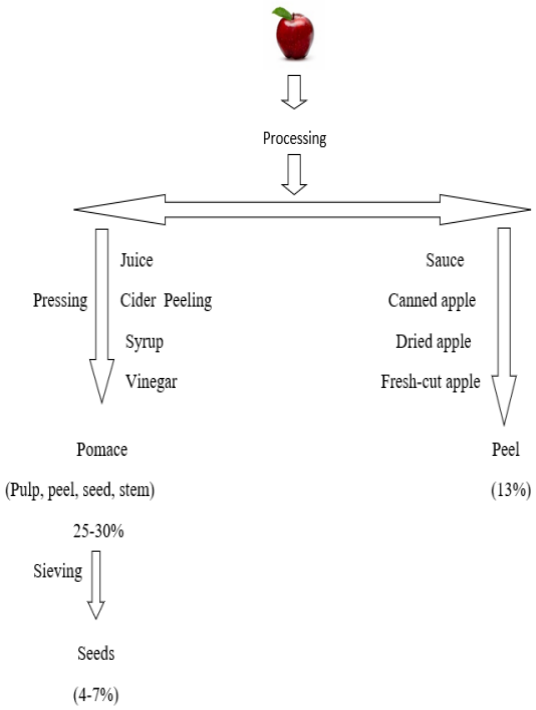


Figure 1. Development of apple derived by-products Adapted from (Rabetafika et al. 2014)

Despite these approaches, there remains a substantial edible portion of this by-product that can serve as a source of essential components. These components include anthocyanins (cyanidin 3-O-galactoside), dihydrochalcones (phloretin 2-O-glucoside and phloretin 2-O-xyloglucoside), hydroxycinnamic acids (chlorogenic acid and p-coumaroylquinic acid), and flavan-3-ols (ranging from monomers like epicatechin to large polymers known as procyanidins) (Fernandes et al. 2019). Research studies have demonstrated that using 70% ethanol as an extraction solvent reveals apple pomace as a rich source of bioactive compounds such as quercetin (1195 μg/g), chlorogenic acid (891 μg/g), phloridzin (678 μg/g), epicatechin (431 μg/g), catechin (314 μg/g), caffeic acid (296 μg/g), and rutin (123 μg/g) (Rashid et al.

2023). Apples, being highly consumed fruits, contain various bioactive compounds such as phenolic compounds, vitamins, dietary fibers, triterpenic acids, oligosaccharides, dihydrochalcones, flavonols, anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids. These compounds provide to the strong qualities of free radical scavengers of apples, with a concentration exceeding 20 mmol TE/kg, as recognized by experts (Asma et al. 2023).

3.Valorization techniques for the synthesis of bioactive constituents from apple pomace as a potential agro-waste residue

The extraction of bioactive substances involves carefully removing beneficial and physiologically active molecules from natural sources like plants, fruits, and marine life. There are several procedures used to achieve this, including solvent extraction, supercritical-fluid extraction, and enzyme-assisted extraction. The selection of a solvent and extraction method is based on the properties of the desired components. The utilization of extracted bioactive compounds has contributed to advancements in health, nutrition, and new product development across various industries, e.g. food processing, cosmetics, and pharmaceutical products. This process is crucial for numerous industries due to sustainability and ongoing research initiatives aimed at improving extraction techniques, reducing waste, and discovering new bioactive chemicals. A complete and standardized screening approach must be developed to filter out substances that are good for the development of human health, considering the significant number of plant species and the diverse range of bioactive chemicals (Azmir et al. 2013).

3.1. Conventional Extraction Methods:

Extraction of bioactive constituents from plant resources comprises of several techniques. The majority of these procedures are dependent on the strength of the solvents used, as well as thermal treatment and/or mixing. Bioactive compounds from plant resources can be extracted by using three classical techniques viz. Soxhlet extraction apparatus, Maceration, and Hydro distillation (Azmir et al. 2013).

3.11.Extraction by Soxhlet Apparatus:

The Soxhlet method of extraction uses a pure solvent to extract solid materials in a way that is effective and affordable. It uses minimal solvent and maintains extraction efficiency by repeatedly extracting solid materials. At first this new extractor was only used for lipid extraction, but it had a lot of potential in extracting other components also. It can be used to extract various components from natural sources. A lot of new techniques uses this old technique to compare the extraction efficiency. This technique follows the following protocol. Dry plant powder is placed inside the porous thimble first and then the thimble is finally introduced inside the distillation assembly where the solvent is poured. Generally, petroleum ether or n-hexane. Once the overflow level is reached, a siphon is used to aspirate the solution from the thimble holder.

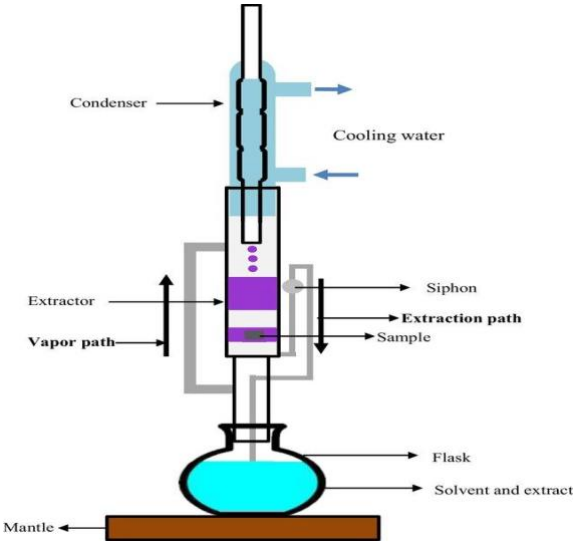


Figure 2. Representation of a Soxhlet extraction apparatus adapted from (Asif et al. 2023)

When the solvent is heated the solvent evaporates and the vapour flows through the extractor and goes to the condensation apparatus and liquifies the solvent. The solvent falls into the distillation flask afterwards. Whenever it reaches the level the siphon transfers the solvent to the flask, which contains the extracted

components. That completes a cycle, and this process continues for several cycles depending upon the requirements and the plant material used.

3.1.2.Maceration:

Maceration is an old technique for extracting valuable components from natural resources. It was commonly used in the past to prepare tonics for various health issues and is still used today due to its convenience and easy to follow protocol. This method can also be employed for essential oils extraction, although the yield is lower compared to other available techniques. By modifying some of the parameters of the maceration process, the yield can be increased. Typically, dried and ground plant materials are mixed with a suitable solvent, which is known as the menstruum. The plant material is then combined with the menstruum and left for one to several days for better absorption of the components, while occasional shaking can increase absorption. After this period, the solution is filtered, and the remaining plant material is called marc. Sometimes, more solvent is added to the older menstruum to increase the yield.

3.1.3.Hydro-distillation:

Bioactive constituents e.g. plant based essential oils, can be extracted by using hydro-distillation as is a conventional method without any requirement of organic solvents. There are three different classes of hydro-distillation: distillation induced by direct steam, water based distillation, and distillation induced by water and steam both. In the process of hydro-distillation, plant materials are kept in a container and water is added to the container and the solution is heated till boiling. In direct steam technique uses hot steam injected directly into the dried plant sample. The main influencing factors that extracts the bioactive chemicals from plant are steam and hot water. The vapor combination condenses by indirect cooling, allowing the oil and bioactive chemicals to be separated from the water. Finally, the essential oils and oil-based bioactive substances are typically dried over anhydrous sodium sulfate (Purkait et al. 2023). During hydro distillation, some volatile ingredients, natural colors, and heat-labile bioactive chemicals may be lost as the process is conducted at higher temperatures than water's boiling point.

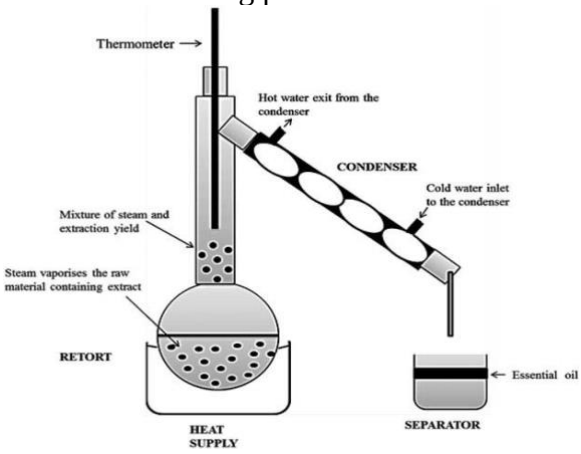


Figure 3. Representation of a hydro-distillation assembly (Cleavenger equipment) (Samadi et al. 2016)

3.2. Novel extraction techniques:

The primary liabilities of traditional extraction techniques include long extraction times, expensive, high-purity solvent requirements, large vaporisation of solvent, low level of selectivity for extraction, and heat degradation of thermostable substances. However, there are novel and intriguing extraction approaches that have been developed to overcome these limitations. These methods are known as novel extraction techniques or emerging processing technologies and the most promising ones include pressurized liquid extraction (PLE), pulsed electric field aided extraction (PFE), microwave assisted extraction (MAE), enzyme assisted extraction (EAE) , supercritical fluid extraction (SCFE), and ultrasound assisted extraction (UAE) (Azmir et al. 2013).

3.2.1.Ultrasound assisted extraction:

The food processing industry utilizes ultrasonic technology for various purposes such as extraction, filtering, freezing, packaging, cutting, and nano-formulation. In addition, significant laboratory research has been conducted to explore its potential in extracting bioactive compounds from plant matrices (Belwal et al. 2020). Ultrasound frequencies

typically range from 20 kHz to 100 MHz. It passes through a medium by causing compression and rarefaction, like other waves. Cavitation, or the implosion of bubbles, is a phenomenon caused by compression and rarefaction that involves the development, expansion, and disintegration of bubbles. Due to the fact that ultrasonic energy facilitates the percolation of both biological and inorganic components from plant matrix, the main benefit of UAE (Ultrasound Assisted Extraction) is observed in plant samples (Purkait et al. 2023). There are two main types of ultrasound applications are high intensity and low intensity.

According to (Putra et al. 2023), the optimum conditions observed for extraction of apple pomace using indirect sonication in an bath type ultrasonicator were liquid-solid ratio of 34.4:1 (v/w), a KOH concentration of 3.3 M, and a 2.5-hour ultrasonic-assisted extraction period.

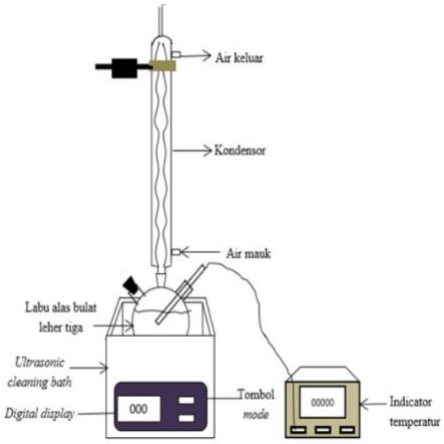


Figure 4. Representation of an ultrasonicator (Qadariyah et al. 2018)

3.2.2.Supercritical fluid extraction:

A CO_2 pump with a pressure cell containing the sample, a mechanism to maintain the system's pressure constant, and a collecting vessel are required to extract substances from a sample. The liquid is heated by pumping it to a heating zone, which boosts the temperature to critical levels. The extracted chemical then dissolves and diffuses quickly into the solid matrix inside the extraction vessel. The recovered component settles out, and the material that has dissolved is carried from the extraction unit into a lower-pressure separator. Finally, CO2 can be released into the atmosphere or cooled, compressed, and reused (Sapkale et al. 2010).

Since carbon dioxide is in gaseous stage at ambient temperature, it is eliminated when the extraction phenomenon is finished and the system is decompressed, producing an extract without any solvent. When carbon dioxide utilization is large on an industrial level, the recycling will be the management strategy. Nevertheless, because of its less polar nature, CO_2 is less successful in removing highly polar chemicals from organic matrices (Da Silva et al. 2020). The dissolution of the chemical components existing on the solid origin and their segregation into the supercritical solvent are the two main phases in the process of supercritical extraction (Da Silva et al. 2020).

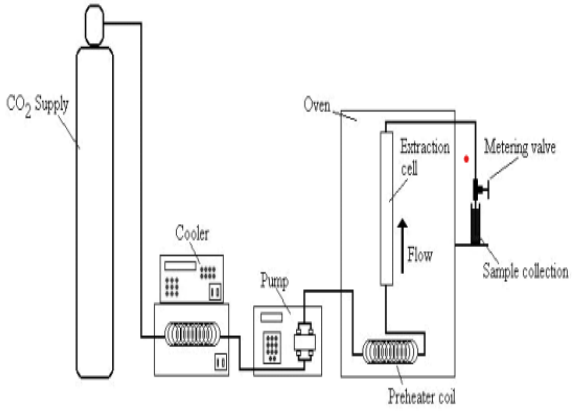


Figure 5. Schematic diagram of SCFE (Sapkale et al. 2010)

According to the experimental observation of (Putra et al. 2023), the effects of SCFE (supercritical fluid extraction) was found at a pressure level of 20 and 30 MPa at 45°C and 55 °C respectively in presence of ethanol (5%) as co-solvent. The highest level of extraction yield was obtained as 5.63 mg/g at 30 MPa pressure, 45 °C temperature and a period of two hours using ethanol (5%) as a co-solvent. However, conventional extraction methods such as Soxhlet technique and boiling water maceration achieved higher levels of extraction yield viz.2.05 mg/g of and 1.14 mg/g respectively.

3.2.3.Microwave-assisted extraction:

MAE is an automated and eco-friendly extraction method that offers numerous benefits. Compared to traditional methods, MAE reduces extraction time and solvent usage, can extract up to 40 samples simultaneously, and significantly increases sample throughput. It provides a highly appealing alternative for extracting organic and organometallic compounds from a broad range of resources while meeting the minimal requirements for sample preparation processes (Kataoka . 2019).

MAE is a standard technique for recovering active compounds from therapeutic plants. It entails heating of both solvents and samples with the application of microwave energy and segregating analytes from a sample matrix into the solvent (D. Sinha et al. 2022). The fundamental advantage of this technique is its ability to accelerate the heating of sample solvent mixtures, making it suited for the rapid extraction of analytes, especially thermally fragile fraction (Kataoka et al. 2019). The beneficial effects of MAE are determined by a variety of factors, which includes the solvent, sample, and extracted elements, particularly their dielectric properties.

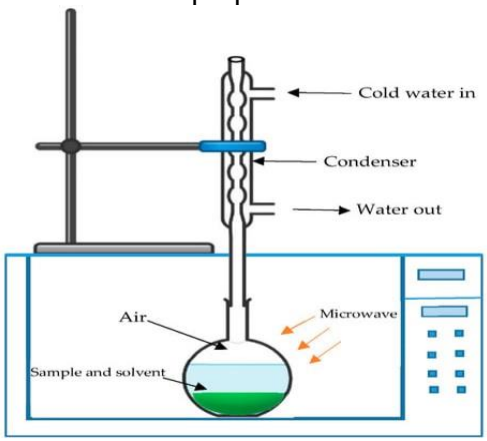


Figure 6. Representation of Microwave-assisted extraction technique (Saifullah et al. 2021)

When developing procedures for extracting medications from plants, various MAE factors, such as size of the sample, temperature of extraction and duration, power of microwave oven, and the polar character and volume of the solvent being extracted, should be optimized (Kataoka. 2019).

According to (Putra et al. 2023), it was found that a solid-liquid ratio of 0.069, a pH of 1.01, a microwave power of 499.4 W, and an extraction period of 20.8 minutes are the optimal parameters for extracting pectin. The use of MAE significantly minimized the time of extraction for dehydrated apple pomace.0.315 grams of pectin were predicted to be extracted optimally from 2 grams of dehydrated apple pomace.

3.2.4.Pressurized-Liquid extraction:

Pressurized-Liquid extraction (PLE) is an economical approach of extracting substances using liquid solvents under high pressures and temperatures. This method yields better results compared to those obtained using room temperature and atmospheric pressure methods (Mustafa and Turner.2011). In PLE, solvents are used at high temperatures and pressures, which keeps them liquid even when they would normally boil. To select appropriate operational factors, the key components must be considered theoretically. The fundamental ideas of PLE for solid samples are included as well. However, since commercial equipment has its limitations, the only way to handle liquid samples is by adding an absorbent to turn them into solids.

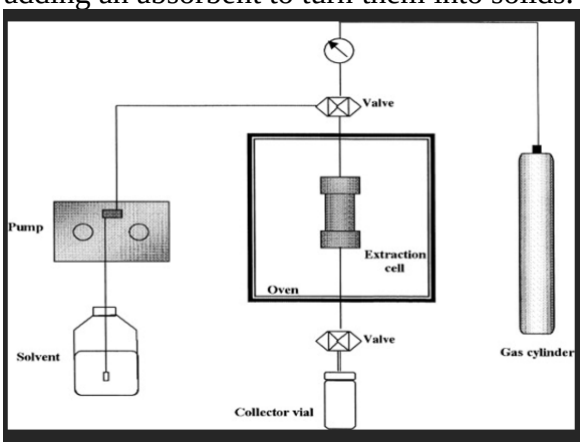


Figure 7. Schematic diagram of instrument used for extraction of honey carbohydrates using PLE (Soria et al. 2012)

3.2.5.Pulsed electric field aided extraction:

The PEF treatment is a non-thermal food processing technique, due to its extremely low temperature rise upon application. A refrigeration device, a high-voltage source of power, a device with energy storage capacity, a sterilization chamber with a pump to transport food through it, and a computing device to manage the system's controls are all part of the test system. PEF technology works on the principle that cells undergo a transformation when exposed to a strong external electric field, which increases the permeability and electrical conductivity of the cellular material (Korma et al. 2016). PEF technology has gained interest in recent years as a way to extract valuable components from food waste and by products. PEF removes the negative effects of traditional heating methods and has been used accurately to stabilize, separate, intensify and dehydrate essential substances while retaining their nutritional qualities. It is a promising alternative to conventional procedures like boiling, microwave and ultrasound-assisted extractions. In addition to enhancing the extraction process, PEF can also stress plant cells and promote the production of active components (Ranjha et al. 2021).

According to the observation of (Putra et al. 2023), apple pomace was suitably utilized for the extraction of antioxidants and polyphenolic compounds using Pressurized Liquid Extraction (PLE) process. Two different temperature ranges, i.e., 160 to 193 °C and 75 to 125 °C was considered for the extraction process. The highest antioxidant activity was attained at a temperature of 200 °C. Therefore;75 to 125 °C was considered the recommended temperature range for extraction. This spectrum of temperature was used to determine the maximum antioxidant activity of 60% ethanol and 102 °C.

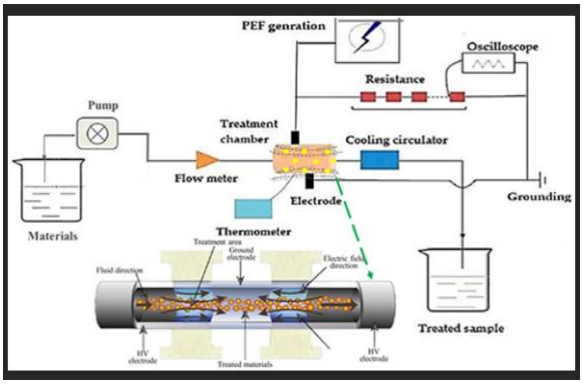


Figure 8.Schematic diagram of PEF system (Fan et al. 2022)

3.2.6.Enzyme assisted extraction:

Several chemical compounds found in plant's bases are distributed throughout the cytoplasm of cells, whereas other substances are bound in the matrix of polysaccharide molecules and complex lignin by hydrophobic or hydrogen bonding and are insoluble in a standard extraction procedure. Pre-treatment with enzymes has been seen as a unique and efficient method of releasing bound molecules and raising total yield (Azmir et al. 2013). In example, treatment of plant material with enzyme is followed before extraction using standard protocol. A versatile type of enzymes are required frequently e.g. cellulases, pectinases, and hemicellulase, to compromise the strength of the plant cell wall and improve the extraction of bioactive constituents from plants. These enzymes increase the permeability of the cell wall by decomposing its constituent parts, which raises the yields of bioactives that may be extracted (Puri et al. 2012). Enzyme assisted aqueous extraction (EAAE) and enzyme assisted cold pressing (EACP) are considered as combined enzymatic method. Generally speaking, EAAE techniques were created primarily for the different oilseed based oil extraction (Azmir et al. 2013).

3.2.7.Liquid-liquid extraction:

Solvent extraction technique used to separate biologically active molecules from natural resources such as plants, herbs, and fruits. In this process, an appropriate organic solvent is used to extract and dissolve the target

chemicals from the source material. The polarity of the bioactive molecules determines the type of solvent used, with nonpolar substances like essential oils being handled by hexane and polar compounds being handled by ethanol or methanol.

The source material is combined with the solvent, which helps the bioactive chemicals transfer into the solvent phase. Combinations of water and alcohol are more effective in extracting phenolic chemicals than mono-component solvent solutions. Particularly, water and ethanol in different proportions were examined, and it was found that the yield of polyphenolic extracted with 50% ethanol (v/v) at various temperature levels (20, 40, and 60°C) was nearly twice as high as that obtained with pure water (Hidalgo &Almajano, 2017). After the completion of extraction process, the solvent is typically eliminated from the extract using methods like evaporation or distillation, leaving behind a concentrated extract full of bioactive components. Solvent extraction is also known as liquid-liquid extraction, and the solvent used for the process is known as "Menstruum". Hexane and Dichloromethane (DCM) are used to extract non-polar compounds.

4.Methods of estimating polyphenols, flavonoids, and tannin content

4.1.Method to determine Total phenolic content (TPC):

Phenolic substances, also known as polyphenols, are molecules containing hydroxyl groups that are prone to oxidation. Numerous studies have shown that polyphenols possess various biological properties, including anti-inflammatory, antiviral, anti-diabetic, anti-cancer, and anti-microbial activities (Cosme et al. 2022). Apple pomace is considered to be a huge resource of polyphenolics and is therefore considered valuable.

The total phenolic (TP) content was measured using the Folin–Ciocalteu (FC) test. To do this, 500 µL of newly diluted 10-fold FC reagent in water and 1 mL of 20% sodium carbonate solution were mixed with 100 L of extract. The absorbance was then measured at 760 nm following a one-hour dark incubation period using a V-630 UV–vis spectrophotometer. The data were expressed as micrograms of gallic acid equivalents (g GAE/g), with gallic acid being used as the benchmark (Boulila et al. 2015).

4.2.Determination of Total Flavonoid Content (TFC):

The most widely distributed and common family of plant phenolics are flavonoids, which may be found in a wide range of foods. Rutin and quercetin are the flavonoids that are most often ingested.

As per the study conducted by (Boulila et al. 2015), the total flavonoid (TF) concentration was measured using the AlCl3 colorimetric technique. To prepare the solution, 1.5 mL of methanol, 0.1 mL of a 10% AlCl3 solution, 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water were mixed with a 500 L sample aliquot. The mixture was incubated for 30 minutes at room temperature and then the absorbance was measured at 415 nm. Quercetin was used as a reference standard, and the TF content was expressed in micrograms of quercetin equivalents (g QE/g).

4.3.Determination of Total Tannin Content (TTC):

To calculate the total tannin content, we followed the methodology described in (Nurdalilah et al. 2018). First, we combined 0.1 ml of methanolic extract, 0.5 ml of Folin-Ciocalteu reactive, 7.5 ml of distilled water, and 1 ml of a 35% aqueous solution of Na2CO3 in a test tube. Then, we added 0.9 ml of purified water and left the mixture in the dark for half an hour at room temperature. We used a UV/Vis spectrophotometer to measure the absorbance at 725 nm and created a standard curve using gallic acid (20-100 ppm). The results were expressed in g of gallic acid equivalents (GAE)/100 g of extract (Purkait et al. 2023).

5.Spectrophotometric assays of determining antioxidant activity

5.1.Antioxidant activity by DPPH Radical Scavenging Activity:

DPPH, which stands for 2,2-diphenyl-1-picrylhydrazyl, is a stable free radical characterized by its purple crystalline appearance. This compound is light-sensitive and not soluble in water, but it can be dissolved in organic solvents such as methanol and ethanol. Due to the lesser harm associated with ethanol compared to methanol, the latter is the preferred solvent (Gulcin and Alwasel . 2023).

The study represented by (Saikia et al. 2016) was used to determine antioxidant activities. Through testing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical's ability to scavenge free radicals, the total antioxidant activity of Ajwain seed extract was examined. The DPPH free radical seeks to rob antioxidants of their hydrogen. The presence of its free radical gives the methanolic DPPH its purple colour. First, 4 mg of DPPH was dissolved in 100 ml of methanol to make DPPH solution. In test tube add with a 400 µl solution of the methanolic extract. The mixture included 5600 µl of DPPH solution. 30 minutes were given for the mixture to remain at room temperature after it had been mixed and kept in the dark. Using a spectrophotometer, the solution's absorbance was calculated at 515 nm.

The percentage inhibition for each sample was determined, and the 50% inhibitory concentration (IC50) for each sample was calculated based on the concentration versus percentage inhibition graph. The IC50 value represents the concentration at which the sample scavenges 50% of the DPPH free radical. The radical scavenging activity was expressed as a percentage of inhibition. Graphical methods were employed to determine the IC50 values for both the extracts and standards, and measurements were conducted in triplicate. The IC50 of the extract signifies the concentration at which the radical scavenging potential is 50% (Phuyal et al. 2020). The percentage of inhibition was calculated using the following formula:

$$I\% = [(AC - AO)/AC] \times 100\%. \quad (1)$$

Where, AC = absorbance of the control (1 mL methanol + 1 mL DPPH solution) and AO = absorbance of the sample solution.

5.2.Antioxidant activity by FRAP method:

This method involves using antioxidants to reduce a ferric-tripyridyl triazine complex to a ferrous state, which is colored. To measure the reducing power of food extracts, their ferric reducing activity was determined. The FRAP reagents were freshly made by combining 300 mM of acetate buffer (pH 3.6), 20 mM FeCl3, and 10 mM TPTZ (2,4,6-Tripyridyl-S-triazine) in a 1:1:10 (v/v/v) ratio. After mixing 1 mg of the apple pomace extract with 2 ml of FRAP reagent, the mixture was incubated for 30 minutes in the dark at 37 °C. The production of the TPTZ-Fe2 + combination in the presence of antioxidant chemicals was measured at 593 nm using a spectrophotometer. The absorbance was calculated using the µM Fe2 + equivalent from the aqueous solution of ferrous sulphate ($FeSO_4 \cdot 7H_2O$) calibration curve (100 ~ 1,000 µM) based on triple analyses. After determining the Trolox methanolic solution's FRAP values, they were represented as TEACs, or Trolox equivalent antioxidant capacity (Moni Bottu et al. 2022). To calculate the Ferric Reducing Activity, the following equations were used:

Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) is measured in micrograms of ascorbic acid per gram of sample. The formula for AEAC is:

$$AEAC(\mu gAA/g) = [(activity) \times (dilution\ factor) \times (V_{extract}\ (ml))] / g(sample). \quad (2)$$

The activity is calculated from the calibration curve of the equation y = ax + C. The formula for calculating activity is:

$$Activity\ (X\mu g/ml) = (y - c) / a. \quad (3)$$

Where y = absorbance of the sample, c = y - intercept, and a = slope.

Finally, we calculate the percentage of Ferric Reducing Activity with the following formula:

$$\%FRAP = [1 - (1 - A_s / A_c)] \times 100. \quad (4)$$

Where A_c is the absorbance of the standard (ascorbic acid) at maximum concentration, and $A_s$is the absorbance of the sample.

5.3.Antioxidant activity by ABTS method:

2,2′-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid)shortly ABTS is a spectrophotometric method that measures the antioxidant activity of plant extract. ABTS radical cation which is blue-green in appearance is produced when ABTS reacts with potassium persulfate. ABTS radical cation is a stable compound. The stable radical is scavenge by the antioxidant molecules present in the plant extract and reduces it's absorbance (Hidalgo and Almajano.2017). In the method described by (Hidalgo and Almajano.2017), an aliquot of sample is mixed with the ABTS solution. After that the mixture is incubated for some amount of time and then absorbance is measured at 734nm. This can be calculated using equation-

$$\%ABTS \text{ radical cation scavenged} = [(A_{control} - A_{sample})/ A_{control}] \times 100 \quad (5)$$

Where '$A_{control}$' is the absorbance of ABTS radical cation solution and 'A_{sample}' is the absorbence of ABTS radical cation with the plant sample.

6.Extraction of Pectin from apple pomace

Apple pomace is a significant source of pectin, and extracting pectin is a reasonable approach to utilizing pomace. Apple pomace contains protopectin, which is a type of acid-soluble polysaccharide that has pectin in it. Pectin is an important constituent that can be extracted from citrus and apple waste. Pectin is a soluble fiber found in fruits and vegetables. It is also used as a gel forming agent, surfactant and thickener in various processed foods such as confectionery, bakery, jellies, yoghurts, and beverages. Pectin has numerous applications in the pharmaceutical and food processing industries. The most well known methods for obtaining pectin from raw materials include aqueous extraction, which involves direct boiling, microwave heating, ultrasonication, autoclaving, and electromagnetic induction. All of these pectin extraction techniques contribute to a certain degree of pectin quality deterioration. The temperature, duration of the extraction process, pH level, and source material all affect the production of pectin. In an acidic solution, pectin is extracted using inorganic acids such as phosphoric acid, hydrochloric acid, or sulfuric acid. Commercial pectin extraction often involves the use of extraction by acid and precipitation by alcohol on an industrial level. The basis for pectin extraction using acid is the higher temperature at which protopectin hydrolyzes. Using strong acids to extract pectin has several benefits, the primary ones being a high pectin production and a shorter period of extraction. However, there are drawbacks as well, including the disposal of acidic wastewater and significant energy and chemical costs (Chandel etal.2022). Pectin extraction from various sources using different acids was compared. Citric acid emerged as the most promising option due to its economic and environmental advantages, yielding the highest pectin output (13.75%) from apple pomace. Additionally, the effect of extraction conditions on pectin production was demonstrated by optimizing temperature (50, 75, 100°C) and duration (30, 50, 80 min) with 5% citric acid on apple pomace. It is important to remember, however, that the presence of residual chelating agents during the extraction process might reduce the final pectin's functionality (Chandel et al. 2022).

The first step in the pectin extraction process involved cleaning and finely chopping apples using an electric grinder. The crushed apple pomace was then pressed to achieve a consistent weight, and the resulting mash was dried at room temperature and then at 50℃ to produce apple flour (Kumar and Chauhan.2010). The apple flour served as the starting material for all pectin extraction experiments. To extract pectin, a reflux method was used in a condensation system at 97°C for 30 minutes, with a solute/solvent ratio of 1:50. The extractant was diluted hydrochloric acid with a pH of 2.5, and

a known weight of apple flour was used as the raw material. The same process was repeated using diluted citric acid with a pH of 2.5, and both acids were diluted with de-ionized water. The procedures used to extract pectin from the flour made from the other type of apple were also repeated (Kumar and Chauhan.2010).

7.Extraction of other bioactive components present in apple pomace

Apple pomace is considered to be a significant resource of biologically active constituents such as quercetin (1195 μg/g), chlorogenic acid (891 μg/g), phloridzin (678 μg/g), epicatechin (431 μg/g), catechin (314 μg/g), caffeic acid (296 μg/g), and rutin (123 μg/g) (Rashid et al. 2023). Apples are a highly consumed fruit and are packed with various bioactive components such as phenolic component, vitamins, dietary fibers, triterpenic acids, oligosaccharides, dihydrochalcones, flavonols, anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids. These compounds give apples strong antioxidant qualities, with a concentration of more than 20 mmol TE/kg, as recognized by many experts (Asma et al. 2023). Table 1 lists the major functional biologically active constituents found in apple pomace and their corresponding bioactivities. Apple pomace is composed of lignins (15.30–23.50%), hemicelluloses (4.26%–24.40%), and fibers, with a high concentration of both soluble (14.6%) and insoluble (36.5%) dietary fiber. These fibers make up 35–60% of the total quantity of non-starch polysaccharides in the material. The primary ingredients in this diet are pectins (5.50%–11.70%), cellulose (7.20%–43.60%), and other fibers. Lignin is generally insoluble in water, Pectin, cellulose (depending on its form), and some hemicelluloses are partially soluble in water. Galacturonic acid, being a monomeric sugar acid, shows its maximum solubility in water. The layer of cutin found in apple pomace also contains triterpenoids, with ursolic and oleanolic acid being the primary ones along with their alcoholic derivatives. Other derivatives include those with extra hydroxyl groups and p-coumaroyloxy- or cinnamoyloxy-moities. Although the number of identified apple triterpenoids is growing, we still don't know their overall abundance in apple pomace (Waldbauer et al. 2017).

Apple pomace is a great resource of hemicelluloses and cellulose, containing up to 18% of the polysaccharides found in apple cell walls, mainly fucogalactoxyloglucans (xyloglucan). Many industrial compounds like methylcellulose, hydroxypropyl cellulose, and carboxymethylcellulose are produced using cellulose, which has extensive industrial applications. However, the extraction of xyloglucan from apple pomace is still in its early stages.

8.Separation and quantification of extracted bioactive compounds

8.1. Separation and quantification by High Performance Liquid Chromatography:

The extract components were separated and detected using HPLC-UV-ELSD analysis in reversed phase. A Varian Pursuit XRs C18 analytical column (150 x 4.6 mm, 5 μm) connected to an RP-C18 protective column was used. A gradient elution program was applied which involved using water as solvent A and methanol as solvent B, both acidified with 0.1% formic acid, at a flow rate of 1 mL/min (Lyu et al. 2020). According to (Da Silva et al. 2020) At 1.1 mL/min, the gradient profile looked like this: 0.05 minutes, 10% B; 0.5 minutes, 15% B; 2.0 minutes, 12.5% B; 3 minutes, 15% B; 4 minutes, 80% B; 5 minutes, 100% B; 6 minutes, 100% B; 7 minutes, 5% B. The injection volume was 5 μL, and there was a 5-minute equilibration period in between runs. Every molecule was identified using a profile that was found by UHPLC-MS/MS tests, co-elution with genuine standards, retention durations and UV spectra comparisons, and other methods. Each compound's standard curve (7 points, 0.1–100.0 mg L−1) was created by graphing the concentration against peak area. These compounds were quantified using the calibration curves of related compounds, and the

results were reported as equivalents (M. A) (Da Silva et al. 2020).

9.Characterization of Bioactive compounds

Nuclear magnetic resonance (NMR) spectroscopy is an advanced analytical technique used extensively in the fields of materials science, biology, and chemistry. It works by using radiofrequency radiation and external magnetic fields to interact with certain atomic nuclei, such as protons and carbon-13. To obtain the 1H NMR spectra, a high-resolution Bruker AVANCE-400 MHz NMR spectrometer running at 9.4 T and 400 MHz frequency was used. According to (Gabriel et al. 2013), the analysis was carried out using a 5.0 mm coil with a 90º pulse, 16 cumulative scans, acquisition points at 64 K, and a spectral window of 10 ppm. The 90º pulse width was 7.5 µs, and the acquisition duration was 7.5 s. The recycle delay was 10 s, and the total time for the process was 17.5 s. These parameters were also applied to other projects (Gabriel et al. 2013). An NMR spectrometer with a frequency of 20 MHz and a 1H nucleus (manufactured by Resonance Instruments, Whitney, U.K.) was used to examine apple powders that had been adjusted to different relative humidity levels. The samples were quickly transferred to glass NMR tubes with an 18 mm diameter and lifted to a height of approximately 20 mm from the controlled humidity chambers. Subsequently, the tubes were sealed to prevent outside moisture from getting in (Lavelli and Kerr.2012). To calculate the T2 timings, the samples were analyzed using free induction decay. A single 90° pulse was used with a recycle delay of 2 seconds at 4.1 µs. All analyses were performed at 22°C (Lavelli and Kerr. 2012)

Table 1. Major biologically active constituents from apple pomace fraction adapted from (Lyu et al. 2020)

Class	Concentration (mg/kg DW)	Major Constituents	Bioactivity and Therapeutic Potential
Carbohydrates	48–62	Pectin and pectin oligosaccharides	Fiber and dietary fiber of properties e.g. solubility, viscosity and fermentable, significant prebiotic characteristics and hypo-cholesterolemic impacts
Phenolic acid	523-1542	Chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid sinapic acid, p-coumaroyl-quinic acid	Antioxidant, antimicrobial, anti-inflammatory, anticancer and cardio-protective effects
Flavonoids	2153-3734	Isorhamnetin, kaempferol, guercetin, rhamnetin, glycoconjugates, procyanidinB2, epicatechin	
Anthocyanins	50-130	Cyanidin-3-O-galactoside	
Dihydrochalcones	688-2535	Phlorizin, phloretein	Antidiabetic, potential in treating obesity, promoting bone-forming, blastogenesis

10.Conclusion

Apple pomace stands as a substantial reservoir of bioactive elements, offering a sustainable remedy to both environmental and economic challenges through the efficient utilization of waste from apple processing. Diverse extraction techniques have been examined to unlock the bioactive potential harbored within apple pomace. Novel approaches, categorized as non-conventional extraction strategies, have demonstrated superior efficiency and sustainability compared to traditional methods involving high temperatures, acidic conditions, and organic solvents. Among these innovative techniques, such as ultrasonic and microwave extraction, have proven effective, potentially supplanting conventional methods. While these advanced strategies show promise in extracting valuable bioactive components, challenges persist in terms of productivity and economic feasibility. Thus, further advancements are warranted to address these challenges and pave the way for environmentally friendly solutions in the future.

11.References

Asma, U., Morozova, K., Ferrentino, G., & Scampicchio, M. (2023). Apples and Apple By-Products: Antioxidant Properties and Food Applications. *Antioxidants*, *12*, 1456. https://doi.org/10.3390/antiox12071456

Asif, M., Javaid, T., Razzaq, Z., Khan, M., Maan, A., Yousaf, S., & Shahid, S. (2023). Sustainable utilization of apple pomace and its emerging potential for development of functional foods. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-023-28479-9

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Belwal, T., Chemat, F., Venskutonis, P. R., Cravotto, G., Jaiswal, D. K., Bhatt, I. D., Devkota, H. P., & Luo, Z. (2020). Recent advances in scaling-up of non-conventional extraction techniques: Learning from successes and failures. *TrAC Trends in Analytical Chemistry*, *127*, 115895. https://doi.org/10.1016/j.trac.2020.115895

Boulila, A., Hassen, I., Haouari, L., Mejri, F., Amor, I. B., Casabianca, H., & Hosni, K. (2015). Enzyme-assisted extraction of bioactive compounds from bay leaves (Laurus nobilis L.). *Industrial Crops and Products*, *74*, 485–493. https://doi.org/10.1016/j.indcrop.2015.05.050

Chandel, V., Biswas, D., Roy, S., Vaidya, D., Verma, A., & Gupta, A. (2022). Current Advancements in Pectin: Extraction, Properties and Multifunctional Applications. *Foods*, *11*(17). https://doi.org/10.3390/foods11172683

Cosme, F., Pinto, T., Aires, A., Morais, M. C., Bacelar, E., Anjos, R., Ferreira-Cardoso, J., Oliveira, I., Vilela, A., & Gonçalves, B. (2022). Red Fruits Composition and Their Health Benefits—A Review. *Foods*, *11*(5), Article 5. https://doi.org/10.3390/foods11050644

Da Silva, L. C., Souza, M. C., Sumere, B. R., Silva, L. G. S., Da Cunha, D. T., Barbero, G. F., Bezerra, R. M. N., &Rostagno, M. A. (2020). Simultaneous extraction and separation of bioactive compounds from apple pomace using pressurized liquids coupled on-line with solid-phase extraction. *Food Chemistry*, *318*, 126450. https://doi.org/10.1016/j.foodchem.2020.126450

Fan, R., Wang, L., Fan, J., Sun, W., & Dong, H. (2022). The Pulsed Electric Field Assisted-Extraction Enhanced the Yield and the Physicochemical Properties of Soluble Dietary Fiber From Orange Peel. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.925642

Fernandes, P. A. R., Ferreira, S. S., Bastos, R., Ferreira, I., Cruz, M. T., Pinto, A., Coelho, E., Passos, C. P., Coimbra, M. A., Cardoso, S. M., & Wessel, D. F. (2019). Apple Pomace Extract as a Sustainable Food Ingredient. *Antioxidants*, *8*(6), Article 6. https://doi.org/10.3390/antiox8060189

Gabriel, L. S., Prestes, R. A., Pinheiro, L. A., Barison, A., &Wosiacki, G. (2013). Multivariate analysis of the spectroscopic profile of the sugar fraction of apple pomace. *Brazilian Archives of Biology and Technology*, *56*, 439–446. https://doi.org/10.1590/S1516-89132013000300012

Grigoras, C. G., Destandau, E., Fougère, L., &Elfakir, C. (2013). Evaluation of apple pomace extracts as a source of bioactive compounds. *Industrial Crops and Products*, *49*, 794–804. https://doi.org/10.1016/j.indcrop.2013.06.026

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, *11*(8),Article8.https://doi.org/10.3390/pr11082248

Hidalgo, G.-I., & Almajano, M. P. (2017). Red Fruits: Extraction of Antioxidants, Phenolic Content, and Radical Scavenging Determination: A Review. *Antioxidants*, *6*(1), Article 1. https://doi.org/10.3390/antiox6010007

Hobbi, P., Okoro, O. V., Hajiabbas, M., Hamidi, M., Nie, L., Megalizzi, V., Musonge, P., Dodi, G., & Shavandi, A. (2023). Chemical Composition, Antioxidant Activity and Cytocompatibility of Polyphenolic Compounds Extracted from Food Industry Apple Waste: Potential in Biomedical Application. *Molecules*, *28*(2), 675.https://doi.org/10.3390/molecules28020675

Kataoka, H. (2019). Pharmaceutical Analysis | Sample Preparation☆. In P. Worsfold, C. Poole, A. Townshend, & M. Miró (Eds.), *Encyclopedia of Analytical Science (Third Edition)* (pp. 231–255). Academic Press. https://doi.org/10.1016/B978-0-12-409547-2.14358-6

Korma, S. A., Alahmad, K., Ali, A. H., Shoaib, M., Abed, S. M., Yves, H., Atindana, J. N., & Qin, J. (2016). Application of pulsed electric field technology in apple juice processing. *Austin Journal of Nutrition and Food Sciences*, *4*(2), 1080. https://doi.org/10.11648/J.IJNFS.20160504.15

Kumar, A., & Chauhan, G. S. (2010). Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor. *Carbohydrate Polymers*, *82*(2), 454–459. https://doi.org/10.1016/j.carbpol.2010.05.001

Lavelli, V., & Kerr, W. (2012). Apple Pomace Is a Good Matrix for Phytochemical Retention. *Journal of Agricultural and Food Chemistry*, *60*(22), 5660–5666. https://doi.org/10.1021/jf3010993

Lyu, F., Luiz, S. F., Azeredo, D. R. P., Cruz, A. G., Ajlouni, S., &Ranadheera, C. S. (2020). Apple Pomace as a Functional and Healthy Ingredient in Food Products: A Review. *Processes*, *8*(3), Article 3. https://doi.org/10.3390/pr8030319

Makris, D. P., & Şahin, S. (2019). Polyphenolic Antioxidants from Agri-Food Waste Biomass. *Antioxidants*, *8*(12), Article 12. https://doi.org/10.3390/antiox8120624

Moni Bottu, H., Mero, A., Husanu, E., Tavernier, S., Pomelli, C. S., Dewaele, A., Bernaert, N., Guazzelli, L., & Brennan, L. (2022). The ability of deep eutectic solvent systems to extract bioactive compounds from apple pomace. *Food Chemistry*, *386*, 132717. https://doi.org/10.1016/j.foodchem.2022.132717

Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta*, *703*(1), 8–18. https://doi.org/10.1016/j.aca.2011.07.018

Nurdalilah, O., Teoh, Y. P., Ooi, Z. X., & Sam, S. T. (2018). Comparative Study on the Extraction of Bioactive Secondary Metabolites from Pomelo and Pineapple Peels Extract. *IOP Conference Series:*

Materials Science and Engineering, *429*(1), 012040. https://doi.org/10.1088/1757899X/429/1/012040

O'Shea, N., A. Ktenioudaki, T.P. Smyth, P. McLoughlin, L. Doran, M.A.E. Auty, E. Arendt, and E. Gallagher. "Physicochemical Assessment of Two Fruit By-Products as Functional Ingredients: Apple and Orange Pomace." *Journal of Food Engineering* 153 (May 2015), 89–95. https://doi.org/10.1016/j.jfoodeng.2014.12.014

Perussello, C. A., Zhang, Z., Marzocchella, A., & Tiwari, B. K. (2017). Valorization of Apple Pomace by Extraction of Valuable Compounds. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 776–796. https://doi.org/10.1111/15414337.12290

Phuyal, N., Jha, P. K., Raturi, P. P., &Rajbhandary, S. (2020). Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of *Zanthoxylumarmatum* DC. *The Scientific World Journal*, *2020*, e8780704. https://doi.org/10.1155/2020/8780704

Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology*, *30*(1), 37–44. https://doi.org/10.1016/j.tibtech.2011.06.014

Purkait, M. K., Duarah, P., & Das, P. P. (2023). *Recovery of Bioactives from Food Wastes*. CRCPress. https://doi.org/10.1201/9781003315469

Purkait, M. K., Haldar, D., & Duarah, P. (2023). Chapter 4—Advancement in bioactive compound extraction from natural products for pharmaceutical applications. In M. K. Purkait, D. Haldar, & P. Duarah (Eds.), *Advances in Extraction and Applications of Bioactive Phytochemicals* (pp. 75–99). Academic Press. https://doi.org/10.1016/B978-0-443-18535-9.00011-9

Putra, N. R., Rizkiyah, D. N., Abdul Aziz, A. H., Che Yunus, M. A., Veza, I., Harny, I., & Tirta, A. (2023). Waste to Wealth of Apple Pomace Valorization by Past and Current Extraction Processes: A Review. *Sustainability*, *15*(1), Article 1. https://doi.org/10.3390/su15010830

Qadariyah, L., Mahfud, M., Sulistiawati, E., & Swastika, P. (2018). Natural Dye Extraction From Teak Leves*(Tectona Grandis)* Using *Ultrasound Assisted Extraction* Method for Dyeing on Cotton Fabric. *MATEC Web of Conferences*, *156*, 05004. https://doi.org/10.1051/matecconf/201815605004

Rabetafika, H. N., Bchir, B., Blecker, C., & Richel, A. (2014). Fractionation of apple by-products as source of new ingredients: Current situation and perspectives. *Trends in Food Science & Technology*, *40*(1), 99–114. https://doi.org/10.1016/j.tifs.2014.08.004

Ranjha, M. M. A. N., Kanwal, R., Shafique, B., Arshad, R. N., Irfan, S., Kieliszek, M., Kowalczewski, P. Ł., Irfan, M., Khalid, M. Z., Roobab, U., &Aadil, R. M. (2021). A Critical Review on Pulsed Electric Field: A Novel Technology for the Extraction of Phytoconstituents. *Molecules*, *26*(16), 4893. https://doi.org/10.3390/molecules26164893

Rashid, R., MohdWani, S., Manzoor, S., Masoodi, F. A., &Masarat Dar, M. (2023). Green extraction of bioactive compounds from apple pomace by ultrasound assisted natural deep eutectic solvent extraction: Optimisation, comparison and bioactivity. *Food Chemistry*, *398*, 133871. https://doi.org/10.1016/j.foodchem.2022.133871

Saifullah, M., McCullum, R., &Vuong, Q. V. (2021). Optimization of Microwave-Assisted Extraction of Polyphenols from Lemon Myrtle: Comparison of Modern and Conventional Extraction Techniques Based on Bioactivity and Total Polyphenols in Dry Extracts. *Processes*, *9*(12), Article 12. https://doi.org/10.3390/pr9122212

Saikia, S., Mahnot, N. K., & Mahanta, C. L. (2016). Phytochemical content and antioxidant activities of thirteen fruits of

Assam, India. *Food Bioscience*, *13*, 15–20. https://doi.org/10.1016/j.fbio.2015.11.003

Samadi, M., Abidin, Z., Yunus, R., Radiah, D., Yoshida, H., & Lok, E. (2016). Assessing the kinetic model of hydro-distillation and chemical composition of Aquilaria malaccensis leaves essential oil. *Chinese Journal of Chemical Engineering*, *25*. https://doi.org/10.1016/j.cjche.2016.09.006

Sapkale, G. N., Patil, S. M., Surwase, U. S., &Bhatbhage, P. K. (2010). Supercritical Fluid Extraction: A Review. *International Journal of Chemical Sciences*, *8*(2), 729–743. https://doi.org/10.1201/b17997-15

Sinha, D., Mukherjee, S., & Chowdhury, S. (2022). Methods of Extraction of Phytochemicals. In *Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds* (pp. 250–279). IGI Global. https://doi.org/10.4018/978-1-6684-7337-5.ch010

Soria, A. C., Brokł, M., Sanz, M. L., &Martínez-Castro, I. (2012). 4.11—Sample Preparation for the Determination of Carbohydrates in Food and Beverages. In J. Pawliszyn (Ed.), *Comprehensive Sampling and Sample Preparation* (pp. 213–243). Academic Press. https://doi.org/10.1016/B978-0-12-381373-2.00135-6

Waldbauer, K., McKinnon, R., & Kopp, B. (2017). Apple Pomace as Potential Source of Natural Active Compounds. *Planta Medica*, *83*(12/13), 994–1010. https://doi.org/ 10.1055/s-0043-111898

Acknowledgment

Authors are grateful to the Department of Food Engineering and Technology Kokrajhar, Kokrajhar, Assam, India or providing the necessary assistance to carry out this review work.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

FUNCTIONAL FERMENTED MILK WITH CAMEL COLOSTRUM FOR HEALTH PROMOTING

Rasha Abdelaal Ibrahim [1,✉], Rehab Hamed Gab-Allah[1]

[1] Dairy Research Department, Food Technology Research Institute, Agricultural Research Center, Giza 12619, Egypt

✉rashamorsy@arc.sci.eg;✉rasha_ibrahim610@yahoo.com

https://doi.org/10.34302/crpjfst/2024.16.4.8

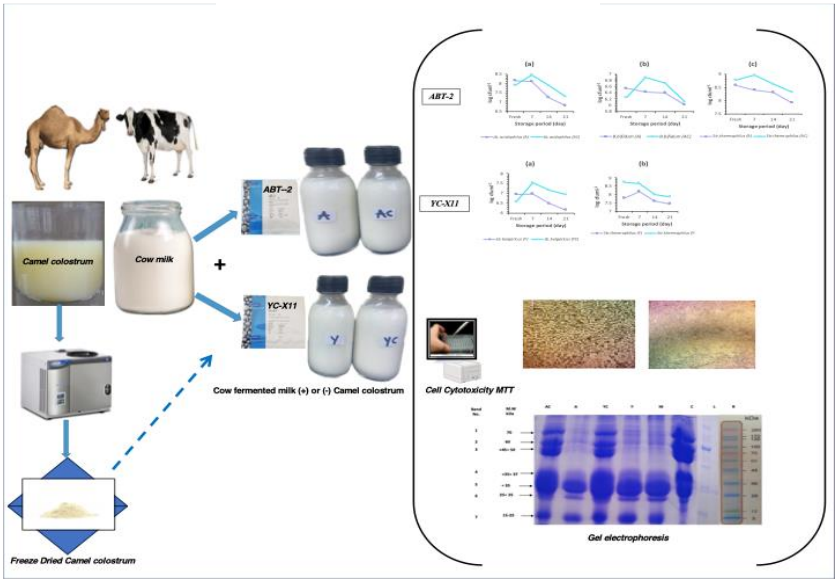
Article history:
Received
October 20th, 2024
Accepted
December 10th , 2024

Keywords:
Camel colostrum;
Cow fermented milk;
Probiotic;
Anticancer;
Sensory acceptability.

ABSTRACT

This study aims to investigate the in vitro anticancer properties of camel colostrum in fermented milk by comparing the antiproliferative activity of fermented cow milk with freeze-dried camel colostrum to fermented cow milk . The probiotic starter cultures DVS ABT-2 (*Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*) and YC- X11 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) were used to prepare fermented cow milk separately. The chemical composition of liquid and freeze-dried camel colostrum and immunoglobulin G (IgG) concentration were analyzed. Furthermore, the analysis of fermented milk samples included physicochemical and microbiological assessments, electrophoresis pattern analysis, and sensory evaluation. The findings showed a significant antiproliferative impact on Caco-2 cells with a lower IC_{50} and a rise in lactic acid bacteria availability in colostrum-fermented milk samples. Furthermore, adding freeze-dried camel colostrum enhanced both the textural and sensory characteristics of the resultant fermented milk.

Graphical Abstract



Fermented cow milk fortified by freeze-dried camel colostrum using DVS ABT-2 or DVS YC-X11 as a starter resulted in significantly increased bacterial count, antiproliferative effectiveness, and lower IC_{50} values against Caco-2 cells.

1.Introduction

Consumers inclination towards healthy food products, such as functional foods, has rapidly increased because of the growing desire for a healthy lifestyle for enhancing overall well-being, which are the main factors generating markets for functional food products. Functional food refers to food that provides additional health benefits, including improved physiological performance, such as the regulation of specific body functions and the manipulation of biological defense mechanisms to prevent and/or treat particular disorders along with basic nutritional properties (Shori *et al*. 2019). Supplements including calcium, fibers, omega-3 fatty acids, plant stanols, prebiotics, and lactic acid bacteria (LAB) that produce bioactive peptides can be used as components in the production of functional foods. One particularly successful model of a functional dairy product type is fermented dairy products, which contain probiotic microflora, including yeast and bacteria, such as lactic acid bacteria (LAB) and bifidobacterium, recognized for their health-promoting properties (Behnsen *et al*. 2013). Which are responsible for enhancing food product digestion and the overall health of the human gastrointestinal system, as well as promoting well-being by improving the responsiveness of immune cells and the capacity to boost interferon (INF) production. It also contributes to lowering blood cholesterol, preventing various forms of cancer, and improving cognitive impairments (Bohlouli *et al*. 2021; Sanders *et al*. 2018). Additionally, fermented milk is comparatively simpler to digest than unfermented milk products and is considered a suitable food for those who are intolerant to lactose (Ezzatpanah 2020). The extended shelf life and appealing sensory acceptability of fermented milk enhance its distribution, consumption, and marketability. There is currently enough data to take into account fermented dairy products that include living bacteria when creating dietary plans to enhance health and the fact that it is frequently included in diets across the globe (Akdeniz and Akalin 2019; Behnsen *et al*. 2013).

Colostrum is the main source of nutrition and immunological components for the newborn. The nutraceutical components of colostrum are mainly represented by nutrients, antibodies, vitamins, immune factors, and growth factor contents; these nutrient profiles differ amongst species (Ceniti *et al*. 2023). Camel colostrum is an "early" milk generated by milking glands of she-camel in the first five days after parturition; over the next two days, it changes into mature milk (El-Hatmi *et al*. 2006). Compared to cow, goat, and sheep colostrum, camel colostrum has less lactose and fat but more protein, peptides, non-protein nitrogen, ash, vitamins, and minerals. A significant feature of camel colostrum is that is free of β-lactoglobulin (β-Lg), the principal whey protein in bovine milk that induces allergies in children, alongside a higher concentration of lactoferrin, which enhances the antimicrobial properties of camel colostrum (Benkerroum *et al*. 2004). Furthermore, contains immunoglobulins, which represent the main component of newborns' immunity to infections and is divided into three major subclasses (IgG1, IgG2, and IgG3). The two immunoglobulin subclasses, IgG2 and IgG3, are light chains and have molecular weights of 42 and 45 kDa, respectively (El-Hatmi *et al*. 2006; Konuspayeva *et al*. 2010). This characteristic of camel colostrum's protein composition represents a specific biological action, such as antibacterial, antioxidant, and antihypertensive effects (Jrad *et al*. 2020).

A new generation of competitive food products can be produced through developments in food ingredients and innovative technology. As fermented milk is an active subject of research, therefore, it is necessary to develop innovative new varieties of specialized foodstuffs based on fermented milk enriched with physiologically functional components. Camel milk and its derived products are increasingly being used in human nutrition. Little-known regarding the usage of camel colostrum in food products. Consequently, the aim of this study was to examine in vitro the antiproliferative action against carcinogenic cell line (Caco-2 cells) of fermented cow milk fortified with freeze-dried camel colostrum.

Additionally evaluated fermented milk made with cow milk containing camel colostrum, whether with DVS ABT-2 (*Str. thermophilus, Lb. acidophilus, and Bifidobacterium bifidum*) or YC-X11 (*Str. thermophilus* and *Lb. delbrueckii subsp. bulgaricus*), in terms of bacterial viability throughout a 21-day storage period at 4°C and bioactive peptide content compared to fermented cow milk.

2.Materials and methods

2.1. Materials

DVS ABT-2 (*Str. thermophilus, Lb. acidophilus, and B. bifidum*) and YC-X11 (*Str. thermophilus and Lb. delbrueckii* subsp. *bulgaricus)* starter cultures were obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. Camel colostrum (CC) was obtained from Berkash Farm, Giza, Egypt. Which were collected from over four multiparous adult female camels (*Camelus dromedarius*) at only one calving season at the beginning of November, and the samples were physically taken from the udder's four quarters after parturition for three days and kept at -18 °C for additional analysis. At the time of colostrum collection, none of the camels that were sampled showed signs of clinical mastitis. Fresh cow milk (11.75% ± 0.05 total solids, 3.30 %± 0.04 fat, 3.41%±0.1 protein, 4.42% ±0.03 lactose, 0.68%± 0.02 ash, 6.77±0.02 pH) was got from the Faculty of Agriculture's dairy unit, Cairo University, Giza, Egypt.

2.2. Methods

2.2.1. Freeze drying camel milk

Camel colostrum is completely dried in a freeze dryer (Snijders Scientific type 2040). The lyophilized camel colostrum was kept at 4°C for additional uses.

2.2.2. Preparation of functional fermented milk

The steps of producing fermented milk are demonstrated in Figure 1. In short, the raw cow milk (5 kg) was heated to 85 °C for 15 minutes, cooled to 5 °C, after that raised to 40 °C. Four different cow fermented milk samples were produced for analysis (Figure 1). The control samples of fermented cow milk included 0.2 g/L milk of two different types of starters (DVS ABT-2 or DVS YC-X11), which represent A and Y samples, respectively. While AC and YC samples differ from the control samples in the addition of colostrum to each of them at a rate of 3.5% (Tajorudin and Hamirudin 2020). Samples of inoculated milk were placed in 100 ml glass containers and incubated for 3 - 4 h at 42 °C until fully coagulation (pH reaches approximately 4.6). The glasses of fermented milk treatments were stored at 4 °C for 21 days and were examined in triplicate on fresh, 7, 14, and 21 days of cold storage.

2.2.3. Physicochemical analysis

Total solids, fat, total protein, ash, and titratable acidity were measured using the procedure outlined in AOAC (2012). The lactose content was analyzed according to Lawrence (1968). The pH of fermented milk was estimated electrometrically by a pH meter with a plastic electrode and a digital pH meter (JENWAY 3510).

2.2.4. Determination of IgG concentrations by enzyme-linked immunosorbent assay (ELISA)

2.2.4.1. Colostrum whey preparation

Colostrum (100 ml) was added to 50 ml saline. The mixture was centrifuged at 100,000 g for one hour at 4 °C to separate fat. The clarified aqueous layer (cleared colostrum, or "lactoserum") between the surface fatty layer and the particle was collected. After stirring and adding hydrochloric acid to get the pH down to 4, the solution was centrifuged at 30,000 g for 30 minutes at 4°C. After discarding the pellet, the supernatant was neutralized with 2M Tris and centrifuged for 30 minutes at 4°C at 30,000 g. The supernatant was run through a 0.45 μm filter, and the filtrate (colostrum whey) was kept at - 20°C until it was needed after (Azwai *et al*.1996).

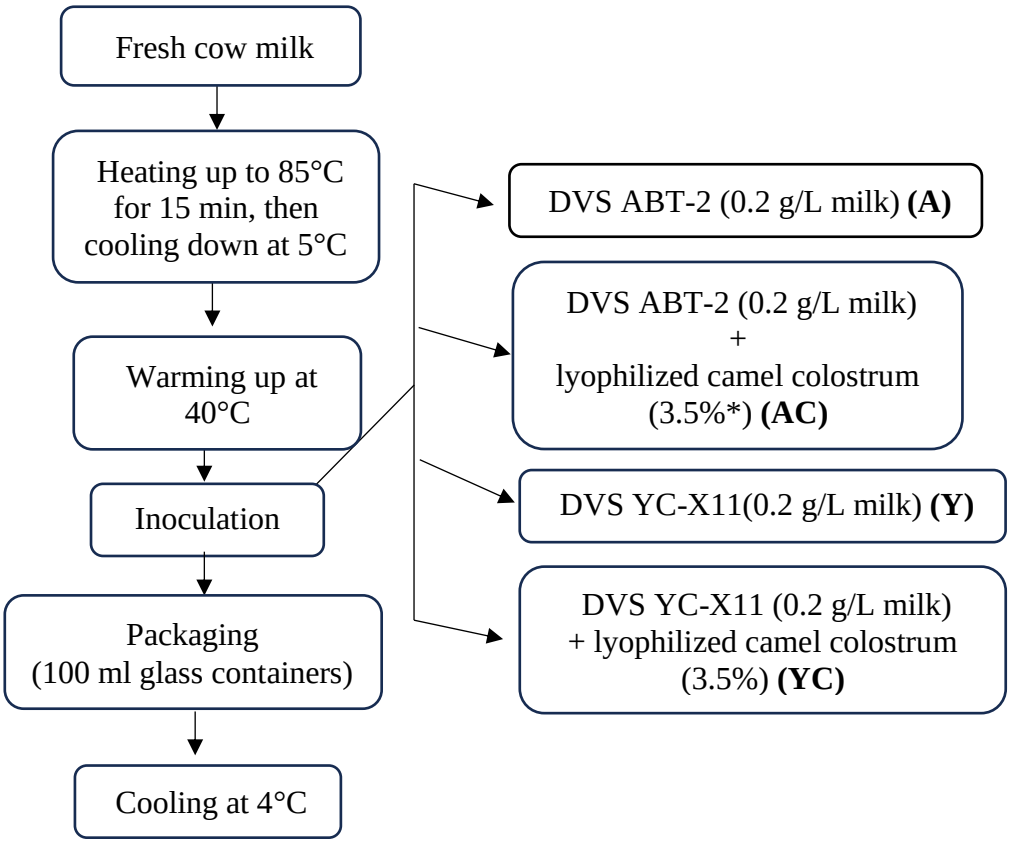


Figure 1. Preparation of functional fermented milk fortified with freeze dried camel colostrum

2.2.4.2. Enzyme-linked immunosorbent assay (ELISA)

For the testing of cross-reactivity of IgG with camel colostrum proteins**.** The antibody generated against IgG was evaluated for its cross-reactivity with a preparation of camel colostrum whey. The samples were diluted 1/800,000 in order to achieve optimal quantification prior to the experiment. The IgG concentration was measured by 2-site ELISA kits (Abcam, Cambridge, UK). The results were interpreted by spectrophotometric measurements at 450 nm wavelength. The antibody level was calculated using 4-parameter logistic curves for quantitative analysis. (Bartkiene *et al*. 2020).

2.2.5. Microbiological changes in functional fermented milk during storage period

The determination of the viable cell count for the *Lb. acidophilus* strain within the mixed culture was conducted by incorporating 1.5% (w/v) bile salt into MRS agar, according to Vinderola and Reinheimer (2000). The enumeration of *Lb. delbrueckii* subsp. *bulgaricus* was conducted utilizing MRS agar, according to Frank and Yousef (2004). Whilst *Str*. *thermophilus* by using M17. The enumeration of *B. bifidum* was conducted using modified MRS agar, which was supplemented with lithium chloride (0.600 g), neomycin sulfate (0.200 g), nalidixic acid (0.030 g), and paromomycin sulfate (0.250 g), all sourced from Merck, Warszawa, Poland, which were then dissolved in 100 ml of distilled water. The pH of the solution was adjusted to 7.3 ± 0.1 using 0.1 M sodium hydroxide (NaOH) and subsequently sterilized via filtration through 0.22 µm millipore filter prior to sterilization. An aliquot of 5 ml from this antibiotic solution was incorporated into 100 ml of MRS agar (pH 6.2 ± 0.1; Oxoid) just before application. The plates were incubated at a temperature of 37 °C for a

duration of 48 hours. Anaerobic conditions were established utilizing anaerobic culture jars with a capacity of 2.5 liters, along with AnaeroGen AN 25 sachets from Oxoid (Dave and Shah 1996). Coliform bacteria were enumerated on Violet Red Bile (VRB) agar following incubation at 37 ˚C for a duration of 48 hours. Colonies exhibiting pink to red-purple coloration, with or without surrounding halos of precipitation, were classified as coliform bacteria (Atlas 2004). Yeast and mold counts were assessed using yeast peptone dextrose agar (Oxoid) that was enriched with chloramphenicol at a concentration of 0.1 g/L (Oxoid) and incubated at a temperature of 30 °C for a duration of 72 hours (Jay *et al*, 2005). All microbial counts were calculated and expressed as log cfu ml^{-1}. The bacteria were counted three times in each experiment.

2.2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis was conducted with minor adjustments in accordance with Laemmli (1970). Initially, samples were ultracentrifuged for 30 minutes at 55,000 g, and the high fat surface layer was removed (Goil *et al*.1998). Fat-free samples along with the sample application buffer, which consisted of 2.5 mM Tris-HCl at pH 6.8, 10.0% glycerol, 4.0% SDS, 0.02% bromophenol blue, and 10% mercaptoethanol, were prepared in a 1:2 ratio. The resulting mixtures were subsequently incubated in a water bath at 100 °C for 5 minutes to facilitate protein denaturation. Following this step, 10 µl of the mixture was loaded into the electrophoresis apparatus. Electrophoresis was performed in 15% polyacrylamide separating gel and 4% stacking gel. The gels were subjected to electrophoresis for a duration of 2 hours at a voltage of 100 V. After that, stained with Coomassie Brilliant Blue (CBB) R-250 (Bio-Rad) for 3 hours at ambient temperature to allow the dye to migrate to the bottom of the separating gel and detection of protein bands. Then, the gel is destained until the background is less dark by repeatedly immersing the sample in a solution of methanol, acetic acid, and water in a ratio of 1:1:8. The sample was run in 4 % stacking and 15% separating gel and used the broad-range protein marker (RIS11-prestained protein ladder, Cat. No. PMI11-0500, Volume: 500 µl, Bio- Helix Co., LTD) as the standard of the protein size.

2.2.7. Cytotoxic activity

2.2.7.1. Water-soluble extract

After adjusting the pH of each fermented milk sample to 4.6 using either 1.0 M hydrochloric acid or 1.0 M sodium hydroxide, the samples were centrifuged at 9,000 x g for a duration of 15 min at 4 °C. The supernatants were filtered through a syringe filter with a pore size of 0.45 µm (mixed cellulose esters membrane, EMD Millipore Corp., Billerica, MA) and were subsequently stored at -20 °C for additional analysis.

2.2.7.2. Cytotoxic effect on human cell line (MTT assay)

Water-soluble extracts were filtered through a Macrosep Advance spin filter (3 kDa; Pall Corp., Port Washington, NY). Filtrates were tested against carcinogenic cell line (Caco-2 cells) using the technique described by Ayyash *et al*. (2018). A 96-well tissue culture plate (Biofloat, Mannheim, Germany) was seeded with 1 x 10^5 cell/ml (100 µl/well) and incubated at 37°C for 24 h to create a complete monolayer sheet. The growth medium was removed from the 96-well microtiter plates when a confluent cell monolayer formed, and the monolayer was subsequently washed twice with wash media. Two-fold dilutions of the tested samples were prepared in RPMI medium containing 2% serum (maintenance medium). 0.1 ml of each dilution was added to separate wells, keeping three wells as controls that contained only maintenance medium. The plate was incubated at 37°C; following that, the cells were examined for any physical indicators of toxicity, such as partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. A 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) solution was prepared by mixing 5 mg of MTT with 1 ml of PBS (Bio Basic Canada INC). After that, 20 µl MTT solution was added to each well. The plate was then put on a shaking table and spun at 150 rpm for five minutes to fully incorporate the MTT

into the medium. To enable the MTT to be metabolized, the incubation procedure was then carried out for four hours at 37°C with a 5% CO_2 atmosphere. After draining the culture medium from the plate, they were then dried on paper towels. 200 µl of dimethyl sulfoxide (DMSO) was used to dissolve the formazan compounds that resulted from the reduction of tetrazolium salt. In order to fully integrate the formazan into the solvent, the mixture was then put on a shaking table and stirred at 3.77 g for 5 min. The optical density was measured at a wavelength of 560 nm and subtracted background at 620 nm. Three separate analyses of each sample were conducted. The impact of samples on cell lines was captured using a digital colour microscope camera connected to a microscope (Leitz Labovert, Germany).

2.2.8. Sensory evaluation

Assessment of sensory attributes of all fermented milk samples was organoleptically tested by a 20-member trained and experienced panel consisting of scientists of the Dairy Research Department, FTRI, using fresh samples and samples on the 21 th day of storage that were maintained at 4 °C until the time of testing. The samples were presented in identical plastic cups that were randomly coded. The evaluation of the fermented milk samples encompassed color and appearance, taste and odor, texture, and overall acceptability, employing a 9-point hedonic scale that ranged from 1 (dislike greatly) to 9 (like greatly), as outlined by Lim (2011).

2.2.9. Statistical analysis

The results obtained were subjected to statistical analysis utilizing a software package (Version 2004, SAS Institute, Cary, North Carolina, USA) that employed analysis of variance. In instances where the F-test yielded significant results, the least significant difference (LSD) was computed according to Duncan's method for mean comparisons at a significance level of $P < 0.05$. The data displayed in the tables represent the mean of three measurements, accompanied by the (± standard deviation).

3.Results and discussion

3.1. Chemical composition of camel colostrum

The chemical composition of camel colostrum is shown in Table 1. The amount of total solids TS in camel colostrum is 13.90% this is nearly consistent with the findings of Omer and Eltinay (2008) regarding United Arab Emirates (UAE) camel colostrum (12.90%), but considerably lower than the values reported by Konuspayeva *et al*. (2010) and Ohri and Joshi (1961) for Kazakhstan (KZ) and Indian (IN) camel colostrum (15.61% and 18.4%, respectively). The protein ratio of 4.92% is lower than that of SA and KZ camel colostrum, as reported by Gorban and Izzeldin (1997) and Konuspayeva *et al*. (2010), which were 5.8% and 6.03%, respectively. While in agreement with the results provided by Omer and Eltinay (2008) regarding UAE camel colostrum. The lactose content is 4.70%, exceeding that of camel colostrum from the UAE (4.4%), SA (2.7%), and KZ (3.63%) (Omer and Eltinay 2008; Gorban and Izzeldin 1997; Konuspayeva *et al*. 2010). Abu-Lehia (1989) noted that the lactose concentration during parturition was 2.68% and progressively increased to 4.4% by the third day. The fat content (3.10%) is higher than that reported by Johnson (1978) at 1.36%, Ohri and Joshi (1961) at 0.2-2.8%, and Yagil and Etzion (1980) at 0.15%, but it is comparable to the percentages reported by Gorban and Izzeldin, (1997) and Omer and Eltinay (2008) at values of 3.01 and 3.10%, respectively. Nevertheless, the research conducted by Konuspayeva *et al*. (2010) revealed a finding of 7.88%. The ash content measured at 1.15% is almost equal to the 1.11% reported by Omer and Eltinay (2008). It exceeds the 0.78% found by Gorban and Izzeldin (1997) but is lower than the 2.60% noted by Ohri and Joshi (1961). The average Ig concentration in liquid colostrum was found to be 4.58±0.08%, which is lower than the value reported by El-Hatmi *et al*. (2006) for Tunisian dromedary camel colostrum, which was 10.07 %. The pH level measured at 6.32 exceeds that of UAE and IN camel colostrum, which were recorded at 6.19 and 5.6, respectively, according to Omer and Eltinay

(2008) and Ohri and Joshi (1961). Conversely, this pH is lower than that of SA camel colostrum, which ranged from 6.40 to 6.60, as noted by Abu-Lehia (1989) and Gorban and Izzeldin, (1997), as well as lower than the 6.52 pH found in KA camel colostrum as reported by Konuspayeva *et al*. (2010). The lower pH values observed in colostrum compared to milk may be attributed to the presence of dihydrogen phosphate, citrate, and carbon dioxide in camel colostrum, which are linked to the reduced pH levels found in colostrum (Starkutė *et al*. 2023). Freeze-dried camel colostrum yield is (84.36%± 1.12). The TS content of this colostrum is 96.8%, as detailed in Table 1. Among its components, protein and lactose account for the largest proportions at 34.52% and 32.57%, respectively. This is followed by fat, which constitutes 21.50%, while ash represents the smallest ratio at 8.00%.

Table 1. Composition of liquid and freeze-dried camel colostrum

Properties	Liquid colostrum	Freeze-dried colostrum
Total solids (%)	13.90±0.05	96.80±0.10
Protein (%)	4.92±0.10	34.52±0.20
Fat (%)	3.10±0.10	21.50±0.10
Lactose (%)	4.70±0.05	32.57±0.03
Ash (%)	1.15±0.04	8.00±0.06
IgG (%)	4.58±0.08	28.00±0.19
pH	6.32±0.10	6.48±0.10

3.2. Physicochemical properties of functional fermented milk

Data displaying in Table 2 are the physicochemical characteristics of fermented milk, influenced by the addition of camel colostrum and the type of bacterial starter culture. The results cleared that supplementing with camel colostrum as lyophilized powder led to a significant increase in TS contents. The TS values of fresh samples A, AC, Y, and YC were 11.98, 15.50, 12.00, 15.51%, respectively. However, the TS contents are uninfluenced by the type of bacterial starter culture. A slightly gradual increase in the TS contents of all samples correlated to the prolonging of the cold storage duration. In general, all TS contents obtained in all treatments agree with the legal standard of EOSQ (2010). The pH values of cow milk fermented with the YC-X11 were found to be significantly lower ($P < 0.05$) compared to those fermented with the ABT-2. Additionally, the titratable acidity (TA%) measurements of the fermented milk from each treatment indicated that the acidity level generated by the YC-X11 was significantly higher than that formed by ABT ones either with or without colostrum. This explains the lower acidity rate when using ABT -2 due to the absence of *Lb. delbrueckii* subsp. *bulgaricus*. Similar findings are observed by Akgun *et al*. (2018). Generally, the pH exhibited a significant reduction ($P< 0.05$) and TA % increased ($P < 0.05$) in both treatments fermented by YC-X11 or ABT-2 during the storage period.

The incorporation of camel colostrum resulted in a fermented milk product exhibiting a higher TA% and a lower pH value compared to the fermented milk that did not contain camel colostrum.

Lactic acid bacteria (LAB) metabolize lactose in milk for their growth and produce metabolites, mainly lactic acid, and energy for their growth and keeping up, resulting in a reduction in the pH of fermented milk (Wang *et al*. 2021). Logically, the prolonging of the cold storage period caused an increase in TA% and hence a corresponding decrease in pH value, as the longer presence time of live bacteria in the product means more time available for the LAB to metabolize the milk lactose, and so more lactic acid was produced, which contributed to a much lower pH surrounding the LAB. Also, the results demonstrate that fermented milk fortified with camel colostrum led to a fermented milk

with TA% slightly higher and pH value lower than those that occurred in fermented milk without camel colostrum. The variations noted in pH and acidity are influenced by the composition of the substrate, a relationship that can be linked to the presence of fermentable sugars in camel colostrum including lactose, along with glucose, fructose, glucosamine, galactosamine, N-acetylneuraminic acid, and oligosaccharides (Fukuda *et al*. 2010). The results regarding the total acidity percentage are consistent with the research conducted by (Ayyash *et al*. 2018), which demonstrated that *Lactococcus lactis* KX881782 exhibited a greater ability to generate organic acids, reflected in a higher total acidity percentage, when fermenting camel milk compared to cow milk. We hypothesize that the elevated percentage of TA in camel colostrum fermented camel milk could be associated with the strain's adaptation to camel colostrum, as well as the presence of specific amino acids and peptides in camel colostrum that promote the growth of this bacteria. On the other hand, colostrum is characterized by high buffering capacity (Lucey *et al*.1993) as the high buffering capacity is related to the increased protein content, which is considered a principal buffering component, similar findings are observed by Varghese and Mishra (2008).

3.3. Microbiological changes in functional fermented milk

To examine the effect of camel colostrum on fermentation, DVS YC-X11 (*Lb*. *bulgaricus* and *Str*. *thermophilus*) or DVS ABT-2 starter (*Lb*. *acidphilus*, *B. bifidium*, and *Str*. *thermophilus*) were inoculated in cow milk. While the same analysis was performed as before, but without adding colostrum as a control. The bacterial numbers (log cfu ml^{-1}) of ABT-2 in colostrum fermented milk or fermented milk without colostrum treatments stored at 5 °C, is shown in Figure 2 (a, b, and c). In fresh samples, significantly elevated number of counts ($P < 0.05$) was observed in ABT fermented milk fortified with colostrum. Generally, there was a slight raise in the count of viable cells during the first seven days of refrigerated storage, following a gradual decrease. Xu *et al*. (2021) observed that the count of viable fermented milk organisms initially rose following production, peaked at a certain point, and subsequently declined during the period of refrigerated storage. *Str*. *thermophilus* emerged as the predominant starter culture, with its population surpassing 7.5 logs at the onset of the storage period. This observation aligns with the manufacturer's specifications for the DVS ABT-2 starter culture provided by Chr. Hansen. A significant rise ($P< 0.05$) in the counts of *Str*. *thermophilus* was observed on the seventh day between control and colostrum-fortified fermented milk. The difference in the number of streptococci between colostrum fermented milk and the control group increased throughout the storage period, eventually reaching a value of 0.4 log cycles by the end of the storage experiment. This may be due to the stimulatory effect of the nutrients found in colostrum, which can enhance the growth of LAB (Fasse *et al*. 2021). However, *Str*. *thermophilus* counts were found to be within the range of (7.92±0.28 to 8.32±0.13 log cfu ml^{-1}) after 21 d of storage at 5 °C. It should be noted that the initial count of *Lb. acidphilus* 0.7 log cycle lower than for *Str. thermophilus*. However, a significantly higher viable count of *Lb*. *acidophilus* was recorded during cold storage at 5 °C in comparison to *B. bifidum*, this is due to the resistance of *Lb. acidophilus* to acidity in contrast to *B.bifidium* (Lourens-Hattingh and Viljoen 2001). Nevertheless, a decrease in *Lb. acidophilus* count was observed after that. The initial counts of *B.bifidium* ranged between 7.25-7.43 log cfu ml^{-1}. The count of bifidobacterium noted between days 7 and 14 was greater than that recorded on day 0 in both types of fermented ABT milks. There were significant differences ($P< 0.05$) between the numbers of bifidobacterium in fermented milk fortified with colostrum compared to fermented milk without it; this may be due to oligosaccharides and lacto-N-biose in camel colostrum which act as prebiotics and stimulate the growth of bifidobacteria (Fukuda *et al*. 2010).

Table 2 Chemical properties of functional fermented milk fortified with freeze-dried camel colostrum

Properties	Cold storage (days)	Treatments			
		A	AC	Y	Y C
Total solids (%)	Fresh	11.98 ± 0.01 b, a, b	15.50 ± 0.01 a, a, b	12.00 ± 1.00 b,a,b	15.51 ±0.01 a,,a,b
	7	12.04 ± 0.04 b, a, ab	15.57 ±0.02 a, a, ab	12.07 ± 0.01 b, a, ab	15.58 ±0.02 a, ,a,ab
	14	12.19 ±0.01 b, a, a	15.74 ±0.04 a, a, a	12.20 ± 0.01 b, a, a	15.73 ± 0.03 a ,a, a
	21	12.27 ±0.01 b, a, a	15.82 ±0.02 a, a, a	12.28 ±1.00 b, a, a	15.81 ±0.01 a, a, a
Fat (%)	Fresh	3.20 ±0.20 b, a, a	4.2 ±0.10 a, a, a	3.20 ±0.20 b, a, a	4.20 ±0.20 a, a, a
	7	3.20 ± 0.20 b, a, a	4.2 ± 0.20 a, a, a	3.20 ± 0.20 b, a, a	4.20 ± 0.10 a, a,a
	14	3.30 ±0.30 b, a,a	4.3 ±0.10 a, a, a	3.30 ±0.30 b, a, a	4.30 ±0.10 a, a,a
	21	3.30 ± 0.10 b, a, a	4.3 ± 0.10 a, a, a	3.30 ± 0.20 b, a, a	4.30 ±0.20 a, a, a
Protein (%)	Fresh	3.40 ±0.03 b, a, b	4.64 ±0.02 a, a, b	3.40 ±0.01 b, a, b	4.64 ±0.04 a, a, b
	7	3.41 ±0.01 b, a, b	4.66 ± 0.01 a,a,b	3.42 ± 0.02 b, a, b	4.67 ± 0.1 a, a, b
	14	3.43 ± 0.03 b, a, ab	4.70 ± 0.04 a, a, ab	3.45 ± 0.01 b, a,ab	4.71 ± 0.02 a, a, ab
	21	3.48 ± 0.04 b, a, a	4.73 ± 0.01 a, a, a	3.49 ±0.01 b, a,a	4.75 ±0.05 a, a, a
Lactose (%)	Fresh	4.62 ±0.02 b, a, b	5.61 ± 0.01 a, a, b	4.63 ± 0.10 b,a,b	5.62 ± 0.02 a, a, b
	7	4.66 ± 0.1 b, a, b	5.64 ± 0.04 a, a, b	4.68 ±0.10 b, a, b	5.65 ± 0.05 a, a, b
	14	4.68 ± 0.02 b, a, ab	5.66 ± 0.01 a, a, ab	4.67 ±0.10 b, a, ab	5.64 ±0.04 a, a, ab
	21	4.69 ± 0.1 b, a, a	5.69 ± 0.01 a, a, a	4.68 ± 0.10 b, a, a	5.67 ± 0.07 a, a, a
Ash (%)	Fresh	0.76 ±0.02 b, a, b	1.05 ±0.01 a, a, b	0.77 ±0.01 b, a, b	1.05 ± 0.05 a,a ,b
	7	0.77 ± 0.01 b, a, b	1.07 ± 0.02 a, a, b	0.77 ±0.01 b, a, b	1.06 ±0.01 a, a, b
	14	0.78 ± 0.02 a, a, ab	1.08 ±0.02 a, a, ab	0.78 ±0.01 b, a, ab	1.08 ± 0.02 b, a,ab
	21	0.80 ± 0.1 b, a, a	1.10 ±0.1 a, a, a	0.81 ±0.10 b, a, a	1.09 ±0.01 a, a, a
pH	Fresh	4.73 ±0.03 a, a, a	4.72 ±0.02 b, a, a	4.63 ±0.03 a, b, a	4.60 ±0.10 b, b, a
	7	4.60 ± 0.05 a, a, ab	4.55 ± 0.01 b,a,ab	4.51 ± 0.01 a, b, ab	4.42 ±0.02 b, b,ab
	14	4.51 ±0.01 a, a, b	4.43 ±0.03 b, a, b	4.40 ±0.1 a, b, b	4.33 ±0.03 b, b, b
	21	4.34 ±0.03 a, a, c	4.28 ±0.02 b, a, c	4.25 ±0.02 a, b, c	4.17 ±0.01 b, b,c
T.A. (%)	Fresh	0.75 ±0.01 b, b, c	0.77 ±0.01 a, b, c	0.81 ±0.01 b, a, c	0.85 ±0.03 a, a, c
	7	0.86 ±0.02 b, b, b	0.88 ±0.01 a, b, b	0.93 ±0.01 b, a, b	0.98 ±0.01 a, a, b
	14	0.91 ±0.01 b, b, ab	0.96 ±0.01 a, b, ab	1.02 ±0.02 b, a,ab	1.12 ±0.01 a, a, ab
	21	1.00 ±0.1 b, b, a	1.09 ±0.01 a, b, a	1.10 ±0.1 b, a, a	1.23 ±0.01 a ,a,a

The letters preceding the comma indicate the presence of camel colostrum factor, whereas those following the comma represent the factors associated with the starter and storage periods, respectively. Means sharing the same letter at any position do not exhibit significant differences (P>0.05). All values are presented as means ±SD, n = 3. (A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum Fermented milk with YC -X11.

Additionally, it was noted that *Str. thermophilus* has the potential to enhance the growth and viability of bifidobacterium when utilized in conjunction within starter cultures through creating an anaerobic environment by acting as an oxygen scavenger (Lourens-Hattingh and Viljoen 2001). However, the decrease in the number of bifidobacterium on the 21st day of storage is due to this low tolerance to acidity, and thus their survival rate decreases at low levels of pH (less than 4.6) (Lee and Salminen 2009).

Data presented in Figure 2 (d & e) shows changes in fermented milk with YC-X11 (*Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*) in fresh samples and during storage period. The survival rate of *Str. thermophilus* is often reported to be high, exceeding 8 log cfu ml^{-1} in fermented milk products that have been refrigerated for a duration of up to 6 weeks. (Varga *et al*. 2014). Research indicates that the relative abundance of *Str*. *thermophilus* within the overall acidifying microflora of yogurt exceeds that of *Lb. bulgaricus*, despite both being inoculated in equal proportions (Bielecka and Majkowska, 1998). The proto-cooperation is partly due to the ability of *Str. thermophilus* to rapidly metabolize lactose, resulting in the production of acids that decrease the pH to a level conducive to the growth of *Lb*. *bulgaricus*. Additionally, *Lb*. *bulgaricus* may be further stimulated by various metabolites generated by *Str. thermophilus*, as previously noted (Sieuwerts *et al*. 2008). Concurrently, *Lb*. *delbrueckii* subsp. *bulgaricus* synthesizes vital amino acids during the fermentation process, which are necessary for the proliferation of *Str. thermophilus* (Lourens-Hattingh and Viljoen 2001). Moreover, *Lb. bulgaricus* was better adapted to the acidic environment and dominated the fermentation process subsequently until the endpoint (Gasser *et al*. 2022). The viable counts of *Str*. *thermophilus* and *Lb*. *bulgaricus* in colostrum fermented milk were significantly higher ($P < 0.05$) compared to those in free colostrum fermented milk. This enhancement may be attributed to the presence of amino acids and peptides in camel colostrum, which are known to promote the growth of these bacterial species (Fenster *et al*. 2019).

The population of *Str. thermophilus* in ABT-fermented samples exceeded that of the YC-X11 starter, both in fresh samples and throughout the storage period. This phenomenon can be attributed to the fact that the ABT culture generated lower acidity in the fermented milk compared to the YC-X11 starter, resulting in a reduced loss of survival values for the former relative to the latter. These findings align with the observations made by Ismail (2015). The diminished bacterial population observed in ABT -2 or YC-X11 within fermented milk containing colostrum in fresh samples may be attributed to the fact that camel colostrum contains more natural antibacterial compounds than cow's milk. These components, which include immunoglobulins, lactoferrin, lactoperoxidase, lysozyme, and cytokines, are known to impede the rapid proliferation of bacterial cultures. Furthermore, they exhibit bacteriostatic rather than bactericidal effects on probiotic strains (Ali *et al*. 2023; González-Navarro *et al*. 2022). So, we assume that both starter cultures take more time to overcome this effect. In general, the counts of different microbial groups for all fermented milk treatments pronounced decreased within storage. The decline was evidently attributed to a reduction in pH below the optimal level, resulting from lactic acid production by proliferating lactic acid bacteria. This alteration affects the intracellular pH of the LAB, thereby inhibiting enzyme activity, ion transport, and nutrient absorption, which subsequently inhibits bacterial growth and reduces LAB populations at elevated concentrations (Soleymanzadeh *et al*. 2016). However, all fermented milk conforms to requirements by food regulations the starter microorganisms must remain viable, active, and in sufficient numbers (at least 10^6) in the product to the date of minimum durability to maintain their health promoting effects (CODEX STAN 243-2003).

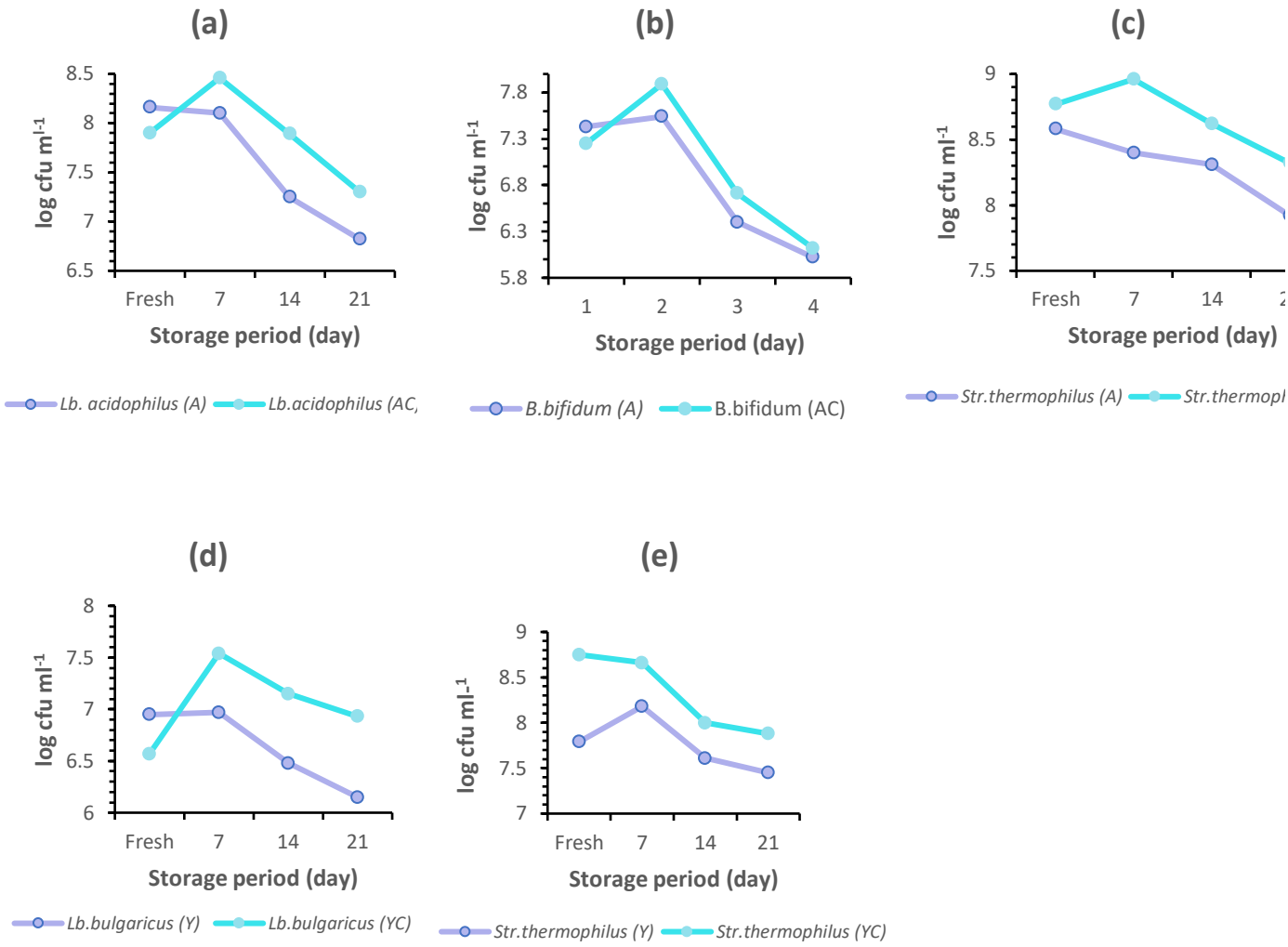


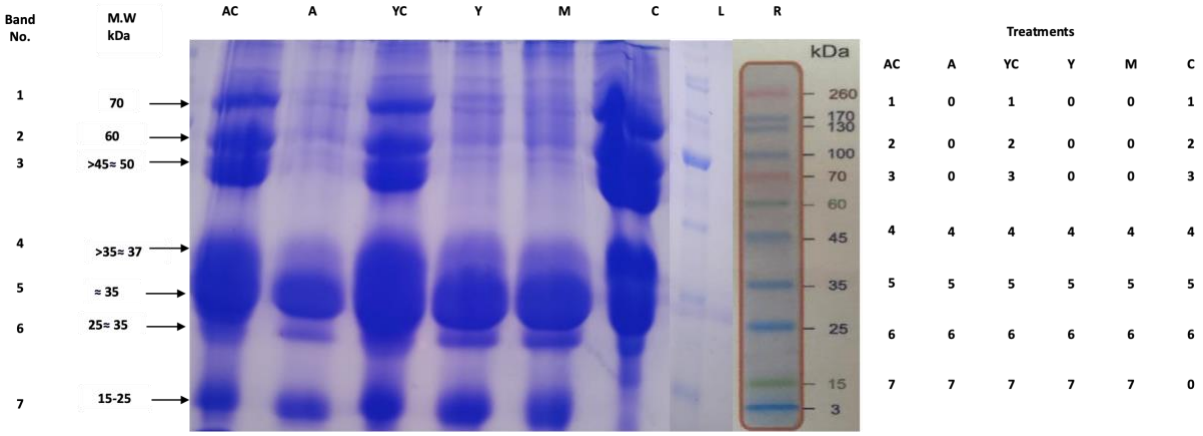
Figure 2 Effect of using camel colostrum on starter bacteria counts of fermented milk.
(A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum fermented milk with YC-X11.

This results from the particular dietary needs that lactic acid bacteria (LAB) and bifidobacteria have for growth and function (Hébert *et al*. 2004). The availability of nutrients in the growth environment, including short peptides, free amino acids, and oligosaccharides, influences the growth and viability of starter cultures (Fenster *et al*. 2019). The camel colostrum provides the fermentation media with the nutrients that starter bacteria need to grow and maintain their viability. Moreover, the decline in viability during storage was significant in the control fermented milk. The findings indicate that colostrum has a beneficial impact on improving the viability of ABT and YC starter cultures.

No proliferation of yeast and mold, or coliform was observed in either the colostrum-fermented ABT milk or the controls throughout the entire storage duration. This can be associated with the characteristics of fermented milks, which include low oxygen levels, high acidity, and the synthesis of antimicrobial compounds by the starter bacteria (Surono and Hosono 2011).

3.4. Electrophoretic patterns of functional fermented milk

The proteins bands of fresh fermented milk treatments as well as cow milk and freeze-dried camel colostrum are presented in descending order based on their relative molecular weights are shown in Figure 3. It is clear that there were more bands of protein in the range of 50 to 70 kDa in colostrum fermented milk and freeze-dried colostrum (treatment C) compared to free colostrum samples and cow milk. These bands may be associated with IgM (band 1), IgA (band 2), IgG1, IgG2 and IgG3 (band 3) with molecular weight of 70, 63, 50, 46, and 42 kDa, respectively. The findings align with those of Azwai *et al*. (1996), who indicated that the molecular weight for IgM, IgA and IgG1, IgG2, IgG3 have the same range of molecular weight. Furthermore, a specific biological activity, such as antibacterial, antioxidant, and antihypertensive properties, was reported for proteins with a molecular weight of 42-45 kDa (Jrad *et al*. 2020). The data indicated that a peptide with MW of 25≈30 kDa present in all tested samples, which may correspond to lactoferrin (Gaspar-Pintiliescu *et al.* 2020). All tested samples except colostrum exhibited a band indicative of light chains within the 15-25 kDa range. These results agree with the observations in the literature (Costa *et al*. 2014), who indicated that the proteins α S2- casein (CN), α S1- CN, β- CN, and κ- CN exhibit molecular weights ranging from 35 to 24 kDa, while β- lactoglobulin and α- lactalbumin display molecular weight bands of 18 kDa and 14.2 kDa, respectively, in cow milk.



Treatments

	AC	A	YC	Y	M	C
1	1	0	1	0	0	1
2	2	0	2	0	0	2
3	3	0	3	0	0	3
4	4	4	4	4	4	4
5	5	5	5	5	5	5
6	6	6	6	6	6	6
7	7	7	7	7	7	0

Figure 3 Electrophoretic patterns of fermented milk samples, cow milk, and freeze-dried colostrum migrated along with prestained protein ladder. **(**Lane A**)** fermented milk with ABT-2; (lane AC) colostrum fermented milk with ABT-2; (lane Y) fermented milk with YC- X11; (lane YC) colostrum fermented milk with YC-X11; (lane C) Freeze-dried colostrum; (lane L) prestained protein ladder; (R) molecular weight marker.

3.5. Cytotoxic activity of functional fermented milk

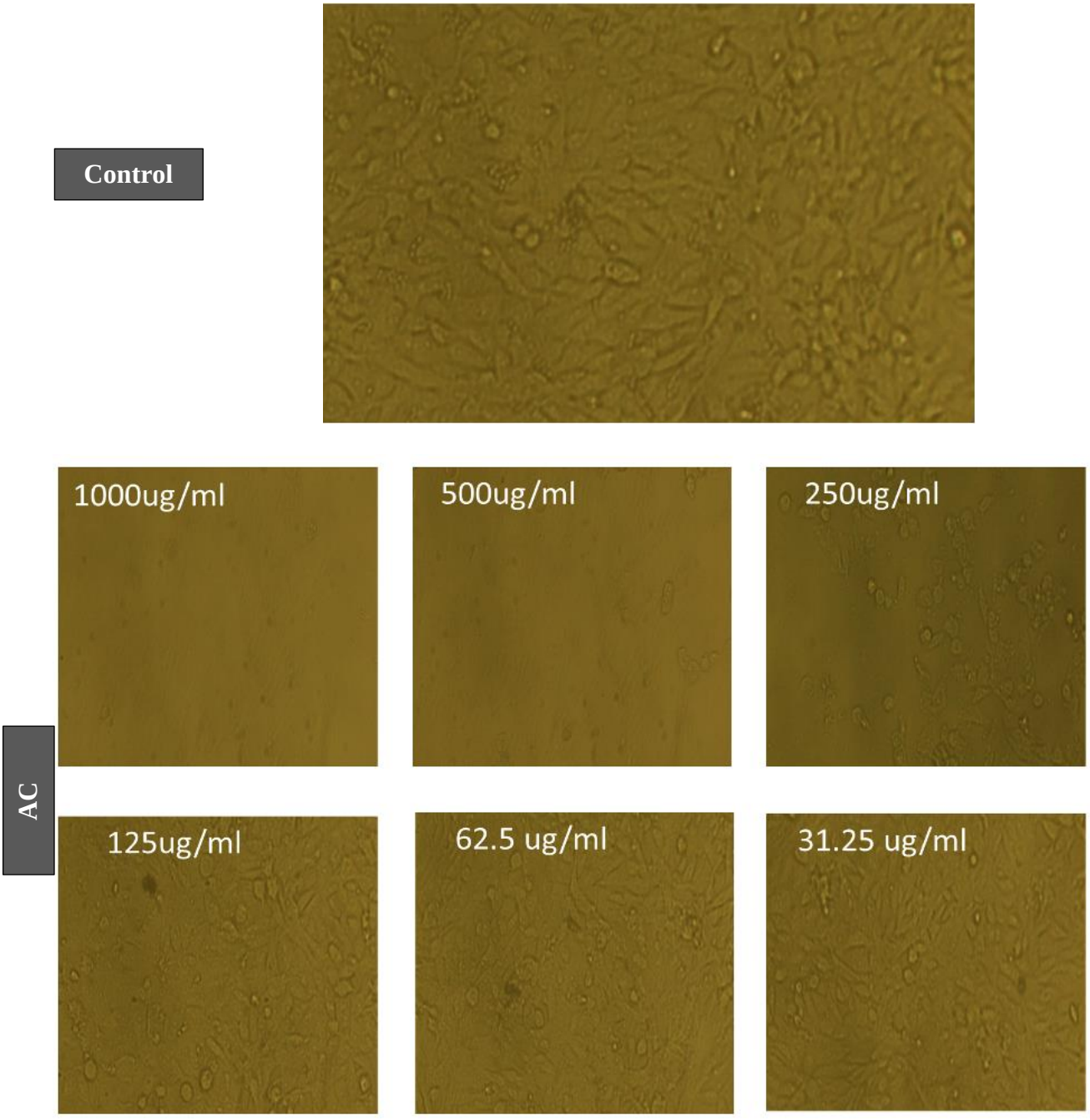
The anticancer effect of different concentrations and the IC_{50} value (refers to the concentration of a substance that results in a 50% mortality rate of cells within a 48-hour exposure period) of fermented milk and colostrum fermented milk samples against the carcinogenic cell lines (Caco-2 cells) are illustrated in Table 3 and Figure 4. The present results confirmed that the carcinogenic cells maintained their viability when cultivated in the standard growth conditions and exhibited no cytotoxic effects. The data further indicated a reduction in the viability of carcinogenic cells when cultured in growth media supplemented with the fermented milk samples. The cytotoxic activity against Caco-2 cells, as demonstrated in Table 3 and Figure 4, generally increased with higher concentrations of each sample. Specifically, the lowest cytotoxic activity was observed at the minimal concentration of 31.25 µg/ml, whereas the most significant antiproliferative cytotoxic effect was recorded at

the maximum concentration of 1000 µg/ml. The findings additionally indicated that the YC sample exhibited the most significant cytotoxic activity ($P < 0.05$), as reflected by a reduced IC_{50} value. Whereas the YC sample inhibited the viability and growth of half of the cultured Caco-2 cells using 74.62 µg/ml of its extract, followed by Y (79.51 µg/ml), AC (158.1µg/ml), and A (204.52 µg/ml). The current results support previously published information about the therapeutic properties of camel colostrum, especially its effectiveness as an anticancer agent (El-Kattawy *et al*. 2021). The findings align with those of Gaspar-Pintiliescu *et al*. (2020), who indicated that fermented colostrum extracts and their associated peptide fractions exhibited good cytocompatibility in fibroblast-treated cells. Nevertheless, additional investigations indicated that the existence of small peptides derived from fermented milk promoted the proliferation of murine spleen cells and augmented their immunomodulatory capabilities (Qian *et al*. 2011).

Table 3. Cytotoxic activity of functional fermented milk on Caco-2 cell lines

ID	µg/ml	Viability %	Toxicity %	IC_{50} µg/ml
Negative control (Caco2)	-------	100.00	0.00	
A	31.25	100.00 ± 1.00 ^{a,a,a}	0.00 ± 0.00 ^{b,b,f}	204.52 ± 1.87^{a}
	62.50	100.00 ±1.00 ^{a,a,b}	0.00 ±0.00 ^{b,b,e}	
	125	93.28 ±0.02 ^{a,a,c}	6.72 ± 0.13 ^{b,b,d}	
	250	30.09 ± 0.17 ^{a,a,d}	69.91 ±0.10 ^{b,b,c}	
	500	10.80 ± 0.01 ^{a,a,e}	89.20 ±0.10 ^{b,b,b}	
	1000	3.99 ± 0.01 ^{a,a,f}	96.01 ± 0.08 ^{b,b,a}	
AC	31.25	99.72 ± 0.20 ^{b,a,a}	0.28 ± 0.01 ^{a,b,f}	158.1 ± 1.63^{b}
	62.5	92.81 ± 0.01 ^{b,a,b}	7.19 ± 0.04 ^{a,b,e}	
	125	51.65 ± 0.05 ^{b,a,c}	48.35 ± 0.17 ^{a,b,d}	
	250	20.17 ± 0.06 ^{b,a,d}	79.83 ± 0.01^{a,b,c}	
	500	2.69 ± 0.04 ^{b,a,e}	97.31 ± 0.10 ^{a,b,b}	
	1000	2.50 ± 0.08 ^{b,a,f}	97.50 ± 0.09 ^{a,b,a}	
Y	31.25	99.17 ± 0.06 ^{a,b,a}	0.83 ± 0.03 ^{b,a,f}	79.51 ± 0.94 ^{c}
	62.5	51.69 ± 0.01 ^{a,b,b}	48.31± 0.11^{b,a,e}	
	125	21.28 ± 0.14 ^{a,b,c}	78.72± 0.09 ^{b,a,d}	
	250	10.20 ± 0.07 ^{a,b,d}	89.80 ± 0.12 ^{b,a,c}	
	500	3.01 ± 0.09 ^{a,b,e}	96.99 ± 0.11 ^{b,a,b}	
	1000	2.92 ± 0.18 ^{a,b,f}	97.08 ± 0.12 ^{b,a,a}	
YC	31.25	92.44 ± 0.16 ^{b,b,a}	7.56 ± 0.16 ^{a,a,f}	74.62 ± 0.38^{d}
	62.5	45.94 ± 0.03 ^{b,b,b}	54.06 ± 0.10 ^{a,a,e}	
	125	9.92 ± 0.19 ^{b,b,c}	90.08 ± 0.17 ^{a,a,d}	
	250	5.70 ± 0.10 ^{b,b,d}	94.30 ± 0.17 ^{a,a,c}	
	500	2.60 ± 0.04 ^{b,b,e}	97.40 ± 0.19 ^{a,a,b}	
	1000	2.55 ± 0.11 ^{b,b,f}	97.45 ± 0.30 ^{a,a,a}	

The letters preceding the comma indicate the presence of camel colostrum factor, whereas those following the comma represent the factors associated with the starter and storage periods, respectively. Means sharing the same letter at any position do not exhibit significant differences (P >0.05). All values are presented as means ±SD, n = 3 (A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum Fermented milk with YC -X11.



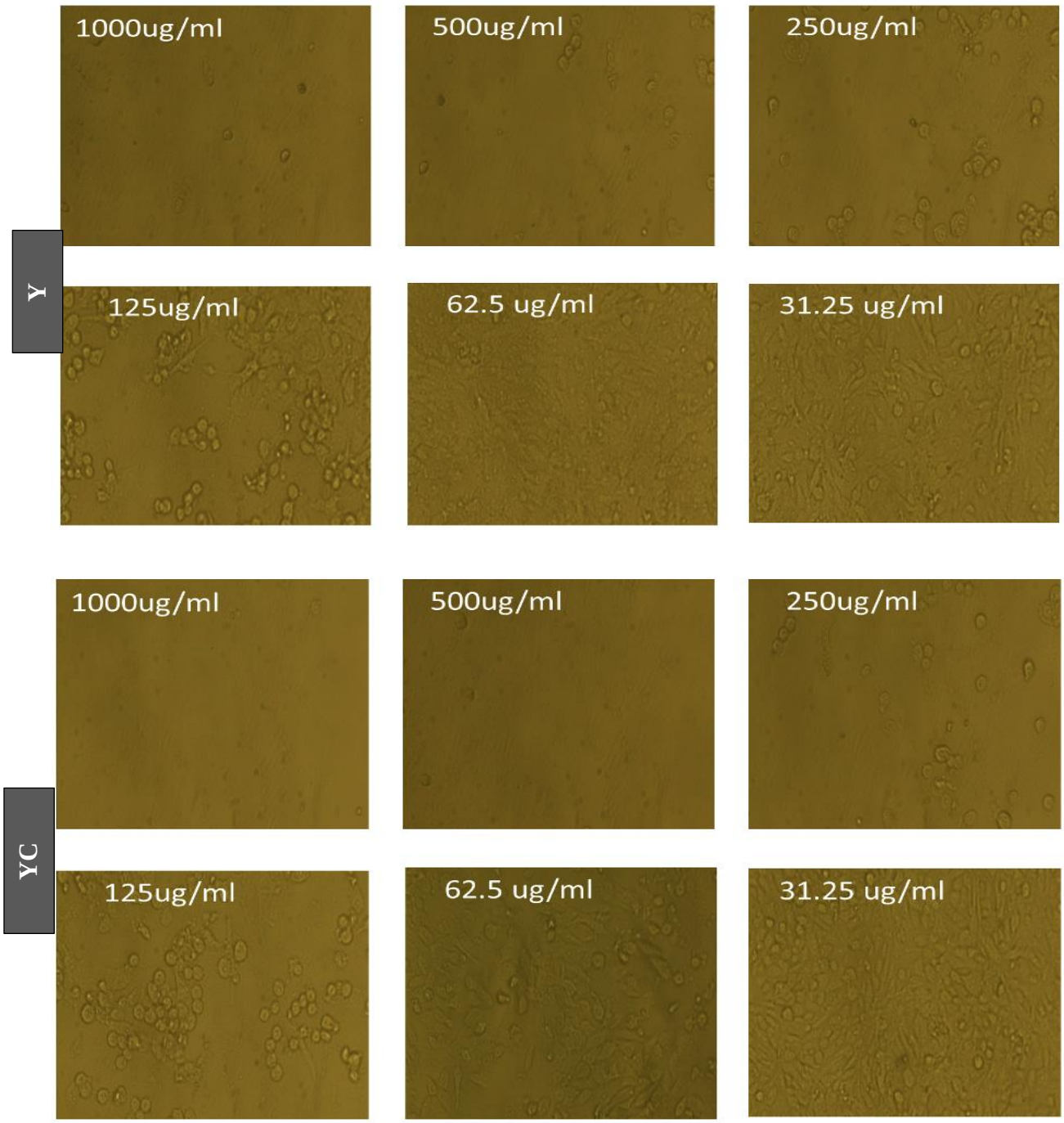


Figure 4. Effect of camel colostrum fermented samples on the Caco-2 cell line. **(**A) fermented milk with ABT-2; (AC) colostrum fermented milk with ABT-2; (Y) fermented milk with YC- X11; (YC) colostrum fermented milk with YC -X11.

3.6. Organoleptic quality of functional fermented milk

Table 4 presents the scores of fermented milks, both in their fresh and following a 21- day cold storage period, highlighting the influence of freeze-dried camel colostrum fortification and the specific type of bacterial starter used (ABT-2 or YC -X11). In terms of color and appearance ratings, all samples exhibited no differences, regardless of whether they were fortified with ABT-2 or YC-X11. However, colostrum fermented milk samples recorded higher scores (P <0.05) compared to the other treatments and the same result was observed both in fresh

samples and those stored for 21 days. Regarding the panelist score of texture data, it indicated that fermented milk containing colostrum received a significantly higher score (P <0.05) compared to fermented milk samples devoid of colostrum. This enhancement in texture is likely attributable to the increased viscosity conferred by the addition of colostrum, a finding that aligns with the results reported by Ayar *et al*. (2016) in their studies on yogurt and kefir formulations. Nevertheless, the texture consistency was affected by the end of cold storage period. Concerning the taste and odor scores, the fortification with colostrum led to a higher score than those without colostrum; it can be a consequence of the higher ratio of TS, fat, and overall protein content present in colostrum, which contribute significantly to the flavor and sensory characteristics of milk products (Silva *et al.* 2022*)*. Moreover, fermented milk made by ABT-2 gained a palatability score higher than those made by YC-X11; these findings align with Gallardo-Escamilla *et al.* (2005). However, a lower flavor score (P<0.05) was observed in the fermented milk produced using the YC-X11 starter after 21 days of storage, which may be attributed to an increase in acidity. For overall acceptability, the statistical analysis indicated that the fermented milk produced with camel colostrum achieved a significantly higher total score (P<0.05) in comparison to samples free of colostrum, regardless of the use of ABT or YC-X11 starter. Generally, the sensory attributes assessed in the camel colostrum samples consistently received scores exceeding the acceptability threshold of 70% (6.30 points), indicating favorable acceptance of this fermented food among consumers (Gularte 2009).

Table 4 Sensory evaluation of functional fermented milk fortified with camel colostrum

Sample	**A**	**AC**	**Y**	**YC**
Storage period (day)	Color and appearance			
Fresh	8.56 ±0.13 ^{b,a,a}	8.68 ±0.02 ^{a,a,a}	8.45 ±0.15 ^{b,a,a}	8.51 ±0.06 ^{a,a,a}
After 21 days	8.10 ±0.09 ^{b,a,b}	8.29 ±0.09 ^{a,a,b}	8.21 ±0.10 ^{b,a,b}	8.35 ±0.11 ^{a,a,b}
	Taste and odor			
Fresh	8.68 ±0.04 ^{b,a,a}	8.72 ±0.08 ^{a,a,a}	8.43 ±0.08 ^{b,b,a}	8.56 ±0.09 ^{a,b,a}
After 21 days	8.13 ±0.03 ^{b,a,b}	8.34 ± 0.10 ^{a,a,b}	7.94 ±0.06 ^{b,b,b}	8.29 ±0.03 ^{a,b,b}
	Texture			
Fresh	8.30 ±0.05 ^{b,b,a}	8.46 ±0.01 ^{a,b,a}	8.52 ±0.09 ^{b,a,a}	8.60 ±0.15 ^{a,a,a}
After 21 days	7.91 ±0.04 ^{b,b,b}	8.21 ±0.04 ^{a,b,b}	8.39 ±0.06 ^{b,a,b}	8.44 ± 0.06 ^{a,a,b}
	Overall acceptability			
Fresh	8.51 ± 0.19 ^{b,a,a}	8.62 ±0.03 ^{a,a,a}	8.47 ± 0.05 ^{b,a,a}	8.56± 0.05 ^{a,a,a}
After 21 days	7.98 ± 0.10 ^{b,a,b}	8.28± 0.14 ^{a,a,b}	8.24 ± 0.13 ^{b,a,b}	8.36 ± 0.08 ^{a,a,b}

The letters preceding the comma indicate the presence of camel colostrum factor, whereas those following the comma represent the factors associated with the starter and storage periods, respectively. Means sharing the same letter at any position do not exhibit significant differences (P>0.05). All values are presented as means ±SD, n = 3. (A) Fermented milk with ABT-2; (AC) Colostrum fermented milk with ABT-2; (Y) Fermented milk with YC- X11; (YC) Colostrum Fermented milk with YC -X11.

4.Conclusion

Freeze-dried camel colostrum CC (3.5%) promoted the ABT-2 and YC-X11 starter counts in fermented cow milk. Significant antiproliferative activity and lower IC_{50} values were demonstrated in CC fermented milk samples against Caco-2. Free CC samples showed higher IC_{50} values compared to using CC. Fermented milk samples with CC displayed low MW peptides, mainly IgG, IgM, and IgA, with potential health effects. The incorporation of freeze-dried camel colostrum enhanced both the texture and the flavor profile, including taste and aroma, of the resulting fermented milk. The findings indicate the potential utilization of

freeze-dried camel colostrum in the formulation of functional dairy products.

5.References

Abu-Lehia, I.H. (1989). Physical and chemical characteristics of camel milk fat and its fractions. *Food Chemistry,* 3(4), 261 - 271. https://doi.org/10.1016/0308-8146(89)90103-9

Akdeniz, V. and Akalin, A. S. (2019). New approach for yoghurt and ice cream production: High-intensity ultrasound. *Trends in Food Science and Technol*ogy, 86, 392–398. DOI:10.1016/J.TIFS.2019.02.046

Akgun, A., Yazici, F., and Gulec, H. A. (2018). The combined effect of probiotic cultures and incubation final on pH on the quality of buffalo milk yogurt during cold storage. *Food Science and Nutrition,* 6,492-502. DOI: 10.1002/fsn3.580

Ali, A. H., Abu-Jdayil, B., Nabulsi A., Osaili, T., Liu, S. Q., Kamal-Eldin, A., and Ayyash M. (2023). Fermented camel milk influenced by soy extract: Apparent viscosity, viscoelastic properties, thixotropic behavior, and biological activities. *Journal of Dairy Science,* 106 (10), 6671–6687. https://doi.org/10.3168/jds.2023-23294

AOAC (2012). Official Method of Analysis: Association of Analytical Chemists, 19th ed. Washington DC.

Atlas, R. M. (2004). Handbook of microbiological media. CRC Press. https://doi.org/10.1201/9781420039726

Ayar, A., Siçrama, H., and Çetin, I. (2016). The effect of bovine colostrum on the lactic flora of yogurt and kefir. *JSM Biotechnology and Biomedical Engineering,* 3, (4), 1-6. https://api.semanticscholar.org/CorpusID:34493512

Ayyash, M., Al-Dhaheri, A. S., Al Mahadin, S., Kizhakkayil, J. and Abushelaibi, A. (2018). In vitro investigation of anticancer, antihypertensive, antidiabetic, and antioxidant activities of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. *Journal of Dairy Sci*ence, 101 (2), 900-911. DOI:https://doi.org/10.3168/jds.2017-13400

Azwai, S. M., Carter, S. D., and Woldehiwet, Z. (1996). Immunoglobulins of camel (Camelus dromedarius) colostrum. *Journal of Comparative Pathology,* 114 (3), 273-282. https://doi.org/10.1016/S0021-9975(96)80049-1

Bartkiene, E., Lele, V., Sakiene, V., Zavistanaviciute, P., Ruzauskas, M., Stankevicius, A., Grigas, J., Pautienius, A., Bernatoniene, J., Jakstas, V., Zadeike, D., Viskelis, P., and Juodeikiene, G. (2020). Fermented, ultrasonicated, and dehydrated bovine colostrum: Changes in antimicrobial properties and immunoglobulin content. *Journal of Dairy Sci*ence, 103(2), 1315-1323. https://doi.org/10.3168/jds.2019-16357

Behnsen, J., Deriu, E., Sassone-Corsi, M., and Raffatellu, M. (2013). Probiotics: properties, examples, and specific applications. *Cold Spring Harbor Perspectives in Medicine,* 3(3), a010074–a010074. DOI: 10.1101/cshperspect.a010074

Benkerroum, N., Makkaoui, B., Bennani N. and Kamal, H. (2004). Antimicrobial activity of camel's milk against pathogenic strains of *Escherichia coli* and *Listeria monocytogenes*. *International Journal of Dairy Technology*, 57 (1), 39-43. https://doi.org/10.1111/j.1471-0307.2004.00127.x

Bielecka, M. and Majkowska, A. (1998). Survival of synergistic sets of *Streptococcus salivarius* subsp. *thermophilus* and *lactobacillus delbruecki* subsp. *bulgaricus* cultures during spray drying of yogurt. *Polish Journal of Food and Nutrition Sciences*,48(2), 267-274.

Bohlouli, J., Namjoo, I., Borzoo-Isfahani, M., Hojjati Kermani, M. A., Balouch Zehi, Z., and Moravejolahkami, A. R. (2021). Effect of probiotics on oxidative stress and inflammatory status in diabetic nephropathy: A systematic review and meta-analysis of clinical trials. *Heliyon*, 7(1), e05925.

https://doi.org/10.1016/j.heliyon.2021.e05925

Ceniti, C., Costanzo, N., Morittu, V. M., Tilocca, B., Roncada, P., and Britti , D. (2023). Colostrum as an emerging food: nutraceutical properties and food supplement. *Food Reviews International*, 39(7), 4636-4664. https://doi.org/10.1080/87559129.2022.2034165

Codex Alimentarius Commission. Codex standard for fermented milks (2003). Codex Stan 243-2003, 11p. FAO-WHO International Food Standards.

Costa, F. F., Vasconcelos Paiva Brito, M. A., Moreira Furtado, M.A., Martins, M.F., Leal de Oliveira, M. A., Mendonça de Castro Barra, P., Amigo Garrido, L., and dos Santos, A. S. (2014). Microfluidic chip electrophoresis investigation of major milk proteins: study of buffer effects and quantitative approaching. *Analytical Methods*, 6, 1666-1673. DOI: 10.1039/c3ay41706a

Dave, R.I. and Shah, N. P. (1996). Evaluation of Media for Selective Enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and Bifidobacteria. *Journal of Dairy Science*, 79 ,1529-1536. https://doi.org/10.3168/jds.S0022-0302(96)76513-X

El-Hatmi, H., Levieux, A., and Levieux, D. (2006). Camel (*Camelus dromedarius*) immunoglobulin G, α-lactalbumin, serum albumin and lactoferrin in colostrum and milk during the early post-partum period. *Journal of Dairy Researc*h, 73(3), 288-293. DOI: 10.1017/S0022029906001713

El-kattawy, A. M., Algezawy, O., Alfaifi, M. Y., Noseer, E. A., Hawsawi, Y.M., Alzahrani, O. R., Algarni, A., Kahilo, K. A. and El-Magd, M.A. (2021). Therapeutic potential of camel milk exosomes against HepaRG cells with potent apoptotic, anti-inflammatory, and anti-angiogenesis effects for colostrum exosomes. *Biomedicine and Pharmacotherapy*, 143, 112220. https://doi.org/10.1016/j.biopha.2021.112220

EOSQ (2010). Egyptian Organization for Standardization and Quality. Egyptian Standard. D-1 Food/2010. Guide Lines for Quality Criteria for Milk and Milk Products.

Ezzatpanah, H. (2020). Traditional food and practices for health: Iranian dairy foods, in: Nutritional and Health Aspects of Food in South Asian Countries, pp. 275-287. Elsevier. https://doi.org/10.1016/B978-0-12-820011-7.00030-7

Fasse, S., Alarinta, J., Frahm, B., and Wirtanen, G. (2021). Bovine colostrum for human consumption-improving microbial quality and maintaining bioactive characteristics through processing. *Dairy*, 2 (4), 556 -575. https://doi.org/10.3390/dairy2040044

Fenster, K., Freeburg, B., Hollard, C., Wong, C., Rønhave Laursen, R., and Ouwehand, A.C. (2019). The production and delivery of probiotics: a review of a practical approach. *Microorganisms*, 7(3), 83-99. https://doi.org/10.3390/microorganisms7030083

Frank, J. F. and Yousef, A.E. (2004). Tests for groups of microorganisms, in: Frank J F (Ed.), Standard Methods for The Examination of Dairy Products. American Public Health Association.

Fukuda, K., Yamamoto, A., Ganzorig, K., Khuukhenbaatar, J., Senda, A., Saito, T., and Urashima, T. (2010). Chemical characterization of the oligosaccharides in Bactrian camel (*Camelus bactrianus*) milk and colostrum. *Journal of Dairy Science*, 93 (12), 5572–5587. https://doi.org/10.3168/jds.2010-3151

Gallardo-Escamilla, F. J., Kelly, A. L., and Delahunty, C. M. (2005). Influence of starter culture on flavor and headspace volatile profiles of fermented whey and whey produced from fermented milk. *Journal of Dairy Science*, 88 (11), 3745-3753. https://doi.org/10.3168/ jds.S0022-0302(05)73060-5

Gaspar-Pintiliescu, A., Oancea, A., Cotarlet, M., Vasile, A. M., Bahrim, G. E., Shaposhnikov, S. Craciunescu, O., and Oprita, E.I. (2020).

Angiotensin-converting enzyme inhibition, antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum. *International Journal of Dairy Technology*, 73(1), 108 -116. https://doi.org/10.1111/1471-0307.12659

Gasser, C., Garault, P., Chervaux, C., Monnet, V., Faurie, J. M. and Rul, F. (2022). Co-utilization of saccharides in mixtures: Moving toward a new understanding of carbon metabolism in *Streptococcus thermophilus*. *Food Microbiology*, 107, 104080. https://doi.org/10.1016/j.fm.2022.104080

Goil, S., Barnes, C., Thibeault, D., and Truog, W.E. (1998). Simplified method for purification of colostrum to obtain secretory component of immunoglobulin A, using secretory component as a reference protein in tracheal aspirate fluid. *Journal of Chromatography B: Biomedical Sciences and Applications*, 705(2), 203-211. https://doi.org/10.1016/S0378-4347(97) 00523-9

González-Navarro, E. J., Beltrán, M. C., Molina, M. P., Pérez-Barbería, F. J., Molina, A. and Berruga, M. I. (2022). Suitability of goat colostrum to produce a fermented yogurt-type product. *Animals*, 12 (21), 3025. DOI: 10.3390/ani12213025

Gorban, A. M. S. and Izzeldin, O. M. (1997). Mineral content of camel milk and colostrum. *Journal of Dairy Research*, 64 (3),471- 474. DOI: https://doi.org/10.1017/S0022029997 002264

Gularte, M.A., (2009). Manual de análise sensorial de alimentos, Universidade Federal de Pelotas, Editora e Gráfica Universitária PREC, Pelotas, RS.

Hébert, E.M., Raya, R. R. and Savoy, G. S. (2004). Nutritional requirements of *lactobacillus delbrueckii* subsp. *lactis* in a chemically defined medium. *Current Microbiology*, 49, 341-345. https://doi.org/10.1007/s00284-004-4357-9

Ismail, M. M. (2015). Improvement of nutritional and healthy values of yoghurt by fortification with rutub date. *Journal of microbiology, biotechnology and food sciences*, 4 (5), 398-406. DOI: 10.15414/jmbfs.2015.4.5.398-406

Jay, J. M., Loessner, M. J., and Golden, D. A. (2005). Modern Food Microbiology: Primary Sources of Microorganisms Found in Foods, pp33-36 ,7th Edition, Springer Science and Business Media, Inc., New York.

Johnson, A.H. (1978). The composition of milk, pp 125–209. in: Webb B H, Johnson A H, Al-ford J (Eds.), Fundamental of dairy chemistry. The AVI Publishing Company Inc., West Port, CT, USA.

Jrad, Z., Oussaief, O., Khorchani, T., and El-Hatmi, H. (2020). Camel colostrum composition, nutritional value, and nutraceuticals, pp. 240–262. DOI: 10.4018/978-1-7998-1604-1.ch011

Konuspayeva, G., Faye, B., Loiseau, G., Narmuratova, M., Ivashchenko, A., Meldebekova, A., and Davletov, S. (2010). Physiological change in camel milk composition (Camelus dromedarius) 2: physico-chemical composition of colostrum. *Tropical Animal Health and Production*, 42(3), 501-505. DOI: 10.1007/s11250-009-9450-4

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. https://doi.org/10.1038/227680a0

Lawrence, A. J. (1968). The determination of lactose in milk products. *Australian Journal of Dairy Technology*, 23, 103.

Lee, Y. K .and Salminen, S. (2009). Handbook of probiotics and prebiotics, 2nd ed. Inc. Hoboken, New Jersey. Canada.

Lim, J. (2011). Hedonic scaling: A review of methods and theory. *Food Quality and Preference*, 22(8), 733-747. https://doi.org/10.1016/j.foodqual.2011.05. 008

Lourens-Hattingh, A. and Viljoen, B. C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal*, 11(1-2), 1-17. https://doi.org/10.1016/S0958-6946(01)00036-X

Lucey, J. A., Hauth, B., Gorry, C. and Fox, P. F. (1993). Acid–base buffering of milk acid–base buffering of milk. *Milchwissenschaft*, 48, 268 -272.

Ohri, S. P. and Joshi, B. K. (1961). Composition of camel milk. *Indian Veterinary Journal*, 38, 514-517.

Omer, H. R .and Eltinay, A.H. (2008). Chemical composition of camel's colostrum and milk in united arab emirates. *Arab Universities Journal of Agricultural Sciences,* 16 (1), 127-134. DOI:10.21608/ajs.2008.14620

Qian, B., Xing, M., Cui, L., Deng, Y., Xu, Y., Huang, M., and Zhang, S. (2011). Antioxidant, antihypertensive, and immunomodulatory activities of peptide fractions from fermented skim milk with *Lactobacillus delbrueckii* ssp. *bulgaricus* LB340. *Journal of Dairy Research,* 78(1), 72-79. DOI: 10.1017/S0022029910000889

Sanders, M. E., Merenstein, D., Merrifield, C. A., and Hutkins, R. (2018). Probiotics for human use. *Nutrition Bulletin* ,43(3), 212-225. https://doi.org/10.1111/nbu.12334

Shori, A. B., Baba, A. S., and Muniandy, P. (2019). Potential health-promoting effects of probiotics in dairy beverages, pp. 173-204. In: Value-Added Ingredients and Enrichments of Beverages. Elsevier. https://doi.org/10.1016/B978-0-12-816687-1.00005-9

Sieuwerts, S., de Bok, F. A. M., Hugenholtz, J., and van Hylckama Vlieg, J. E. T. (2008). Unraveling microbial interactions in food fermentations: from classical to genomics approaches. *Applied and Environmental Microbiology*, 74 (16), 4997-5007. DOI: 10.1128/AEM.00113-08

Silva, E. G. dos S. O., Anaya, K., Bezerra, M. de F., Macêdo, C. S., Urbano, S. A., Borba, L. H. F., Barbosa, I. de M., Ramalho, H. M. M., Cipolat-Gotet, C., Galdino, A. B. da S., de Oliveira, J. P. F., and Rangel, A. H. D. N. (2022). Physicochemical and sensory evaluation of greek style yoghurt with bovine colostrum. *Food Science and Technology* (Brazil), 42, e22121. https://doi.org/10.1590/fst.22121

Soleymanzadeh, N., Mirdamadi, S., and Kianirad, M. (2016). Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). *Dairy Science and Technology,* 96, 443- 457. https://doi.org/10.1007/s13594-016-0278-1

Starkutė, V., Mockus, E., Klupšaitė, D., Zokaitytė, E., Tušas, S., Mišeikienė, R., Stankevičius, R., Rocha, J. M., and Bartkienė, E. (2023). Ascertaining the influence of lacto-fermentation on changes in bovine colostrum amino and fatty acid profiles. *Animals*, 13(19), 3154. DOI: 10.3390/ani13193154

Surono, S. and Hosono, A. (2011). Fermented milks | starter cultures, pp. 477–482. In: Encyclopedia of dairy sciences. Elsevier. https://doi.org/10.1016/B978-0-12-374407-4.00181-3.

Tajorudin, N. and Hamirudin, A. H. (2020). Effects of bovine colostrum supplement intake to health: a systematic literature review. Special Issue: *Current Research in Nutrition and Dietetics,* 4 (20). DOI: https://doi.org/10.31436/ijahs.v4i3.477

Varga, L., Süle, J., and Nagy, P. (2014). Short communication: Survival of the characteristic microbiota in probiotic fermented camel, cow, goat, and sheep milks during refrigerated storage. *Journal of Dairy Science*, 97 (4), 2039–2044. http://dx.doi.org/10.3168/jds.2013-7339.

Varghese, K. S. and Mishra, H. N. (2008). Modelling of acidification kinetics and textural properties in *dahi* (Indian yogurt) made from buffalo milk using response surface methodology. *International Journal of Dairy Technology*, 61(3), 284-289. https://doi.org/10.1111/j.1471-0307.2008.00411.x

Vinderola, C. G. and Reinheimer, J. A. (2000). Enumeration of *Lactobacillus casei* in the presence of *L. acidophilus*, bifidobacteria and lactic starter bacteria in fermented dairy products. *International Dairy Journal*, 10(4), 271-275.

https://doi.org/10.1016/S0958-6946(00)00045-5

Wang, Y., Wu, J. Lv M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., and Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology*, 9, 612285. https://doi.org/10.3389/fbioe.2021.612285

Xu, W., Li, C., Guo, Y., Zhang, Y., Ya, M., and Guo, L. (2021). A snapshot study of the microbial community dynamics in naturally fermented cow's milk. *Food Science and Nutrition*, 9(4), 2053-2065. DOI:10.1002/fsn3.2174.

Yagil, R. and Etzion, Z. (1980). Effect of drought condition on the quality of camel milk. *Journal of Dairy Research*, 47(2) ,159-166. DOI: https://doi.org/10.1017/S0022029900021026

Conflict of interest statement

There are no conflicts of interest declared by the Authors.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECT OF DATE FLOUR AS SUGAR SUBSTITUTE ON THE TEXTURAL, PASTING PROPERTIES AND QUALITY CHARACTERISTICS OF CHINCHIN

BOLAJI OLUSOLA TIMOTHY[1]✉, AKEJU MARY TOMISIN[2] and APOTIOLA ZACCHEAUS OLASUPO[1]✉

[1]Department of Food Science and Technology. Lagos State University of Science and Technology, Ikorodu, Lagos, Nigeria

[2]Department of Food Technology, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria

✉olusholat@yahoo.com; ✉bolaji.o@lasustech.edu.ng

https://doi.org/10.34302/crpjfst/2024.16.4.9

Article history:
Received
February 22th, 2024
Accepted
November 12th, 2024

Keywords:
Chinchin;
Snacks date flour;
Textural properties;
Pasting properties;
Sugar substitute;
Colour.

ABSTRACT

This paper studied the textural and quality characteristics of Chinchin produced from wheat with date flour substituted at varying proportion as sugar replacer(FTB (100%), BYT (90:10), BGD (80:20), FYB (70:30), MOA (60:40) and BOT (50:50). These were deep fried at 180ºC for 8min. Pasting, Functional properties, mineral content, colour and consumer acceptability were also evaluated. The result revealed that significant difference (P<0.05) exited between values obtained for pasting, functional and textural properties. The values of cohesiveness, gumminess, resilience and springiness ranged from 0.11-0.30, 2788-6920, 0.09-3.34 and 0.27-1.38, respectively. There was significant impact (P<0.05) of Date fruits on the mineral content and particularly the colour of the composite flour and the chinchin. The organoleptic attributes significantly decreased with increase in the inclusion of date flour. The possibility of Chinchin produced with moderate date fruits flour inclusion as sugar substitute was established.

1.Introduction

Snacks as part of human diet has a reputation of contributing tremendously to global and national food security and economy (Thakur and Saxena, 2000; Lasekan and Akintola, 2002; Abegunde et al. 2014). Snack is a portion of food, often smaller than a regular meal and most times eaten between meals. The demand for snacks is often connected with population growth and urbanization of both developed and developing countries. Snacks are usually prepared from ingredients commonly available in the home (Opara et al, 2013; Adegunwa et al., 2014). They are typically designed to be portable, quick, and satisfying (Thakur and Saxena, 2000) and Chinchin is one of these important products usually produced from wheat flour (Opara et al, 2013; Adegunwa et al., 2014). Chinchin is sweet to taste and slightly hard. This depends on the materials used (Adegunwa et al., 2014; Adeyeye et al., 2020).

Dates are abundant sources of carbohydrates, with sucrose, maltose, glucose and fructose which constitute more than 80% of its dry matter. According to Maqsood et al. (2019), compositions and sugar content of date fruits differ with variety and fruit maturation. Dates have been described as a good source of essential amino acids. Histidine and arginine have been reported necessary in the proper physiological functioning of the human body (Al-Farsi et al., 2005;Idowu et al., 2019). About twenty-three different amino acids were detected in date fruits compared with some notable fruits (Al-Farsi et al., 2005; Idowu et al., 2019). The carbohydrates in dates are mainly

simple sugars and are a mixture of sucrose, glucose and fructose, with small amounts of polysaccharides such as cellulose and starch while Mannose and maltose are present in the seeds (Manickavasagan et al., 2013). It has been reported that significant quantities of potassium, calcium, sodium, phosphorus, magnesium, iron, zinc, copper and manganese were discovered in the date fruits along side significant quantities of water-soluble and oil-soluble vitamins (Manickavasagan et al., 2013). Apart from date fruits providing essential nutrients when consumed, it has also reported to be a good source of dietary fibre (Manickavasagan et al., 2013; Al-Farsi et al, 2007). Dietary fibre is considered good for health and is claimed to have a preventative effect against many diseases such as diabetes, obesity, hyperlipidaemia, coronary heart disease, hypertension, intestinal disorders, prostate cancers, and colorectal cancer (Vyawahare et al, 2009). The texture of dates depends on interaction between water and other components such as protein, carbohydrate, lipids and salts.

In the recent years there has been a lot of concern about the excessive consumption of sugar, and its effect on health (National Heart Lung and Blood Institute 2004; Hutchinson et al., 2018;Lustig et al., 2012; Hutchinson et al., 2018).The World Health Organization recommends limiting added sugar intake to less than 10% of total energy (World Health Organization, 2015). Date fruit contains more than 70% sugar mainly glucose and fructose and therefore are high energy food sources (Dada et al., 2012), thus making it an ideal replacement for sugar (sucrose) in the confectionery recipe, which is also of great nutritional benefit to diabetics and other metabolic health related patients (Dada et al., 2012; Bolaji et al., 2022).

2.Materials and Methodology

The wheat flour, date fruit, egg, nutmeg, salt, milk, pineapple, baking powder, vegetable oil and butter used in this study were purchased from Sabo Market, Ikorodu. The production of the Chinchin was carried out in the Food Processing Laboratory of the Department of Food Technology and biotechnology, Lagos state University of Science and Technology. The method of Platat *et al.* (2015) with little modification was used in the preparation of the date fruit powder. The date was rinsed with water to remove adhering dirt. Seeds were removed the pulp with pericarp was oven dried at 75^0C for 8h and subsequently milled using blender and sieved through a 0.35 µm mesh sieve to obtain fine homogenized particles. The date fruit flour was packaged for further analysis. The flow chart to produce date fruit is as shown in Figure 1. Whole wheat flour was obtained by cleaning to remove dirts, stone and other extraneous material, milled into powder and sieving through a sieve to obtain fine homogenised flour (Figure 1). The composite flour of wheat-date flour blend was formed in the varying proportion (FTB (100%), BYT (90:10), BGD (80:20), FYB (70:30), MOA(60:40) and BOT(50:50)

2.1. Preparation of Chinchin

The production of Chinchin was inline with method reported by Adeyeye *et al.* (2020) as shown in Figure 1, column 3. Flour, salt and nutmeg were first sieved into a bowl and margarine (125/500g) was mixed together with blended flour wheat and date flour evenly. Egg (3/500g) and other ingredients: baking powdered (2 table spoon/500g), about 42.325g of Milk/500g), Nutmeg(10g/500g) and water 100 mL/500g flour) were added to make fairly stiff dough. The stiff dough was rolled tightly to 1cm thickness on a board and cut into cubes. The cut dough was fried in deep hot vegetable oil at 180^{o}C for 8min. The chin-chin was then drained, cooled and packaged in an air tight container.

2.2. Proximate analysis

The moisture content, ash content, protein and crude fibre of the chinchin samples were determined according to the standard method of AOAC (2010). The carbohydrate content of the samples was determined by difference.

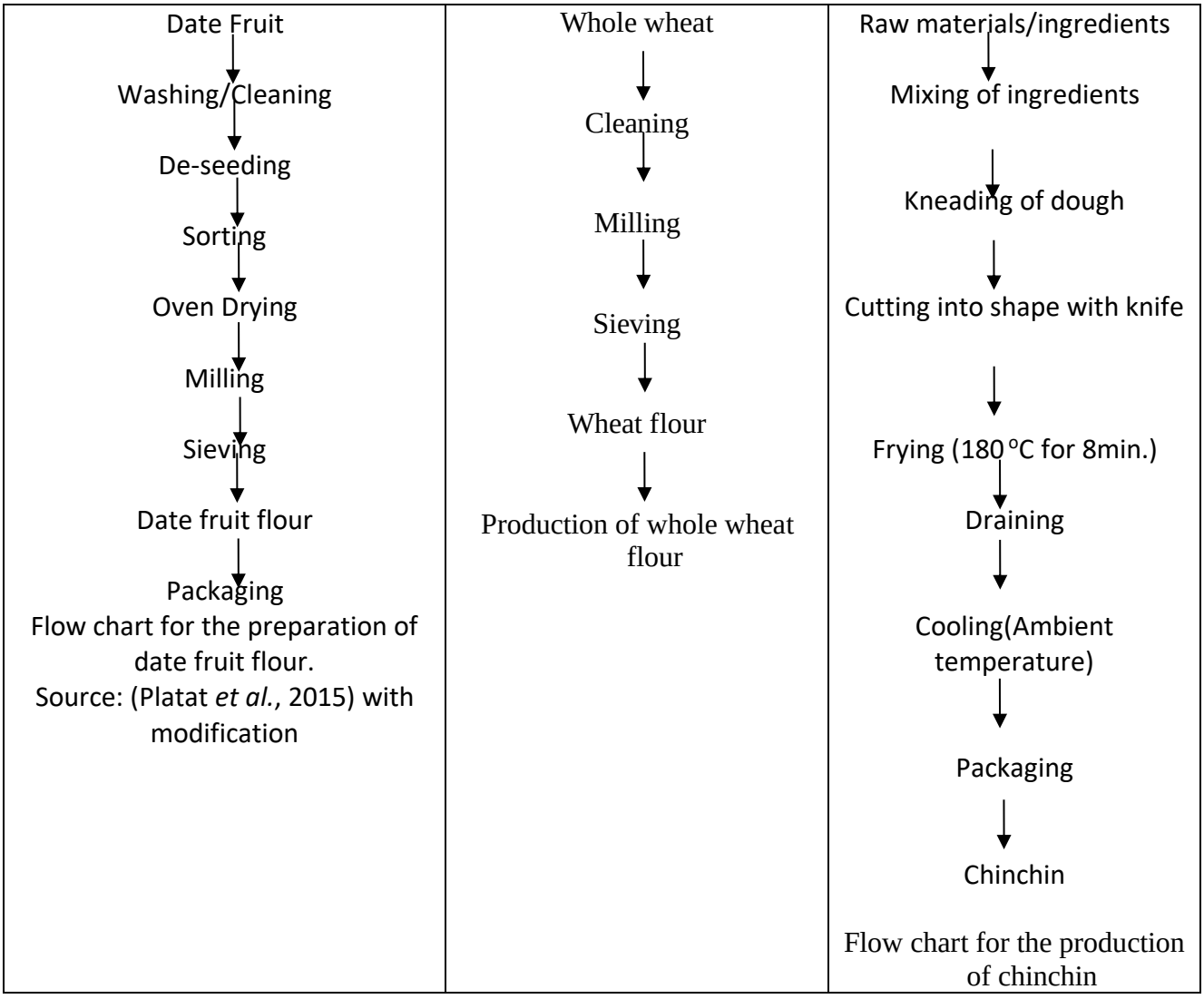


Figure 1. Flow chart for the production of date flour, wheat flour and Chinchin

2.3. Determination of Functional Properties of the Flour

The bulk density, Swelling Power and Solubility of the flour samples were determined using the method described by Nwosu, (2011), Water/Oil Absorption Capacity were determined following methods of Horsfall *et al.* (2007).

2.4.Determination of Mineral Content of the Flour

The mineral content of the flour samples was determined using the method described by AOAC (2005).

2.5. Pasting Properties of the Flour

The pasting properties of the flour samples were determined using the rapid visco analyzer (RVA) as described by Bolaji *et al.* (2011).

2.6.Determination of Colour Attributes

The colour intensity of the samples was measured using a Konica Minolta Colour Measuring System (Chroma Meter CR-410, Minolta LTD Japan).The lightness (L*), redness or greenness (a* or –a*), and yellowness or blueness (b* or –b*) values were obtained after calibrating the instrument using a white tile. Three replicate readings were taken for each

samples and the average value were reported. The results were expressed in accordance with the CIE lab system where L* is known as the lightness (L = 0 (black), L = 100 (white), a* (-a = greenness, +a = redness) andb* (-b = blueness, +b = yellowness)

2.7.Textural Analysis of the Chinchin

Texture of the Chinchin samples was determined with the aid of texture analyser (Model CT310K Texture Analyzer, USA).Texture profile analysis was performed on central Chinchin cubes by TPA test with a 3mm cylindrical stainless steel (TA 44 probe).Downward speed was 0.5mm/s and upward speed 0.5mm/s with a trigger load of 4.0g. The relevant textural properties were recorded

2.8.Sensory Evaluation

Sensory evaluation of the chinchin was carried out using a 9-point Hedonic scale. Samples were rated alongside the control sample (100% wheat flour chinchin). All analysis were performed in triplicate.

2.9.Statistical Analysis

The analysis of variance (ANOVA) was performed to examine the significant level in all parameters measured and where this exited, dumcan multiple range test (DMRT) was used to separate means.

3. Results and Discussion

3.1.Pasting Properties of Wheat and Date Flour

The pasting properties of the flour are shown in Table 1. There was significant influence ($p<0.05$) in the pasting properties of samples of flours with increase in the inclusion of date flour. The addition of date flour as natural sugar replacer caused significant reduction in the peak, trough, breakdown, final and setback viscosities, including the cooking time. However, there was increase significant increase in the pasting temperature with increase in the inclusion date flour. The peak viscosity was within the range of values reported for wheat, breadfruit, and cassava starch(Bolarinwa *et al.*, 2015). Trough viscosity is the ability of the phase to withstand breakdown during cooling (Awolu and Oseyemi, 2016). The reduced trough viscosity with increased date flour suggested that the flour blends may not find good application in the food system where high paste stability during cooling is required (Adegunwa *et al.*, 2015; Awolu and Oseyemi, 2016). The values of trough viscosity in this study were higher compared with values reported by Offia-Olua (2014) who worked on chemical, functional and pasting properties of wheat and walnut flour. Trough and breakdown measure how long a food item can withstand high temperatures without experiencing any disruptions (Adebowale et al., 2011; Bolaji et al., 2022). According to Adebowale et al. (2011), Breakdown viscosity of a food material is the capacity to tolerate heat and shear stress during cooking. Shimels *et al.* (2006) reported that final viscosity gives an idea of the ability of a material to gel after cooking. The final viscosities in this study was higher when compared with the values reported by Anosike *et al.* (2020). The Setback values have been reported to correlate with ability of starches to gel into semi solid pastes. This stage involves re-association, retrogradation or re-ordering of starch molecules (Michiyo *et al.*, 2004). The Peak time is a measure of the cooking time. The value of peak time ranged from 5.84-6.84min. The peak time values in this study were similar to that reported for flours made from wheat and okra by Akoja and Coker (2018). Pasting temperature gives an indication of the energy cost for preparing a product (Punia *et al.*, 2019;Abegunde et al., 2014). However did not follow the pattern reported by Bolaji et al. (2022). This decreased with increase in the Date flour inclusion.

3.2. Textural Properties of the Chinchin Produced from Wheat and Date Flour

Textural properties play a significant role in the perception and acceptability of any processed food product (Serdaroglu *et al.*, 2005). The textural properties of the chinchin

are as shown in Table 2. The peak force varies from 17770-28398.5g. Obomeghei and Ebabhamiegbebho, (2020) reported range of lower values of peak force for chinchin from orange fleshed sweet potato and red bambara groundnut. According to reports, adhesiveness indicates the first bite's negative force area as well as the effort needed to resist the attraction forces between a food's surface and the surfaces of other objects it comes into touch with (Kasapis, 2009). The values of adhesiveness in this study was lower compared with values reported by Obomeghei and Ebabhamiegbebho, (2020). A product with high cohesive force is more resistant to packaging, production and transportation stresses and so can be delivered to the consumers in the intended state (Texture Technologies, 2016). Gumminess, according to Dar and Light (2014), is the amount of energy needed to break up a semisolid meal into pieces that are ready to swallow. The value of gumminess ranged from 2788-6920. Springiness depends on different agents such as heat treatment, protein interaction, elasticity, and degree of unfolding of protein (Delikanli and Ozcan, 2017). High springiness suggest that more mastication energy will be needed in the mouth when chewing (Rahman and Al-Mahrouqi, 2009). The value of stickiness in this study was higher than (-1.6) – (-3.1) reported by Thara and Nazni (2021) for foxtail millet and semolina incorporated ready to cook upma mix. Stickiness is the important factor that affects the texture of products due to the presence of the soluble fibre content as reported by (Aisoni et al., 2018).

3.3. Functional Properties of Wheat and Date Flour

Table 3 displays the flour's functional attributes. Packed bulk density (PBD) and loose bulk density (LBD) were found to range between 0.54 and 0.69 g/ml and 0.76 and 0.85 g/ml, respectively. Bulk density has important applications in raw material handling, packing, and transportation. It is often influenced by the density and particle size of the flour blend (Adegunwa et al., 2015; Bolaji et al., 2022). However, the low bulk density of the flour blends observed in this study would be of an advantage in the formulation of complementary food (Bolaji et al., 2022). The value of water absorption capacity (WAC) and oil absorption capacity (OAC) ranged from 71.80-114.23 and 41.23-67.33, respectively. Water absorption capacity is the ability of flour to take up water and swell for improved consistency in food. This is usually useful in confectionary products that need hydration to improve handling features (Adegunwa *et al.*, 2014; Oppong *et al.*, 2015; Adeyeye *et al.,* 2020). The water absorption capacity was significantly reduced as the level of date flour substitution increased. Thus, this implies that the addition of date flour may be reduced the reconstitution ability of the composite flour. Folorunsho *et al.* (2018) reported the value (137.63-161.29%) for wheat-date palm fruit flour. These values were higher when compared to this present study. The oil absorption capacity significantly($p<0.05$) reduced as the level of date flour substitution increased. Oil absorption capacity tends to cause an increase in the functionality of product by retaining flavour and improvement on the mouth feel (Akinwale *et al.*, 2017). The value of water absorption and oil absorption capacity in this work were lower when compared with values reported by Abioye *et al.* (2020) who worked on chinchin produced from composite flours of wheat and germinated finger millet flour.

3.4. Proximate Composition of Chinchin Produced from Wheat and Date Flour

The proximate composition of the chinchin is as shown in Table 4. The moisture content varies from 3.29-7.82%. The moisture content observed in this study are within range of values reported by some researchers for snacks from composite flour (Adebayo-Oyetoro *et al.*, 2017; Deedam *et al.,* 2020) however, lower compared with values reported by Bolaji et al. (2022).

Table 1. Pasting properties of Chinchin produced from wheat and date flour

Sample	Peak (cP)	Trough (cP)	Breakdown (cP)	final viscosity (cP)	Setback (cP)	Peak time (min)	Pasting temperature (°C)
FBT	1853.50±149.19^{a}	1541.50±150.61^{a}	312.00±1.41^{a}	1893.00±151.32^{a}	351.50±0.71^{a}	6.84±0.05^{a}	69.03±3.14^{b}
BYT	1405.50±7.78^{b}	1139.50±2.12^{b}	267.00±7.07^{b}	1499.00±8.49 ^{b}	359.50±6.36^{a}	6.73±0.00^{a}	89.08±0.04^{a}
BOD	1101.00±18.38^{c}	869.00±21.21^{c}	232.00±2.82^{c}	1202.50±16.26 ^{c}	333.50±4.955^{b}	6.44±0.05^{b}	89.83±0.04^{a}
FYB	794.00±18.38^{d}	613.00±14.14^{d}	181.00±4.24^{d}	885.00±22.63^{d}	272.00±8.49^{c}	6.27±0.09^{c}	91.20±0.00^{a}
MAO	579.50±27.58^{e}	430.50±21.92^{e}	149.00±5.66^{e}	657.50±30.41^{e}	227.00±8.48^{d}	6.04±0.05^{d}	91.25±0.07^{a}
BOT	337.00±4.24^{f}	243.50±3.54^{f}	93.50±0.71^{f}	386.00±1.41^{f}	142.50±4.95^{e}	5.84±0.05^{e}	90.90±0.28^{a}

*Mean ± standard deviation with same superscripts along the column are not significantly different at (p>0.05)

Table 2. Textural properties of the Chinchin produced from wheat and date flour

Sample	PF	Height	Weight	Adhesiveness	area	Chewiness	Cohesiveness	CF	Gumminess	Resilience	Springiness	Stickiness	Stringiness
FBT	22133.5	13.02	1.50	1.18	77489.29	1135	0.24	22059.5	5380	0.11	0.22	-8.00	2.05
BYT	17770	13.01	0.00	0.58	56331.12	766.50	0.19	17750.5	3593	0.09	0.21	-3.00	0.88
BOD	24731	12.54	0.50	1.60	78123.38	3387	0.11	14622	2788	3.34	1.38	-8.50	1.65
FYB	28398.5	10.76	1.50	4.35	83161.89	3127.50	0.18	21382	5015.5	0.56	0.61	-4.50	1.87
MAO	18680	11.42	11.42	0.47	40272.16	1170	0.30	16995	5548.	0.32	0.27	-4.00	0.41
BOT	21294.5	13.92	0.50	4.84	48486.79	1859	0.32	20198.5	6920	0.34	0.30	-5.00	2.16

KEY: FTB (100%), BYT (90:10), BOD (80:20),FYB (70:30), MOA(60:40) and BOT(50:50)

Table 3. Functional properties of Chinchin produced from wheat and date flour

Sample	LBD (g/mL)	PBD (g/mL)	WAC (%)	OAC ((%)
FBT	0.54±0.00^{a}	0.84±0.00^{b}	114.23±0.48^{a}	67.33±0.04^{a}
BYT	0.60±0.00^{a}	0.85±0.00^{a}	93.75±0.50^{b}	63.29±0.01^{b}
BOD	0.60±0.00^{a}	0.83±0.00^{b}	76.00±0.03^{d}	58.62±0.16^{c}
FYB	0.69±0.00^{a}	0.80±0.00^{c}	81.20±0.01^{c}	53.66±0.01^{d}
MAO	0.63±0.00^{a}	0.76±0.01^{d}	75.69±0.01^{d}	51.95±0.07^{e}
BOT	0.69±0.19^{a}	0.83±0.01^{b}	71.80±0.31^{e}	41.23±0.35^{f}

*Mean ± standard deviation with same superscripts along the column are not significantly different at (p>0.05)

Table 4. Proximate composition of Chinchin produced from wheat and date flour

Sample	MOISTURE (%)	PROTEIN (%)	FAT (%)	FIBER (%)	ASH (%)	CHO (%)
FBT	5.88±0.06^{b}	3.42±0.01^{f}	24.12±0.03^{b}	0.01±0.00^{b}	2.94±0.08^{ab}	63.65±0.13^{b}
BYT	5.81±0.02^{b}	3.48±0.03^{e}	24.08±0.02^{b}	0.01±0.00^{b}	3.54±0.62^{a}	63.10±0.64^{bc}
BOD	7.82±0.44^{a}	3.55±0.01^{d}	17.58±0.03^{c}	0.01±0.00^{b}	2.73±0.08^{b}	68.33±0.37^{a}
FYB	5.87±0.05^{b}	3.81±0.01^{b}	18.51±0.04^{c}	0.49±0.01^{a}	2.59±0.01^{b}	68.75±0.16^{a}
MAO	3.29±0.04^{c}	3.92±0.01^{a}	27.59±0.01^{a}	0.25±0.35^{ab}	2.62±0.08^{b}	62.34±0.40^{c}
BOT	6.20±0.01^{b}	3.71±0.01^{c}	27.58±0.03^{a}	0.01±0.00^{b}	2.72±0.01^{b}	59.76±0.06^{d}

*Mean ± standard deviation with same superscripts along the column are not significantly different at (p>0.05)

KEY: FTB (100%), BYT (90:10), BOD (80:20),FYB (70:30), MOA(60:40) and BOT(50:50)

The relative low moisture content of product below 13% may aid longer shelf-life, if stored under low relative humidity. There was significant difference (P<0.05) in the value of protein. The result showed that the addition of date flour aided an increase in the protein content of the samples and this suggested that the chinchin produced from wheat and date blend could provide additional protein to consumers in developing countries where many can hardly afford costly sources.

The values of protein in this study was lower when compared with values reported by Deedam *et al.* (2020) for chinchin developed from wheat and African walnut flour blends. However still relevant compared with the recommended daily allowance (RDA) for adults, adolescents and children which is 0.8, 1.0 and 1.5 g protein/kg, respectively (Kafatos and Hatzis, 2008).

The findings in this work revealed that the consumption of about 100g of the Chinchin produced from wheat-date flour blend in this study will provide adequate amount of daily protein needed in the body. The value of fat varies from 17.58-27.59%. There was significant difference (p<0.05) among the samples. The values of fat in this study are comparable with the value reported by Adebayo-Oyetoro *et al.* (2017) for chinchin from wheat-tigernut flour blends. While the crude fibre were significantly influenced by date flour inclusion. The crude fibre values obtained in this work were within the range of recommendation -5% (Ndife *et al.*, 2020). The value of ash obtained ranged from 2.59-3.54%. Value recorded for sample FYB was significantly different (p<0.05) from other samples and however all higher than values reported by Baljeet *et al.* (2014).The

carbohydrate contents of the chinchin samples from composite flours of varying wheat-date flour blends were within values reported by some researchers (Adegunwa *et al.*, 2014; Adebayo-Oyetoro, 2017; Adeyeye *et al.*, 2020)

3.5.Mineral Composition of Chinchin Produced from Wheat and Date Flour

Table 5 presents the mineral makeup of the chinchin. A noteworthy variation ($p<0.05$) was seen in the mineral makeup of the chinchin samples. Fasogbon et al. (2017) found that chinchin made with wheat encriched with leftover veggies had a greater calcium content. The presence of egg, milk, and margarine among other preparation-related components may have contributed to the high magnesium content of chinchin. The recommended dietary allowance of magnesium for an adult and children are 350 and 170 mg/day, respectively (Akindele *et al.*, 2017). The values of iron were within 5.21 and 6.55 mg/100g. According to Mason (2008), the recommended dietary allowance of iron for men and postmenopausal women was 8 mg/day, while 11, 15 and 30 mg/day were recommended for adolescents, premenopausal women and pregnant women, respectively. This present study showed that these chinchin can supply the daily recommended iron in the diet, most especially when about 200g of the product is consumed daily. The value of sodium ranged from 117.50-138 mg/100g. The results showed that chinchin produced from composite flour of wheat and date flour had higher value of sodium when compared to the control. The value of zinc (0.02-0.05 mg/100g) revealed that these chinchin samples can be consumed without exceeding the maximum Zinc limit daily intake level, since the upper level of Zinc intake according to FAO/WHO is 60 mg/day (Sarikurkcu *et al.*, 2015). The results revealed that the addition of date flour contributes to the increase in phosphate content. Hence, the chinchin produced could be a good source of phosphate.

Table 5. Mineral composition of Chinchin produced from wheat and date flour

SAMPLE	CALCIUM (mg/100g)	MAGNESIUM (mg/100g)	IRON (mg/100g)	SODIUM (mg/100g)	ZINC (mg/100g)	PHOSPHATE (mg/100g)
FBT	117.25 ± 0.35^{a}	36.01 ± 0.01^{a}	6.55 ± 0.07^{a}	117.50 ± 3.54^{c}	0.02 ± 0.01^{c}	42.50 ± 3.54^{b}
BYT	115.49 ± 1.00^{ab}	35.01 ± 0.01^{ab}	5.88 ± 0.03^{b}	130.50 ± 0.71^{ab}	0.03 ± 0.01^{bc}	52.50 ± 3.54^{ab}
BOD	114.69 ± 1.69^{b}	33.50 ± 0.00^{b}	5.63 ± 0.04^{c}	122.50 ± 3.54^{bc}	0.02 ± 0.00^{bc}	52.50 ± 3.54^{ab}
FYB	114.94 ± 0.06^{b}	34.72 ± 1.44^{ab}	5.76 ± 0.08^{bc}	122.50 ± 3.54^{bc}	0.03 ± 0.00^{b}	60.00 ± 7.07^{a}
MAO	109.35 ± 0.64^{c}	33.77 ± 0.01^{b}	5.68 ± 0.11^{c}	126.00 ± 5.66^{bc}	0.05 ± 0.01^{a}	55.00 ± 0.00^{a}
BOT	105.23 ± 0.32^{d}	35.00 ± 0.00^{ab}	5.21 ± 0.01^{d}	138.50 ± 2.12^{a}	0.05 ± 0.01^{a}	52.50 ± 3.54^{ab}

*Mean ± standard deviation with same superscripts along the column are not significantly different at ($P>0.05$)

3.6. Colour Properties of Chinchin Produced from Wheat and Date Flour

Table 6 indicates the chinchin's colour characteristics. The range of the L* value was 18.38–38.19.

A statistically significant ($P<0.05$) variation was seen in the reported values for the samples. As the ratio of date flour grew, the value of L* decreased.

This demonstrated that the samples' inclination to become lighter was greatly diminished by the addition of date flour.

A* has a value between 0.63 and 1.95. As the date flour replacement level grew, so did the a (redness) values.

The range of values for b* was 5.91 to 16.46. As the date flour replacement level increased, the b (yellowness) values declined.

This work revealed colour which is one of the first property that consumers considers in accessing and assessing for will be critical in the chinchin produced from these samples (Kumar *et al.*, 2012).

Table 6. Colour properties of Chinchin produced from wheat and date flour

Sample	L*	a*	b*	ΔE
FBT	38.19±0.14^{a}	0.63±0.23^{d}	16.46±0.32^{a}	42.95±0.14^{a}
BYT	32.14±0.18^{b}	1.06±0.02^{c}	13.51±0.35^{b}	32.46±0.83^{b}
BOD	29.67±0.08^{c}	1.27±0.04^{bc}	10.42±0.21^{c}	26.93±0.63^{c}
FYB	26.66±0.74^{d}	1.41±0.12^{b}	7.58±0.34^{d}	17.92±0.04^{d}
MAO	23.55±0.47^{e}	1.71±0.02^{a}	6.52±0.66^{e}	15.32±0.46^{e}
BOT	18.38±1.07^{f}	1.95±0.06^{a}	5.91±0.02^{f}	11.97±1.34^{f}

*Mean ± standard deviation with same superscripts along the column are not significantly different at (P>0.05)

Table 7. Sensory scores of chinchin produced from wheat and date flour

Sample	Taste	Appearance	Flavour	Colour	Overall Acceptability
FBT	7.50±1.27^{a}	6.80±0.79^{a}	7.90±1.29^{a}	8.80±1.03^{a}	8.00±0.82^{a}
BYT	6.91±1.68^{b}	6.78±1.54^{a}	6.75±1.21^{b}	6.55±1.82^{b}	7.88±1.45^{b}
BOD	6.75±1.38^{bc}	6.65±1.23^{b}	6.58±1.42^{c}	6.25±1.52^{bc}	7.20±1.49^{bc}
FYB	6.69±1.66^{c}	6.55±1.61^{b}	6.60±1.47^{c}	6.20±1.67^{bc}	6.65±1.23^{bc}
MAO	5.61±1.43^{d}	6.00±1.14^{c}	6.42±1.09^{d}	6.45±1.39^{b}	5.86±1.63^{c}
BOT	4.55±1.27^{e}	4.85±1.04^{d}	4.86±1.63^{e}	4.15±1.73^{d}	4.65±1.26^{d}

*Mean ± standard deviation with same superscripts along the column are not significantly different at (p>0.05)

3.7. Sensory evaluation of Chinchin Produced from Wheat and Date Flour

All sensory parameters examined showed a significant change (P<0.05). Table 7 presents the sensory properties of the chinchin. The more date flour added, the worse the panelists' scores were for flavor and color. Sample BOT was ranked lower than all the other samples, with a taste that varied from 4.55-7.50. This was due to the effect that a larger amount of date fruit flour had on the appearance. The results of this study demonstrated that, with the exception of sample BOT, which had its color affected by the significant amount of date flour employed, most of the samples were favored by the panelists.

4.Conclusion

The study showed that utilization and possibility of date flour in preparation of chinchin improved the quality attributes of the product in terms of protein, fibre, and mineral contents. The pasting properties, textural characteristics, water absorption capacity, oil absorption capacity, bulk density, were all affected by the increased in the substitution of date flour. The scores for organoleptic attributes: taste, colour, flavour, appearance and overall

acceptability were generally acceptable. The appearance and colour of the chinchin were impacted by the level of date flour inclusion. Chinchin produced from wheat flour substituted with date, sample FYB (90% Wheat flour; 10% Date Flour) compared favourably well with chinchin produced from 100% wheat flour in all sensory attributes.

5.References

Abegunde, T., Bolaji, O., & Adeyemo, T. (2014). Quality evaluation of maize chips (Kokoro) fortified with Cowpea flour. *Nigerian Food Journal*, 32(1), 97-104. https://doi.org/10.1016/s0189-7241(15)30101-6

AbdelMoneim, E., Sulieman-Itimad A., Abd Elhafise & Awad, M., Abdelrahim (2012), Comparative study of five sudanose Date (Phoenix dactylifera L) fruit cultivars. Food and Nutrition Sciences (3) 1245-1251. https://doi.org/10.4236/fns.2012.39164

Abioye, V.F., Olodude, O.A., Atiba, V & Oyewo, I.O. (2020). Quality Evaluation of Chinchin produced from composite flours of wheat and germinated finger millet flour. *Agroserarch*, 20(1), 13-22. https://doi.org/10.4314/agrosh.v20i1.2s

Adebayo-Oyetoro, A.O., Ogundipe, O.O., Lofinmakin, F.K., Akinwande, F.F., Aina, D.O. & Adeyeye, S.A.O. (2017). Production and acceptability of chinchin snack made from wheat and tigernut (*Cyperus esculentus*) flour. *Cogent Food & Agriculture*,3(1),1282185.

https://doi.org/10.1080/23311932.2017.1282185

Adebowale, A. A., Adegoke, M. T., Sanni, S. A., Adegunwa, M. O., & Fetuga, G. O. (2011). Functional properties and biscuit making potential of sorghum – wheat flour composite. *American Journal of Food Technology*, *7*, 372–379.

Adegunwa, M. O., Adeniyi, O. D., Adebowale, A. A., & Bakare, H. A. (2015). Quality evaluation of Kokoro produced from maize–pigeon pea flour blends. *Journal of Culinary Science & Technology*, 13(3), 200-213. https://doi.org/10.1080/15428052.2015.1015665

Adegunwa, M.O., Ganiyu, A.A., Bakare, H.A. & Adebowale, A.A. (2014). Quality evaluation of composite millet-wheat chinchin. *Agriculture Biology Journal of North America*, 5(1), 33-39.

Adeyeye, S. A., Idowu-Adebayo, F., Bolaji, O. T., Abegunde, T. A., Adebayo-Oyetoro, A. O., & Tiamiyu, H. K. (2020). Quality characteristics and acceptability of chin-chin prepared from rice and high quality cassava composite flour. *Current Nutrition & Food Science*, 16(6), 963-971. https://doi.org/10.2174/1573401315666191003153807

Aisoni, J. E., Yusha'U, M., & Orole, O. O. (2018). Processing effects on physicochemical and proximate composition of finger millet (Eleusine coracana). *Greener Journal of Biological Sciences*, 8(2), 014-020. https://doi.org/10.15580/gjbs.2018.2.032018048

Akindele, O., Gbadamosi, O., Taiwo, K., Oyedele, J. & Adebooye, C. (2017). Proximate, Mineral, Sensory Evaluations and Shelf Stability of Chinchin Enriched with Ugu and Indian Spinach Vegetables. *International Journal of Biochemistry Research and Review*, 18(4), 1-14

Akinwale, T. E., Shittu, T. A., Adebowale, A. A., Adewuyi, S., & Abass, A. B. (2017). Effect of soy protein isolate on the functional, pasting, and sensory acceptability of cassava starch-based Custard. *Food Science and Nutrition*, 5(6), 1163-1169. https://doi.org/10.1002/fsn3.507

Akoja, S.S. and Coker, O.J. (2018). Physicochemical, functional, pasting and sensory properties of wheat flour biscuit incorporated with Okra powder. *International Journal of Food Science and Nutrition*, 3(5), 64-70

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., & Al-Rawahy, F. (2007). Compositional and functional

characteristics of dates, syrups, and their by-products. *Food Chemistry*, 104(3), 943-947. https://doi.org/10.1016/j.foodchem.2006.12.051

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactyliferaL.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry*, 53(19), 7592-7599. https://doi.org/10.1021/jf050579q

Anosike, F.C., Nwagu, K.E., Nwalo, N.F., Ikegwuu, J.O., Onyeji, G.N., Enwere, E.N. and Nwoba, S.T. (2020). Functional and Pasting Properties of Fortified Complementary Foods Formulated from Maize (Zea mays) and African Yam Bean (*Sphenostylis stenocarpa*) Flours. *Legume Science*, 2(4), 62. https://doi.org/10.1002/leg3.62

AOAC, (2005). Official method of analysis: Association of official of analytical chemists. Gaithersburg, Maryland, USA: International (18 Ed).

AOAC, (2010). Official Method of Analysis. Association of Official Analytical Chemists, International Edition, Washington D.C.,U.S.A

Awolu, O. O., & Oseyemi, G. F. (2016). Physicochemical & Rheological properties of optimised cocoyam-based composite flour comprising cassava starch. Acta Universitatis Cibiniensis. Series E: *Food Technology*, 20(2), 65-84. https://doi.org/10.1515/aucft-2016-0016

Baljeet, S.Y., Ritika, B.Y. and Reena, K. (2014). Effect of incorporation of carrot pomace powder and Germinated chickpea flour on the quality characteristics of biscuits. *International Food Research Journal*, 21(1), 217-222.

Bolaji, O.T, Awonorin, S.O. Olalusi, P.A, Idowu, M.A Henshaw, F.O. and Adepoju, P.A (2011) Evaluation of Changes in Pasting Properties of Ogi during Storage. *Electronic journal of Environment, agriculture and Food chemistry 10(1),* 1865-1872.

Bolaji, O. T., Adeyeye, S. A., & Akinboyo, B. E. (2022). Quality evaluation of bread produced from wheat flour (Triticum aevistivum) fortified with date flour (Phoenix dactylifera L.). *Asian Journal of Dairy and Food Research*,. https://doi.org/10.18805/ajdfr.drf-258

Bolarinwa, I.F., Olaniyan, S.A, Adebayo, L.O. & Ademola, A.A. (2015). Malted Sorghum-Soy Composite Flour: Preparation, Chemical and Physicochemical Properties. *Journal Food Process Technology*, 6, 467. https://doi.org/10.4172/2157-7110.1000467

Dada, M, Nwawe, C.N, Okere, R.A & Uwubanmwen, I.O (2012) Potentials of Date Palm Tree to the Nigerian Economy. *World Journal of Agricultural Sciences* 8(3), 309-315

Dar, Y.L. and Light, J.M. (2014). Food texture design and optimization. Hoboken, NJ: John Wiley & Sons. https://doi.org/10.1002/9781118765616

Deedam, N. J., China, M. A., & Wachukwu, H. I. (2020). Proximate composition, sensory properties and microbial quality of chin-chin developed from wheat and African walnut flour blends for household food security. ++, 45-53. https://doi.org/10.9734/ejnfs/2020/v12i830260

Delikanli, B., & Ozcan, T. (2016). Improving the textural properties of yogurt fortified with milk proteins. *Journal of Food Processing and Preservation*, 41(5), e13101. https://doi.org/10.1111/jfpp.13101

Fasogbon, B.M., Taiwo, K.A.& Oyedele, D.J. (2017). Nutritional assessment and consumer acceptability of snacks (chin-chin and cookies) enriched with underutilized indigenous vegetables. *International Journal of Food and Nutritional Science*, 6(3), 97-108.

Folorunsho, A.A., Sabitu, A. and Omoniyi, S. (2018). Functional and Sensory Qualities of cookies and Chinchin Produced from Wheat-Date Fruit Flour Blends. *Annals*

Food Science and Technology, 19(4), 657-666

Horsfall, D.M., Lucy, E. and Nwaojigwa, S.U. (2007). Chemical com-position, functional and baking properties of wheat-plantain composite flours. *African Journal Food Agricultural Nutritional Development*. 7, 1–23.

Hutchinson, J. M., Watterworth, J. C., Haines, J., Duncan, A. M., Mirotta, J. A., Ma, D. W., & Buchholz, A. C. (2018). Snacking patterns of preschool-aged children: Opportunity for improvement. *Canadian Journal of Dietetic Practice and Research*, 79(1), 2-6. https://doi.org/10.3148/cjdpr-2017-022

Idowu, A.T., Igiehon, O.O., Adekoya, A.E. and Idowu, S. (2019). Dates palm fruits: A review of their nutritional components, bioactivities and functional food applications. *AIMS Agriculture and Food*, 5(4), 734-755. https://doi.org/10.3934/agrfood.2020.4.734

Kafatos, A. and Hatzis, C. (2008) Clinical nutrition for medical students. University of Crete, Rethimno, Greece Murray RK, Granner DK, Mayes PA, Rodwell VW (2000) Harper's biochemistry, 25th edn. Health Profession Division, McGrawHill, USA

Kasapis, S. (2009). Developing minced fish products of improved eating quality: An interplay of instrumental and sensory texture. *International Journal of Food Properties,* 12, 11–26. https://doi.org/10.1080/10942910802252171

Kumar, N., Sarkar, B.C., Sharma, H.K. and Jha, S.K.(2012). Colour kinetics and storage characteristics of carrot, pulse and rice by-product based extrudates. *British Food Journal*, 114 (9), 1279-1296. https://doi.org/10.1108/00070701211258826

Lasekan, O.O. and Akintola, A. M. (2002). Production and nutritional evaluation of puffed soy maize snacks. *Journal of Cereal Science,* 24, 79-85.

Lustig, R.H., Schmidt, L.A. and Brindis, C.D. (2012). The toxic truth about sugar. *Nature*, 482(7383), 27-29.

Manickavasagan, A., Mathew, T.A., Al-Attabi, Z.H. and Al-Zakwani, I.M. (2013). Dates as a substitute for added sugar in traditional foods-a case study with idli. *Emirates Journal of Food and Agriculture*, 899-906. https://doi.org/10.9755/ejfa.v25i11.14920

Maqsood, S., Adiamo, O. Q., Ahmad, M. & Mudgil, P. (2019). Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients. *Food Chemistry*. 308, 125-522. https://doi.org/10.1016/j.foodchem.2019.125522

Mason, J.B. (2008). Vitamins, trace minerals and other micronutrients. In: Goldman L, Ausiello DA (eds) Cecil medicine, 23rd edn. Saunders Elsevier, Philadelphia. 1–10

Michiyo, W., Tomoko, M., Kikuchi, T., Hiroshi, K. and Naofumi, M. (2004). Application of pre-germinated brown rice for Bread making. *Cereal Chemistry,* 81, 450 - 455. https://doi.org/10.1094/cchem.2004.81.4.450

National Heart Lung and Blood Institute (2004). An ecological model of diet, physical activity, and obesity. Predictors of obesity, weight gain, diet, and physical activity workshop; Bethesda, MD http://www.nhlbi.nih.gov/research/reports/2004-obesity (2004 Aug 4-5).

Ndife, J., Abasiekong, K.S., Nweke, B., Linus-Chibuezeh, A. and Ezeocha, V.C. (2020). Production and comparative quality evaluation of chin-chin snacks from maize, soybean and orange fleshed sweet potato flour blends. *FUDMA Journal of Sciences*, 4(2), 300-307. https://doi.org/10.33003/fjs-202-0401-220

Obomeghei, A.A. and Ebabhamiegbebho, P. (2020). Productionn, Proximate Composition and Sensorial Attributes of Flour Blend Chinchin froom orange fleshed sweet potato and red bambara groundnut. *International Journal of Recent Advances in*

Multidisciplinary Research, 7(7), 6017-6023

Offia-Olua, B. (2014). Chemical, Functional and Pasting Properties of Wheat (Triticum spp)-Walnut (*Juglan sregia*) Flour. *Food and Nutrition Sciences*, 5, 1591-1604. https://doi.org/10.4236/fns.2014.516172

Oppong, D., Arthur, E., Kwadwo, S. O., Badu, E. andSakyi, P. (2015). Proximate composition and some functional properties of soft wheat flour. *International Journal of Innovative Research in Science Engineering and Technology,* 4(2), 753–758

Platat, C., Habib, H.M., Hashim, I.B., Kamal, H., AlMaqbali, F., Souka, U. and Ibrahim, W.H. (2015). Production of functional pita bread using date seed powder. *Journal of Food Science Technology,* 10, 6375–6384. https://doi.org/10.1007/s13197-015-1728-0

Punia, S., Sandhu, K.S. and Siroha, A.K. (2019). Difference in Protein Content of Wheat (*Triticum Aestivum* L.): Effect on Functional, Pasting, Color and Antioxidant Properties. *Journal Saudi Agricultural Science*, 18, 378–384.

Rahman, M.S. and Al-Mahrouqi, A.I. (2009). Instrumental texture profile analysis of gelatin gel extracted from grouper skin and commercial (bovine and porcine) gelatin gels. *International Journal of Food Sciences and Nutrition,* 60, 229–242. https://doi.org/10.1080/09637480902984414

Sarikurkcu, C., Tepe, B., Kacak, M.S. and Uren, M.C. (2015) Metal concentration and antioxidant activity of edible mushrooms from Turkey. *Food Chemistry* 175,549–555.

Serdaroglu, M., Yildiz-Turp, G. and Abrodimov, K. (2005). Quality of low fat meatballs containing legume flour as extenders. *Meat Science*, 70, 99-105. https://doi.org/10.1016/j.meatsci.2004.12.015

Shimels, E.A., Meaza, M. and Rakshit, S.K. (2006). Physico-chemical properties, Pasting Behavior and Functional characteristics of flour and starches from improved Bean (Phaseoluus vulgaris L). Varieties Grown in East Africa. *CIGRE Journal,* 8, 1-18. https://doi.org/10.1016/j.lwt.2004.07.002

Texture Technologies (2016). An Overview of Texture Profile Analysis TPA, http://texture-technologies.php. Assessed 23/06/2017

Thakur, S. and Saxena, D.C. (2000). Formulation of extruded snack food (gum based cereal pulse blend): optimization of ingredients levels using response surface methodology lebensmittel-Wissenschaft-Technologie-. *Food Science and Technology*, 33, 354-361. https://doi.org/10.1006/fstl.2000.0668

Thara, D. and Nazni, P. (2021). Fomulation and quality evaluation of foxtail millet and semolina incorporated ready to cook upma mix. *Bioscience Biotechnology Research Communication,* 14, 4. https://doi.org/10.1006/fstl.2000.0668

Vyawahare, N., Pujari, R., Khsirsagar, A., Ingawale, D., Partil, M. and Kagathara, V. (2009). Phoenixdactylifera: An update of its indigenous uses, phytochemistry and pharmacology. *International Journal of Pharmacology*,1(3), 225 https://doi.org/10.4103/0975-1483.57069

World Health Organization (2015). Global Health Observatory data repository. Geneva: World Health Organization.

Authors' contributions

Dr. Bolaji Olusola Timothy: initiated ideas, concept and research design, project planning and execution, finance, statistical analysis, manuscript draft, editing and review the final manuscript for publication; Akeju Mary Tomisin: finance, experimental analysis, data/evidence collection, data curation and research report; Apotiola Zaccheaus Olasupo: project planning, validation, prepared the draft research report.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

PHYSICO-FUNCTIONAL, CHEMICAL, NUTRITIONAL AND ANTIOXIDANT PROPERTIES OF FLOUR FROM THREE VARIETIES OF UNRIPE BANANA (*MUSA* SP.) CULTIVATED IN SRI LANKA

Mendis, B.H.K.[1], Perumpuli, P.A.B.N.[1,✉] and Fernando, G.S.N.[1]

[1]*Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka.*
✉*buddhikap@fst.ruh.ac.lk*

https://doi.org/10.34302/crpjfst/2024.16.4.10

Article history:
Received
October 23rd, 2023
Accepted
November 30th, 2024

Keywords:
Green banana flour;
Physico-functional properties;
Nutritional properties;
Antioxidant properties.

ABSTRACT

Banana, scientifically known as *Musa acuminate* L., is a valuable source of nutrients and widely grown in tropical and subtropical regions. They can be consumed either in their natural state or after undergoing various processing methods. Green banana flour is an excellent alternative to keep the nutritional value of fresh bananas while minimizing post-harvest losses. The present study aimed to evaluate the physico-functional, chemical, nutritional, and antioxidant properties of flour obtained from three different banana varieties (*Seeni* banana, *Ambul* banana, and Cavendish banana) grown abundantly in Sri Lanka. Different properties of banana flour from selected banana varieties were measured using standard methods. Green banana flours showed significant differences ($p<0.05$) in colour parameters (CIE L*a*b*), swelling capacity, transparency, gelatinization temperature, pH, and titratable acidity, with no significant difference ($p>0.05$) in bulk, and tapped densities, water holding, oil holding and foaming capacities. There was no significant difference ($p>0.05$) in the amount of crude fiber content among all three flour types, while *Seeni* banana flour showed a significant amount of carbohydrate (88.65±0.39 g per 100 g wet weight), polyphenols (31.41±0.61 mg GAE/100 g of sample) and flavonoids content (337.7±31.11 mg QE/100 g of sample). Cavendish banana flour showed the highest antioxidant activity (576±0.028 mg of TE/g of the dried sample) compared to the other two banana varieties. The obtained results confirmed that green banana flour is a good source of nutrients and can be effectively used in developing value added products.

1. Introduction

Banana is one among the most produced, traded, and consumed fruits in the world. Bananas are classified under genus Musa of the family Musaceae. More than 1000 varieties of banana are produced worldwide and Cavendish reached about the 45% of the global market due to its high production and tolerance to mechanical damages than other varieties. There have been recorded to be 29 banana cultivars in Sri Lanka, along with two wild species (Department of Agriculture Sri lanka, 2012). Five of these species can be used for cooking, while all the others, with the exception of two wild species, are eaten as desert. In Sri Lanka, banana production is estimated to be 780,000 metric tons per year, with an average yield of 13 Mt/ha (Department of Census and Statistics, 2014). The banana can be grown and harvested throughout the year without seasonal barriers, so there is an abundance of bananas available throughout the year.

A banana is one of the most popular fruits among consumers due to its affordability, abundance, and nutritional value. A single 100 g serving of dessert banana contains 368

kilojoules of calories, while the same amount of plantain provides 556 kilojoules (Ranjha et al., 2022). The firm and fleshy banana fruit is packed with antioxidants, minerals such as iron, magnesium, manganese, and phosphorus, and vitamins such as vitamin B1, B2, B3, choline, vitamin C (Nadeeshani et al., 2021). Unripe banana contain resistant starch that supports digestive health and can serve as a healthy option for individuals with non-communicable diseases such as diabetes and obesity. Unripe banana flour offers several health benefits that are not found in wheat flour, making it a nutritious and beneficial alternative. Green banana flour is a gluten-free substitute that is ideal for patients with celiac disease. Besides their nutritional value, bananas offer several medicinal benefits such as lowering the risk of high blood pressure, restoring normal bowel activity, protecting against ulcers, reducing cholesterol levels, maintaining kidney health, boosting immunity, and promoting weight loss. Compared to wheat flour (GI- between 56 and 69), unripe banana flour has a lower glycemic index (around 30) and can be considered as good alternative food to regulate blood sugar levels and reduce the risk of diabetes (Gómez *et al*., 2020).

Bananas are highly perishable and decay quickly due to their high moisture content and rapid metabolic activity that continues after harvest. Therefore, harvested banana become overripe and senescent before being delivered to markets due to unavoidable transportation delays, inadequate post-harvest technologies, and fluctuating market demand. However, it is possible to reduce postharvest losses by converting them into forms with a lower moisture content as it can lengthen the shelf-life and prevent a significant amount of post-harvest loss (Falade & Oyeyinka, 2015). Among the different types of bananas highly consumed by Sri Lankans, the post-harvest loss of *Ambul* and *Seeni* bananas are high compared to the other types. Cavendish banana is one of the main cultivars of banana grown in Sri Lanka targeting the export market. A significant amount of Cavendish bananas that do not meet the export standards are wasted. Therefore, this has prompted research into the processing and application of mature green bananas of *Seeni*, *Ambul*, and Cavendish to diversify the uses while minimizing the post-harvest loss.

Therefore, the objective of this research was to evaluate and compare the physico-functional, chemical, nutritional, and antioxidant properties of banana flours obtained from most abundant three banana varieties grown in Sri Lanka in order to determine their potential for further application in processed food industry.

2. Materials and methods

2.1. Materials

In this study, three banana varieties, namely *Ambul, Seeni* and Cavendish were selected considering the abundance and popularity. Banana in first stage of ripening process which has green peel and sharp edges were chosen. Hard green (unripe) *Seeni* and *Ambul* banana fruits were purchased from the village market located in Kamburupitiya, Matara, Sri Lanka and Cavendish banana from Ambalanthota, Sri Lanka.

2.2. Production of Banana flour

The banana flour was prepared according to the method described by Fatemeh *et al.* (2012) with some modifications. Bunch of matured (green peel and sharp edges) banana fruits were separated into individual fingers and their weight was taken, Banana fingers were washed and dipped in 0.5% (w/w) citric acid solution for 5 min. Then individual banana fingers were peeled and cut in to 2 mm thickness slices and the cut pieces were dipped in citric acid solution. After draining, the pieces were dried at 60 ± 2 °C for eight hours to obtain the dried chips, and they were grounded using a home scale grinder to obtain the banana flour. Finally, banana flour was sieved (60 Mesh) and packed in impermeable plastic bags and stored under room temperature (30 ± 2 °C) until further use.

2.3. Evaluation of the physico-functional properties of banana flour

Prepared banana flour was separately subjected to examination of functional properties such as bulk and tapped densities, compressibility index, Hausner ratio, water absorption and oil absorption capacity, swelling capacity, transparency, foaming capacity, gelatinization temperature, colour parameters, and all the readings were taken in triplicates.

2.3.1. Bulk and Tapped Densities

The bulk and tapped densities of the banana flour were measured using the methods described by Okaka & Potter, (1977). The banana flour samples (50 g) were weighted and gently poured through a glass funnel into a 100 mL cylinder separately. The volume occupied by each sample was recorded, and the bulk density (Bd) was calculated. Then, the measuring cylinders with powders were tapped on a wooden surface until a constant volume was observed, and the tapped density (Td) was calculated. The Bd and the Td were calculated as the volume (mL) per unit weight of a sample.

2.3.2. Compressibility Index (CI) and Hausner Ratio (HR)

The values of bulk (Bd) and tapped densities (Td) were used to calculate the compressibility index (equation 1) and the Hausner ratio (equation 2) of the flour samples according to the method described by Olayemi *et al.*, (2008).

$$\text{Hausner ratio (HR)} = Td / Bd \quad (1)$$

$$CI = [(Td - Bd)/ Td] \times 100 \quad (2)$$

2.3.3.Water Absorption Capacity (WAC) and oil absorption Capacity (OAC)

Water absorption and oil absorption capacities of banana flour were examined using the method described by Sosulski *et al.* (1976). In order to measure the WAC and OAC, one gram of flour sample was mixed with 10 mL of distilled water and coconut oil respectively. Then, the mixtures wer allowed to stand for settling at 30 ± 2 °C for 30 min and it was centrifuged at 2000 rpm for 30 min. The weight of water/ oil absorbed by the flour was measured using the difference of the final weight of the sample after centrifuging and the orginal sample weight. WAC and OACs were expressed as grams of water/oil bound per gram of flour.

2.3.4. Swelling Capacity

The swelling capacity of each flour sample was measured as described by Okaka & Potter (1977). The flour samples were filled in a graduated cylinder separately, and water was added and mixed. The volume occupied by the sample was recorded after 30 min.

2.3.5. Transparency

Transparency of banana flour samples were measured using the method described by Wang *et al.*, (2017) where an aqueous flour solution was prepared, heated and cooled to room temperature. The transparency was measured at 620 nm using the spectrophotometer (CT-8600 double beam spectrophotometer, Spain).

2.3.6. Foaming capacity

Foaming capacity of banana flour was determined according to the method described by Chandra & Samsher, (2013) which involves adding 1 g of flour into 50 mL of distilled water, shaking for 5 min and measuring the volume.

2.3.7. Gelatinization temperature

Gelatinization temperature of the flour samples were measured using the method described by Chandra & Samsher, (2013) where 1 g of flour sample with water was heated slowly in a water bath until a solid gel formed and the temperature at the point of complete gel formation was recorded.

2.3.8. Color Parameters

The CIE tristimulus L*, a*, and b* parameters were determined using colorimeter (BCM-200, China). Chroma (ΔC), hue angle, Yellowness index and whiteness index were calculated according the formulas described by Falade & Oyeyinka (2015).

2.4. Evaluation of the Proximate Composition of banana Flour

Proximate analysis was conducted to determine the amount of moisture, ash, crude fiber, crude protein, and crude fat according to the AOAC methods (2000) while carbohydrate

content was determined by the difference method (Pearson, 1970).

2.5. Evaluation of the chemical properties of banana flour

2.5.1. Titratable acidity and pH

The titrimetric method described by Falade & Oyeyinka, (2015) was used to measure the titratable acidity of the banana flour samples while the pH values of the banana flour were determined using a pH meter (Model AD 132, Romania).

2.6. Evaluation of the nutritional and antioxidant properties of the banana flour

2.6.1. Determination of Vitamin C content

Two grams of banana flour was weighed and mixed with 100 mL of distilled water in a conical flask. The mixture was filtered, and 5 mL of the filtrate was taken and volumed up to 100 mL with distilled water. Five drops of phenolphthalein indicator was added, and the mixture was titrated with 0.01 N NaOH until the color of the solution turned into pink. The vitamin C content present in 100 g of the sample was calculated using formula presented by Ndayambaje *et al.*,(2019).

$$\text{Vit. C} = \text{Vol. of NaOH} \times 0.01\ \text{N} \times 100\ \text{ml} \times 100\ \text{g}/2 \times 176.13 \times 10^{-3} \quad (3)$$

2.6.2. Determination of Total polyphenol content

The total polyphenol content of the prepared banana flour was measured using the method given by Singleton *et al.*, (1999) with some modifications. The banana flour extracts using 95% (v/v) ethanol (400 µL) were mixed with 2 mL of Folin-Ciocalteu reagent which has been diluted (ten-fold) using distilled water. After a period of one minute, 2 mL of 7.5% (w/v) sodium bicarbonate solution was added to stop the reaction. Then the mixture was volumed up to 10 mL using distilled water. This mixture was placed at dark for 120 min, and the absorbance was measured at 760 nm. Results were expressed as mg of Gallic acid equivalents per 100 g of the sample (mg GAE/100 g) using the equation obtained by linear regression of Gallic acid standard curve prepared by serially diluted Gallic acid solutions.

2.6.3. Determination of total flavonoid content

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample according to the methods described by Zhishen *et al*., (1999) and Chang *et al.*(2020) with some modifications using quercetin as the standard. One milliliter from properly diluted methanolic fractions of the samples or quercetin standard solutions or (water or methanol) the blank solution was added to 10 mL volumetric flask. Then 4 mL of distilled water was added to the solution followed by 0.3 mL of 5% $NaNO_2$ at the beginning, and 0.3 mL of 10% $AlCl_3$ after 5 min. At the sixth minute, 2 mL of 1 M NaOH was added, and the solution was volumed up to 10 mL with distilled water, and the mixture was mixed well. After a period of 15 min, the absorbance was measured at 510 nm. The total flavonoid content was expressed as milligrams of quercetin equivalent per 100 g of the sample.

2.6.4. Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was assessed by using the method suggested by Petlevski *et al.*, (2013) with some modifications. A control sample was prepared by mixing 4 mL of DPPH solution in 0.4 mL of methanol to obtain an absorbance at 517 nm using UV-Vis Spectrophotometer. Initially, 4 mL of 0.1 mM DPPH methanolic solution was added to 0.4 mL methanolic sample and mixed well using the vortex. Samples were kept in the dark for 30 min and the absorbance was taken at 517 nm using a spectrophotometer. The results were expressed as Trolox equivalent (TE) in milligrams per g of dried sample.

2.7. Data analysis

All the experiments were conducted in triplicate and data are presented in mean ± standard deviation. One-way ANOVA with Tukey's post hoc test was applied to determine

the statistical significance among the three banana varieties at $p < 0.05$.

3.Results and discussions

3.1. Physico-functional properties of the banana flour

The results of physico-functional properties of flour from three different banana varieties (*Ambul*, *Seeni* and Cavendish) are summarized in Table 1.

Table 1 Physico-functional properties of the banana flour

Functional property	***Ambul***	***Seeni***	**Cavendish**
Bulk density (g/mL)	0.51± 0.00[a]	0.51±0.01 [a]	0.51±0.01 [a]
Tapped density (g/mL)	0.72± 0.01 [a]	0.74±0.01 [a]	0.70±0.01 [a]
Compressibility index	29.83 ± 1.78 [a]	31.28 ± 1.62 [a]	28.56 ± 1.44 [a]
Hausner ratio	1.43 ± 0.04 [a]	1.46 ± 0.03 [a]	1.40 ± 0.03 [a]
Swelling capacity (mL)	27.25± 0.31 [b]	20.50±2.12 [a]	24.00±0.00 [ab]
Transparency	84.00± 0.21 [b]	71.70±0.00 [a]	89.60±0.00 [c]
Water Absorption Capacity (%)	302.00± 8.49 [a]	298.50±2.12 [a]	279.50±0.71 [a]
Oil Absorption Capacity (%)	267.00±1.41 [a]	143.00±1.41 [a]	152.50±3.54 [a]
Foaming capacity	7.00± 1.41 [a]	1.00±0.00 [a]	11.00±1.41 [a]
Gelatinization temperature (˚C)	74.10± 0.01 [a]	75.00±0.00 [b]	75.30±0.00 [b]
Color parameters			
Color – L*	69.64±0.13 [b]	72.18±0.50 [c]	60.40±0.13 [a]
a*	3.33±0.18 [a]	3.15±0.35 [a]	3.55±0.07 [a]
b*	26.90±0.57 [a]	27.26±1.05 [a]	27.88±0.32 [a]
Hue angle	1.45±0.01 [a]	1.46±0.01 [a]	1.44±0.00 [a]
Yellowness index	55.18±0.89 [a]	53.94±1.90 [a]	65.94±0.82 [b]
Whiteness index	59.26±0.10 [b]	60.92±0.59 [c]	51.44±0.02 [a]
Chroma c	27.11±0.54 [a]	27.44±1.09 [a]	28.10±0.31 [a]

Data are expressed in mean ± SD of three independent measurements. Values with different superscripts within the same row are significantly different at $p<0.05$.

The bulk density of flour obtained from all three banana varieties was 0.51±0.01 g/mL and it was in the range (0.459 g/mL to 0.67 g/mL) which reported in literature (Falade & Oyeyinka, 2015 and Pragati *et al*., 2014). The bulk density of banana flour of the present study showed slightly higher value compared to the bulk density of wheat flour (0.49 g/mL) reported by Amankwah *et al*., (2022). Bulk density of flours is mainly influenced by their initial moisture content and particle size (Chandra & Samsher, 2013) where it decreases along with the increase of maturity of fresh material. The bulk density is a good indicator of heaviness of the flour (Ocloo *et al.*, 2010) and it is important to decide the package specifications, material handling, and application in wet processing in the food industry. Flour with lower bulk density occupies more space than a denser product of the same weight, which can affect the amount of packaging material required, and the overall size and weight of the package (Anderson, & Atnip, 2013).

Tapped density is another important physical parameter of flours to determine its powder characterization such as compressibility index and Hausner ratio due to its simplicity and rapidity of measurement. The results of the present study showed that Cavendish banana flour had the lowest tapped density (0.7±0.014 g/mL), while *Seeni* banana flour showed the highest tapped density (0.74±0.01 g/mL). Tapped density for *Ambul* banana flour was about 2.7% lower than for *Seeni* banana flour. Tapped density of wheat flour (0.74 g/mL) obtained from Kumasi-Ashanti region, Ghana was reported as 0.74 g/mL (Amankwah *et al*., 2022), and the tapped densities of banana flour of the present study were also in the same range. The tapped density of flour plays a significant role in its flowability, compressibility, and bulk volume. When the tapped density is lower, it indicates that the particles are loosely packed and have a higher volume, which can result in more dusting and settling during storage and transport. In contrast, higher tapped density indicates tightly packed particles with lower volume, which reduces dusting and settling during storage and transport but also leads to decreased flowability (Rosell & Collar, 2012).

Compressibility index and Hausner ratio are two other important indicators which showing the significance of inter-particulate interactions and important to forecast the propensity of a given powdered sample to be compressed (SherVington & Sherrington, 1998). The relationship among compressibility index, Hausner ratio and flowability of flour is shown in Table 2 (Carr, 1965).

Table 2. The relationship among Compressibility index, Hausner ratio, and flowability of flour

Compressibility index %	Flow character	Hausner ratio
≤10	Excellent	1.00 - 1.11
11-15	Good	1.12 - 1.18
16-20	Fair	1.19 - 1.25
21-25	Passable	1.26 - 1.34
26-31	Poor	1.35 - 1.45
32-37	Very poor	1.46 - 1.59
>38	Very very poor	>1.60

Source : Carr, (1965)

The above-mentioned scale in Table 2 is widely used by researchers to determine and compare the flow characters of flours used in the bakery industry. Highest compressibility index (31.28 ± 1.62) and Hausner ratio (1.46 ± 0.03) recorded from *Seeni* banana flour compared to the other two banana flour types. However, the banana flours of the present study showed very poor flow properties according to the given scale (Table 2). The study conducted by Thanyapanich *et al.*, (2021) reported that the compressibility indexes of banana flour from *Musa acuminata* L. (Musa AAA; Hom Khieo) and *Musa sapientum* L. (Musa ABB; Namwa) were 31.70 ± 2.13 and 34.84 ± 1.59, respectively, and the Hausner ratios of those varieties as 1.47 ± 0.05 and 1.54 ± 0.04, respectively which are in line with the results of the present study. The reason for showing very poor flow characters may be due to the fine particle size distribution of flour. Fine particle size can lead to poor flow characteristics in flour and other powders due to increased inter-particle friction and cohesion. Small particles tend to stick together, forming clumps that impede flow. The irregular shapes and surface features of fine particles can also contribute to increased cohesion. Poor flow can negatively impact the handling and processing of powders (mixing, blending or dispersing of ingredients), leading to downtime, waste, or inconsistent product quality (Vredenburgh, 2017).

The Hausner ratio of the wheat flour (1.53), which were obtained from Kumasi-Ashanti

region, Ghana (Amankwah *et al*., 2022), is slightly higher than the Hausner ratio values reported by the banana flour in the present study (1.40-1.46). This may be due to the lower bulk density of wheat flour than the banana flour. The Hausner ratio is directly related to the bulk density of flour and increase in bulk density usually results a higher Hausner ratio. This is due to the decrease of space between particles and the particles become more tightly packed, making it more difficult for them to flow. Therefore, a material with a higher bulk density is likely to have a higher Hausner ratio, indicating poorer flowability. However, the relationship between Hausner ratio and bulk density may not always be straightforward, as other factors such as particle size, shape, and surface properties can also affect for the flowability.

The swelling capacity of flour is directly related to their binding ability (Lawal *et al.*, 2011). *Seeni* banana flour showed the lowest swelling capacity while *Ambul* banana flour showed the highest swelling capacity. Further, there was a significant difference ($p<0.05$) between the swelling capacities of *Ambul* and *Seeni* banana flour. However, swelling capacity of both flour types were not significantly difference ($p<0.05$) from that of Cavendish banana flour. The study conducted by Ocheme *et al.* (2018) reported that wheat flour has 12.71% swelling capacity and the banana flour of the present study showed around 1.8 times higher swelling capacity than the wheat flour. The reason of showing higher swelling capacity could be the presence of high amount of protein and carbohydrate in the banana flour (Gull *et al*., 2015). In addition, swelling capacity of flours can be depend on the size of particles, types of variety and types of processing methods or unit operations (Chandra & Samsher, 2013).

The transparency of the banana flour from three tested varieties was found to be varied in the sequence of Cavendish > *Ambul* > *Seeni*, and a significant difference ($p<0.05$) was observed among the varieties. Measuring the transparency of flour is important because it can provide information about the quality and purity of the flour, which can affect the final product. Transparency can be influenced by several factors, including the particle size distribution, protein content, and ash content of the flour (AACC International., 2010; Giraldo & Aguilera, 2017).

The highest Water Absorption Capacity (WAC) was showed by the *Ambul* banana flour and the lowest by the Cavendish banana flour. Banana flour showed a higher WAC compared to that of wheat flour (140%) which was reported by Chandra *et al*., (2015). Further, these findings showed that addition of banana flour to wheat flour will be affected on the water holding capacity of the composite flour mixture. Water absorption capacity of banana flour is greatly depend on the starch concentration, composition, granule shape and temperature (Falade and Oyeyinka, 2015). According to Pragati *et al*., (2014) and Falade & Oyeyinka, (2015), WAC of unripe banana flour was greater than ripe banana flour as unripe banana has higher starch content that increase the water absorption capacity. Water absorption capacity of flour affects the texture and quality of the bakery products. Flour with high WAC can absorb more water, resulting in stickier and more elastic dough that can be difficult to handle and shape. Furthermore, a low WAC results in dry and crumbly dough that can be tough and hard to chew. The increase or decrease of WAC can have both beneficial and detrimental effects on the bakery industry.

The Oil Absorption Capacity (OAC) of three types of banana flour ranged between 143 to 267% and there was no significant difference ($p>0.05$) among the flours from three varieties. As compared to wheat flour (146%) (Chandra *et al*., 2015), *Ambul* and Cavendish banana flour showed higher OACs while *Seeni* banana flour showed a lower OAC. This may be due to the composition and granule shape of the flours. OAC is an important parameter in flour quality assessment, with a significant impact on the texture, volume, and overall quality of baked goods. Flours with higher OACs tend to produce more moist, tender, and fine-textured products,

while flours with lower OACs can result in dry, crumbly products.

The gelatinization of starch is a critical process in the production of baked goods, as it provides the structure and texture of the final product. The gelatinization temperature of flour determines how easily the starch can be gelatinized, and therefore, affects the final texture and quality of the baked goods. The temperature at which the starch granules rapidly swell and yield a thickened paste, depends upon the water content, type of starch and the ratio of amylose and amylopectin present in the flour. In the present study, Cavendish banana showed the highest gelatinization temperature (75.30 °C) while *Ambul* banana showed the lowest gelatinization temperature (74.10 °C). The results were in agreement with a previous study that found the gelatinization temperatures of banana flour which was in a range of 70–79 °C (Bello-Pérez *et al.*, 2005; De la Torre-Gutiérrez *et al*., 2008 and Utrilla-Coello *et al.*, 2014). According to the Matsuki *et al.*, (2003), the peak gelatinization temperatures of wheat flour varieties (Norin 3, Norin 29, Haruhikari, and Haruyutaka) at daytime temperature (30 °C) was 60.2 °C, 59.7 °C, 59.8 °C, and 60.0 °C, respectively. Those values are around 20% lower than the gelatinization temperatures of banana flour varieties used in the present study (74.10 - 75.30 °C). This may be due to the variation in stable crystalline structure and length of amylopectin chains between these two flour types (wheat and banana) (Matsuki *et al.*, 2003). Baked goods made from flour with a lower gelatinization temperature tend to have a softer and moister crumb while extremely higher gelatinization temperature may lead to a firmer or drier texture in baked products (Aguilera & Kruger, 2001; Zhang *et al*., 2008).

A particle made up of numerous gas bubbles that have been trapped in a liquid or solid is known as a foam (Fennama, 1996). The foaming capacity of flour is important to evaluate the flours capacity for foaming, which depends on the availability of flexible protein molecules that reduce the surface tension of water (Asif-Ul-Alam *et al.*, 2014). Foam capacities of tested banana flours were found to be varied from 7 mL to 11 mL where the highest foam capacity was observed from Cavendish banana flour (11 mL) while lowest from *Ambul* banana flour (7 mL). However, the foam capacities of banana flour were lower than the reported foam capacity of the wheat flour (12.92%) (Akubor & Badifu, 2004). This may be due to the differences in available flexible protein molecules in wheat flour and banana flour. Further, reduced or negative foam capacities may affect the shelf-life of the flour (Kaushal *et al*., 2012).

The Commission on Illumination (CIE) L*, a* and b* color parameters and the calculated Whiteness index, Chroma C, Hue angle and Yellowness index of the three different banana flour are summarized in Table 1. The L* value shows the lightness parameter which ranged from 0 to 100 (0 is black and 100 is white) whereas a* and b* values shows color direction from green to red and blue to yellow, respectively. There was a significant difference ($p<0.05$) among banana flours for the L* parameter while there was no significant difference ($p>0.05$) for a* and b* values. Wheat flour (variety Khadija) showed higher L* value (94.8) than banana flour while, banana flour had higher a*(3.15-3.55) and b*(26.90-27.88) value ranges than wheat flour (a*-0.54, b*-8.66) (Bouhlal *et al.*, 2019). These changes are probably due to the presence of different degree of colour pigments such as carotenoids in the banana flour.

The whiteness index (WI) represents the degree of whiteness of the product, which is susceptible to change during storage. As a result, materials with a lower level of whiteness are regarded as neutral, greyish, or creamier (yellowish), and materials with a higher level of whiteness are perceived as having bluish tones. According to the results of present study, there were significant differences ($p<0.05$) of whiteness index of flour obtained from three different banana varieties. This may be due to the degree of color pigments present in banana variety, astringency level and the level of controlling browning effect. The study

conducted by Bouhlal *et al*., (2019) reported that wheat flour (variety Khadija) had a whiteness index of 89.89 which was higher than the whiteness index of the banana flour varieties (51.44 – 60.92) used in the present study.

Color parameters of flour especially the CIE L*, a* and b* values were greatly affected by the drying method, variety and the maturity of the banana fingers (Falade & Oyeyinka, 2015). Generally, dried banana samples showed higher L* value and lower a* and b* values than the fresh banana samples (Chandra *et al*., 2015). Moreover, use of pretreatment methods such as adding ascorbic acid, mixed with water or apply lime juice or salt water to unripe banana before processing into flour may help to reduce the effect of enzymatic browning and improve the color quality (Anyasi *et al*., 2014).

3.2 Chemical properties of the banana flour

The comparison of the results of chemical properties of flour from three different banana varieties is shown in Table 3.

Table 3 Chemical properties of the banana flour

Chemical property	***Ambul***	***Seeni***	**Cavendish**
pH	5.32±0.01 ^{c}	4.97±0.01 ^{a}	5.08±0.02 ^{b}
Titratable acidity	0.007±0.00 ^{b}	0.005±0.00 ^{a}	0.01±0.00 ^{b}

Data are expressed in mean ± SD of three independent measurements. Values with different superscripts within the same row are significantly different at $p<0.05$.

Table 4 Nutritional and antioxidant properties of the banana flour

Nutritional property	***Ambul***	***Seeni***	**Cavendish**
Proximate composition of banana flour (g per 100 g Wet Basis (Wb))			
Moisture content	7.10 ± 0.42 ^{a}	7.20 ± 0.00 ^{a}	7.90 ± 0.14 ^{a}
Ash content	2.80 ± 0.28 ^{a}	2.50 ± 0.14 ^{a}	4.60 ± 0.00 ^{b}
Crude fat	0.50 ± 0.23 ^{a}	0.83 ± 0.24 ^{a}	0.66 ± 0.00 ^{a}
Crude protein	0.97 ± 0.01 ^{b}	0.82 ± 0.03 ^{a}	0.97 ± 0.04 ^{bc}
Crude fiber	1.17 ± 0.23 ^{a}	0.67 ± 0.00 ^{a}	0.84 ± 0.23 ^{a}
Carbohydrate	88.64 ± 0.39 ^{b}	88.65 ± 0.39 ^{bc}	85.87 ± 0.39 ^{a}
Antioxidant properties of banana flour			
Total flavanoid content mg QE/100 g	162.20 ±16.26 ^{a}	337.70 ± 31.11 ^{b}	180.70 ± 18.39 ^{a}
Total polyphenol content mg GAE /100 g	30.45 ±0.22 ^{b}	31.41 ± 0.61 ^{b}	14.02 ± 0.26 ^{a}
DPPH radical scavenging activity (mg TE/g)	476.00 ±0.06 ^{ab}	324.00 ±0.04 ^{a}	576.00 ± 0.03 ^{b}
Vitamin C (mg per 100 g sample)	11.20 ± 0.75 ^{a}	10.83 ± 0.12 ^{a}	10.44 ± 0.06 ^{a}

Data are expressed in mean ± SD of three independent measurements. Values with different superscripts within the same row are significantly different at $p<0.05$.

The Organic acids such as malic, citric, and oxalic are the titratable acids found in banana. As per the results in Table 3, Cavendish banana flour had the highest titratable acidity while *Seeni* banana flour had the lowest value. Titratable acidity of flour is important in several baking operations, including leavening, flavor development and product formulation (Cauvain, 2015).

pH is another important parameter which decide the physical and chemical quality of the flour. The water absorption rate, dough

formation, and texture of the final product can be influenced by the pH of flour. Flour with a low pH is more acidic, and thus tends to absorb less water, resulting in firmer and drier dough. Conversely, flour with a high pH is more alkaline, and tends to absorb more water, resulting in softer and stickier dough (Hoseney, 1994). Results of the present study confirmed the above-mentioned statement showing that the *Ambul* banana flour had highest pH value as well as higher water absorption capacity. There was a significant difference ($p<0.05$) among pH values of different banana varieties and the results of the present study are in agreement with the previously reported pH values of banana flour, where it ranged between 5.79–6.18 (Bakar *et al*., 2018). Moreover, the study conducted by Bakar *et al*., (2018) showed that the pH levels of banana flour was affected by the stage of ripeness. The pH of banana flour (4.97 ±0.01 - 5.32 ±0.01) was slightly lower than that of reported pH value of wheat flour (6.00 ± 0.00) (Zhang *et al.*, 2021).

3.3. Nutritional and antioxidant properties of the banana flour

The proximate composition and the antioxidant properties of the flour from three banana varieties were also compared and the obtained results are summarized in the Table 4.

3.3.1. Proximate composition of banana flour

The moisture level of food products has a significant impact on the textural quality, chemical and biochemical responses, shelf-life as well as the rates of microbial development (Aurore *et al.*, 2009). The moisture content of banana flours studied in the present study were within the acceptable limits of moisture (<20.0%) to reach a stable shelf-life (Amarasinghe *et al.*, 2021). Further, moisture content of the banana flour in the present study (7.1-7.9%) was lower than the moisture content of wheat flour (10%) in Kumasi-Ashanti region, Ghana (Amankwah *et al*., 2022) and the moisture content of wheat flour Golden Penny (9.1%) obtained from Minna, Niger (Ocheme *et al.*, 2018). According to Codex Alimentarius standards if the moisture content of a flour is less than 14%, it can resist microbial growth and contribute to longer storage life (Mahloko *et al.*, 2019).

The fat content of the banana flour was ranged in 0.5-0.83 g per 100 g Wb and it is considerably low when compared to that of other common legumes and flours such as pearl millet (7.6%), quinoa (6.3%) (Oshodi *et al*., 1999), pigeon pea flour (1.80%) (Okpala & Mamah, 2001), and wheat flour (3.10%) (Akubor & Badifu, 2004).

Measurement of ash content aims to determine the amount of mineral matter which include in the three different banana varieties. The highest ash content (4.60 g per 100 g Wb) was recorded from Cavendish banana flour while *Seeni* banana flour showed the lowest ash content (2.50 g per 100 g Wb). The deviations between ash contents of banana flour may be due to the differences in the variety and the location. Banana flour showed higher amount of ash content compared to the reported ash content of wheat flour (0.45%) in Kumasi-Ashanti region-Ghana (Amankwah *et al*., 2022).

Considering the crude protein content, both *Ambul* and Cavendish banana flour showed 0.97 g per 100 g Wb protein content while *Seeni* banana flour showed 0.82 g per 100 g Wb protein content. However, the result of the present study was lower than the crude protein content (3.8 - 4.1%) reported by Morton, (1987) for banana flour. The varietal differences, fruit maturation, and environmental factors could be the reasons for showing different crude protein content in different studies. The crude protein content of the wheat flour which were obtained from Kumasi-Ashanti region, Ghana was 10.67% (Amankwah *et al*., 2022), and it was higher compared to the crude protein content of green banana flour.

The highest crude fiber content was showed by *Ambul* banana flour followed by Cavendish and *Seeni* banana flours. The results of present study was lower than the reported value of crude fiber content (4.2 g per 100 g Wb) by Asif-Ul-Alam *et al.*, (2014) for flour obtained from *Musa sapientum*. The disparity may be due to the differences in variety and location. The fiber

content of the banana flour was ranged in 0.67-1.11 g per 100 g Wb and it is considerably high amount when compared to the crude fiber content of wheat flour (0.49%), obtained from Kumasi-Ashanti region, Ghana (Amankwah *et al*., 2022).

The major component of the flour was carbohydrate, and the values obtained from the present study were in a range of 85.87-88.65 g per 100 g Wb. These values are comparably high to those reported by Morton, (1987) which was 79.6% for green banana flour. When comparing the carbohydrate contents of banana flour and wheat flour, banana flour had higher carbohydrate content (85.87- 88.65 g per 100 g Wb) than wheat flour (76.51%), obtained from Kumasi-Ashanti region, Ghana (Amankwah *et al*., 2022), and wheat flour - Golden Penny (72.73%) which was obtained from Minna, Niger (Ocheme *et al.*, 2018). The high carbohydrate content of banana flour suggests that it is a suitable source of energy for humans to consume, particularly in breakfast and weaning formulas.

3.3.2. Antioxidant properties of banana flour

Antioxidants are indispensable to protect the human cells from the negative health effects such as cancer, inflammation, diabetic, and cardiovascular disease (Sulastri *et al.,* 2018). Phenols are one of the most common phyto-constituents of different fruits and vegetables which directly related to their antioxidant properties. Free radicals can be scavenged by phenolic compounds, which can serve as hydrogen donors and reducing agents (Phuyal *et al.*, 2020). The total polyphenol content (TPC) of the tested banana flours were ranged from 14.02 to 31.41 mg GAE/100 g of dry matter (Table 4). The TPC indicated significant variations across all samples ($p<0.05$). The study conducted by Fatemeh *et al.*, (2012) reported that dream banana flour showed 94.25 mg CEQ/100 g TPC value. These results are slightly higher than the results of TPC of banana flours in the present study which could be due to the difference in variety, maturity stage, growing conditions, and climate variations.

The total flavonoid content (TFC) of different types of banana flour exhibit significant difference ($p<0.05$) where the *Seeni* banana flour had the highest TFC (337.70 mg QE/100 g), while the *Ambul* banana flour had the lowest TFC (162.20 mg QE/100 g). The primary flavonoids that can be found in bananas are quercetin, myricetin, and kaempferol (Amarasinghe *et al.*, 2021). Some studies have shown that the banana flour had higher TFC compared to wheat flour (Amarasinghe *et al.*, 2021).

The results of the DPPH radical scavenging activity showed that there were significant differences ($p<0.05$) among the antioxidant activity of different types of banana flour (Table 4). The Cavendish banana flour had the highest DPPH radical scavenging activity (576 ± 0.03 TE mg/g), while the *Seeni* banana flour had the lowest (324 ± 0.04 TE mg/g). Studies by Pasko *et al*., (2009) and Kim *et al*., (2013) have investigated the DPPH radical scavenging activity of different types of wheat flour and reported that it was ranged from 0.50 to 2.8 TE mg/g depending on the variety. Further, Kim *et al*. (2013) found that red wheat flour had DPPH activity ranged from 2.2 to 5.5 TE mg/g. The results of the present study showed that green banana flour had a higher antioxidant activity compared to wheat flour. Moreover, Mahloko *et al.* (2019) stated that antioxidant capacity of flour may decrease, during baking due to reactions such as polymerization. However, they mentioned that the microwave roasting and baking both can improve the antioxidant activity of baked goods.

Vitamin C is one of the abundant nutrients present in banana. It is a thermal sensitive vitamin and can be easily destroyed by any heat treatment. Generally, edible bananas contain 11 mg of vitamin C per 100 g of the fresh sample (Ndayambaje *et al*., 2019). In the present study, vitamin C content of green banana flour range in 10.44 to 11.20 mg/100 g Wb of sample. The vitamin C content in wheat flour (Golden penny) was reported as 0.0924 mg/g (Abdulkadir, 2014) and green banana flour showed comparatively higher amount of vitamin C content than wheat

flour. The highest vitamin C content showed by *Ambul* banana flour (11.20 mg/100 g Wb) while the lowest vitamin C content showed by Cavendish banana flour (10.44 mg/100 g Wb). Mallawaarchchi *et al*., (2021) reported that the vitamin C content of ripen *Seeni* banana was 3.13 mg/100 g, and it was lower than the vitamin C content of raw *Seeni* banana flour (10.83 mg/100 g) found in the present study.

4. Conclusions

The present study aims to characterize the physico-functional, chemical, nutritional and antioxidant properties of green banana flour. The green banana flour has good potential to be developed as an alternative to wheat flour and successful way of minimizing the post-harvest losses. It can be concluded that the green banana flour is an excellent source of antioxidants and good source of carbohydrates. *Seeni* banana flour had the highest polyphenol and flavonoid content while Cavendish banana flour had the highest antioxidant content. Comparing the three different banana flours, *Ambul* banana showed the highest nutritional properties and antioxidant activity. Bulk density and tapped density of banana flours were quite similar to that of the wheat flours. The present study revealed that banana flour has a greater potential to be used as a functional ingredient in food formulations especially in bakery industry.

5.References

AACC International (2010). Approved Methods of Analysis (11th ed.). Method 10-05: Examination of wheat and flour for extraneous matter. St. Paul, MN: AACC International.

Abdulkadir, A. (2014). A Study on Some Vitamins and Minerals Content of Wheat Flour Samples Commonly Sold Within Kano Metropolitant. *International Journal of Science and Research (IJSR)* **3**(9): 1074-1078 Available at: https:// www.ijsr.net /archive/v3i9/ CVNFUDE0MTE5.pdf.

Aguilera, J.M. & Kruger, R.H. (2001). Role of starch gelatinization in the quality of bakery products. *Food Science and Technology International* **7**(4): 275-285.DOI.org/10.1177/108201320100700401.

Akubor, P. & Badifu, G. (2004). Chemical composition functional properties and baking potential of African breadfruit kernel and wheat flour blends. *International Journal of Food Science* **39**(2): 223–229.DOI: 10.1046/j.0950-5423.2003.00768.x.

Amankwah, N.Y.A., Agbenorhevi, J.K. & Rockson M.A.D. (2022). Physicochemical and functional properties of wheat-rain tree (Samanea saman) pod composite flours. *International Journal of Food Properties* **25**(1): 1317–1327. DOI: 10.1080/10942912.2022.2077367.

Amarasinghe, N.K., Wickramasinghe, I., Wijesekara, I., Thilakarathna, G. & Deyalage, S.T. (2021). Functional Physicochemical and Antioxidant Properties of Flour and Cookies from Two Different Banana Varieties (Musa acuminata cv. Pisang awak and Musa acuminata cv. Red dacca). *International Journal of Food Science* :**9**. DOI: 10.1155/2021/6681687.

Anderson, C.A. & Atnip, G.R. (2013). Packaging of Flour and Bakery Products. *Handbook of Food Powders* pp. 569-582.

Anyasi, T.A., Jideani, A.I.O. & Mchau G.R.A. (2014). Effect of organic acid pretreatment on some physical functional and antioxidant properties of flour obtained from three unripe banana cultivars. *Food Chemistry*. DOI: 10.1016/j.foodchem.2014.09.120

Asif-Ul-Alam, S.M., Islam, M.Z., Hoque, M.M. & Monalisa, K. (2014). Effects of Drying on the Physicochemical and Functional Properties of Green Banana (Musa sapientum) Flour and Development of Baked Product. *American Journal of Food Science and Technology* **2**(4): 128–133. DOI: 10.12691/ajfst-2-4-4.

Aurore, G., Parfait, B. & Fahrasmane, L. (2009). Bananas raw materials for making processed food products. *Trends in Food Science and Technology* 20(2): 78–91. DOI: 10.1016/J.TIFS.2008.10.003.

Bakar, S.K.S.A., Ahmad, N. & Jailani, F. (2018). Chemical and Functional Properties of Local Banana Peel Flour. *Journal of Food and Nutrition Research* 6: 492–496.

Bello-Pérez, L.A., De Francisco, A., Agama-Acevedo, E., Gutierrez-Meraz, F. & García-Suarez, F.J.L. (2005). Morphological and molecular studies of banana starch. *Food Science and Technology International* 11(5): 367–372.DOI: 10.1177/1082013205058409.

Bouhlal, O., Boutekrabt, A., Lecheb, S., Hamini-Kadar, N., Boussaha, F. & Zidani, S. (2019). Wheat-lentil fortified flours: health benefits physicochemical nutritional and technological properties. *Journal of Materials and Environmental Science* 10(11): 1098–1106.

Carr, R.L. (1965). Evaluating Flow Properties of Solids. *Chemical Engineering* 72 : 163–168.

Cauvain, S. (2015). Wheat Milling & Flour Testing. *Technology of Breadmaking*: Springer International Publishing. DOI: 10.1007/978-3-319-14687-4_12.

Chandra, S. & Samsher, (2013). Assessment of functional properties of different flours. *African Journal of Agricultural Research* 8(38). DOI: 10.5897/AJAR2013.6905.

Chandra, S., Singh, S. & Kumari, D. (2015). Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology* 52(6): 3681. DOI: 10.1007/S13197-014-1427-2.

Chang, C.C., Yang, M.H., Wen, H.M. & Chern, J.C. (2020). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis* 10(3): 3. DOI: 10.38212/2224-6614.2748.

De La Torre-Gutiérrez, L., Chel-Guerrero, L.A. & Betancur-Ancona D. (2008). Functional properties of square banana (Musa balbisiana) starch. *Food Chemistry* 106(3): 1138–1144. DOI: 10.1016/J.FOODCHEM.2007.07.044.

Department of Agriculture Sri Lanka (2012). Banana. Retrieved from http://www.doa.gov.lk/agbiz/images/agri_com/Banana.pdf

Department of Census & Statistics (2014). Economic census 2013/14 agricultural activities Available at: http://www.statistics.gov.lk/Economic/Final_Report_Agri.pdf (accessed: 11 September 2022).

Falade, K.O. & Oyeyinka, S.A. (2015). Color Chemical and Functional Properties of Plantain Cultivars and Cooking Banana Flour as Affected by Drying Method and Maturity. *Journal of Food Processing and Preservation* 39(6): 816–828. DOI: 10.1111/jfpp.12292.

Fatemeh, S.R., Saifullah, R., Abbas, F.M.A. & Azhar, M.E. (2012). Total phenolics flavonoids and antioxidant activity of banana pulp and peel flours : Influence of variety and stage of ripeness. *International Food Research Journal* 19(3): 1041–1046.

Fennama, R.O. (1996). *Food Chemistry* (3rd ed). New York, NY: Marcel Dekker, pp. 36–39

Giraldo, L.F. & Aguilera, J.M. (2017). Effects of flour particle size and flour type on the quality of expanded extruded snacks. *Journal of Food Science and Technology* 54(13): 4165-4174.

Gómez, M., Gutkoski, L.C., & Bravo-Núñez, Á. (2020). Understanding whole-wheat flour and its effect in breads: A review. *Comprehensive Reviews in Food Science and Food Safety* 19(6) : 3241–3265. DOI: 10.1111/1541-4337.12625.

Gull, A., Jan I.U., Khan M.R., Farooq U., Naz S., & Zeb A. (2015). Physico-chemical and nutritional evaluation of unripe banana flour. *Journal of food science and technology* 52(12): 7797-7804.

Hoseney, R.C. (1994). Principles of cereal science and technology. *American Association of Cereal Chemists.*

Kaushal, P., Kumar V. & Sharma H. (2012). Comparative study of physicochemical functional antinutritional and pasting properties of taro (Colocasia esculenta) rice (Oryza sativa) flour pigeonpea (Cajanus).

Food Science and Technology 48(1): 59–68.Available..at: https://www.sciencedirect.com/science/article/pii/S0023643812001065 (accessed: 13 September 2022).

Kim, M.J., Lee, Y.J., Kim, Y.J., Lee, J.H. & Kim, J. (2013). Antioxidant activity and phenolic content of white and red wheat flour as affected by ionizing radiation. *Journal of the Korean Society of Food Science and Nutrition 42(*6): 975-982.

Lawal, O., Lapasin, R., Bellich, B. & Olayiwola, T.O. (2011). Rheology and functional properties of starches isolated from five improved rice varieties from West Africa. *Food Hydrocolloids* 25(7): 1785–1792. Available at: https://www.sciencedirect.com/science/article/pii/S0268005X11001226 (accessed: 21 September 2022).

Mahloko, L., Silungwe, H., Mashau, M.E. & Kgatla, T.E. (2019). Bioactive compounds, antioxidant activity, and physical properties of honeybush tea (Cyclopia spp.) extracts as affected by processing methods. *Journal of Food Processing and Preservation* 43(12).

Mallawaarachchi, M.A.L.N., Somachandra, K.P. & Costa M.D.D. (2021). Determination of nutritional composition of four banana varieties available in Sri Lanka. *International Symposium on Agriculture and Environment* p. 2021.

Matsuki, J., Wada, T., Takeda, Y. & Suzuki, T. (2003). Effects of environmental temperature on structure and gelatinization properties of wheat starch. *Cereal Chemistry* 80(4): 476–480. DOI: 10.1094/CCHEM.2003.80.4.476.

Morton, J. (1987). The Jackfruit (Artocarpus heterophyllus Lam .): Its Culture Varieties and Utilization 53: 336–344. Available at: https://journals.flvc.org/fshs/article/download/ 100549/96504 (accessed: 17 September 2022).

Nadeeshani, H., Samarasinghe, G., Silva, R., Hunter, D. and Madhujith, T. (2021). Proximate composition, fatty acid profile, vitamin and mineral content of selected banana varieties grown in Sri Lanka, *Journal of Food Composition and Analysis*, 100, 103887, ISSN 0889-1575, https://doi.org/10.1016/j. jfca.2021.103887.

Ndayambaje, J.P., Dusengemungu, L. & Bahati, P. (2019). Nutritional Composition of Plantain Flour of (Musa Paradisiaca): the Effect of Various Drying Methods in Rwanda. *American Journal of Food Science and Technology* 7(3): 99–103. DOI: 10.12691/ajfst-7-3-5.

Ocheme, O.M., Abulude, F.O., Adetuyi, F.O. & Adejumo B.A. (2018). Proximate composition functional and pasting properties of wheat and groundnut protein concentrate flour blends. *Food Science and Nutrition* 6(5): 1173–1178. DOI: 10.1002/fsn3.670.

Ocloo, F., Bansa, D., Boatin, R., Adom, T. & Agbemavor, W. (2010). Physico-chemical functional and pasting characteristics of flour produced from Jackfruits (Artocarpus heterophyllus). Food Science and Technology Research, 16(6): 557-563.

Okaka, J.C. & Potter, N.N. (1977). Functional and Storage Properties of Cowpea Powder-Wheat Flour Blends in Breadmaking. *Journal of Food Science* 42(3): 828–833. DOI: 10.1111/J.1365-2621.1977.TB12614.X.

Okpala, L. & Mamah, E. (2001). Functional properties of raw and processed pigeonpea (Cajanus cajan) flour. *International Journal of Food Sciences* 52(4): 343–346, DOI: 10.1080/09637480120057549.

Olayemi, O.J., Oyi, A.R. & Allagh T.S. (2008). Comparative evaluation of maize, rice and wheat starch powders as pharmaceutical excipients. *Nigerian Journal of Pharmaceutical Sciences* 7:131-138.

Oshodi, A.A., Ogungbenle, H.N. & Oladimeji, M.O. (1999). Chemical composition nutritionally valuable minerals and functional properties of benniseed (Sesamum radiatum) pearl millet (Pennisetum typhoides) and quinoa (Chenopodium quinoa) flours. *International Journal of Food Sciences and Nutrition*

50(5): 325–331. DOI: 10.1080/096374899101058.

Pasko, P., Zagrodzki, P., Barton, H., Chlopicka, J. & Gorinstein S. (2009). Effect of different wheat flours on the antioxidant activity of ready-to-eat breakfast cereals. *Journal of Food Science* 74(4): C258-C263.

Pearson, D. (1970). The Chemical analysis of foods (7th ed.). Edinburgh: Churchill London.

Petlevski, R., Flajs, D., Kalođera, Z. & Koncic, M.Z. (2013). Composition and antioxidant activity of aqueous and ethanolic Pelargonium radula extracts. *South African Journal of Botany* 85: 17–22.

Phuyal, N., Jha, P.K., Rana M.S. & Bhattarai U. (2020). Total phenolic flavonoid contents and antioxidant activities of fruit seed and bark extracts of Zanthoxylum armatum DC. *International Journal of Food Science* : p.7.DOI: 10.1155/2020/9840620.

Pragati, S., Genitha, I. & Ravish, K. (2014). Comparative Study of Ripe and Unripe Banana Flour during Storage. *Journal of Food Processing and Technology* 5(11). DOI: 10.4172/2157-7110.1000384.

Ranjha, M., Irfan, S., Nadeem, M. & Mahmood, S. (2022). A Comprehensive Review on Nutritional Value, Medicinal Uses, and Processing of Banana, *Food Reviews International*, 38. 199-225. 10.1080/87559129.2020.1725890.

Rosell, C.M. & Collar, C. (2012). Flour Testing Methods and Their Impact on Bakery Products. *Bakery Products Science and Technology* : 29-55.

SherVington, L.A. & Sherrington, A. (1998). Guaifenesin. *Analytical Profiles of Drug Substances and Excipients* 25(C): 121–164.DOI: 10.1016/S0099-5428(08)60754-6.

Singleton, V., Orthofer, R. & Raventós Lamuela, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin- ciocalteu reagent. *Methods in enzymology* 299 pp. 152-178.

Sosulski, F., Garratt, M.D. & Slimkard, A.E. (1976). Functional Properties of Ten Legume Flours. *Canadian Institute of Food Science and Technology Journal* 9(2): 66–69. https://DOI.org/10.1016/s0315-5463(76)73614-9.

Sulastri, E., Zubair, M.S., Anas, N.I., Abidin S., Hardani R., Yulianti, R., Sulastri, E., Zubair, S., Anas, I. & Hardani, R. (2018). Total Phenolic Total Flavonoid Quercetin Content and Antioxidant Activity of Standardized Extract of Moringa oleifera Leaf from Regions with Different Elevation. *Pharmacognosy Journal* 10(6): 104–108.

Thanyapanich, N., Jimtaisong, A. & Rawdkuen, S. (2021). Functional properties of banana starch (Musa spp.) and its utilization in cosmetics *Molecules* 26(12): 1–16. DOI: 10.3390/molecules26123637.

Utrilla-Coello, R.G., García-Suárez, F.J., Morales-Contreras, J.J., Jiménez-Aparicio, A.R., Bello-Pérez, L.A. & Paredes-López, O. (2014). In vitro digestibility physicochemical thermal and rheological properties of banana starches. *Carbohydrate Polymers* 101(1): 154–162.DOI: 10.1016/j.carbpol.2013.09.019.

Vredenburgh, A. (2017). Particle Size and Shape Handbook of Powder Technology, pp. 31-67.

Wang, J., Huang, H.H. & Chen, P.S. (2017). Structural and physicochemical properties of banana resistant starch from four cultivars. *International Journal of Food Properties* 20(6); 1338–1347. DOI: 10.1080/10942912.2016.1209517.

Zhang, W., Wu, J. & Wei, Y. (2008). Effect of flour gelatinization on dough rheology and bread quality. *Journal of Cereal Science* 48(2): 526-532. https:// DOI.org/10.1016/j.jcs.2008.03.005.

Zhang, W., Xiong, X., Chen, Y., Chen, Q., Zhang Y. & Li H. (2021). Selected Quality Attributes of Wheat Flour Added with Overozonized Wheat Flour. *Journal of Food Quality*. DOI: 10.1155/2021/5559884.

Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry* 64(4): 555–559.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

ENRICHMENT OF SANGAK BREAD WITH CARROT POMACE POWDER AND ITS EFFECT ON DOUGH RHEOLOGY, BREAD QUALITY, AND SHELF LIFE

Milad Asadi, Ebrahim Ahmadi[1]✉, Ahmad Nourmohammadi[1]

[1]*Department of Biosystems Engineering, Faculty of Agriculture, Bu-Ali Sina University, Hamadan, Iran.*
✉*eahmadi@basu.ac.ir*

https://doi.org/10.34302/crpjfst/2024.16.4.11

Article history:
Received
May 17th, 2024
Accepted
November 18th, 2024

Keywords:
Dietary fiber;
Sangak bread;
Carrot;
Rheology;
Shelf life

ABSTRACT

The study explores the use of antioxidant dietary fiber (ADF) from vegetable and fruit by-products in bread production to enhance nutritional value and reduce food waste. Specifically focusing on Sangak bread, a popular flatbread in Iran, the research investigates the effects of supplementing soluble fiber from carrot powder on bread quality and shelf life. By adding carrot pomace powder at different levels to the bread dough formulation, improvements in protein levels, reduced carbohydrate content, and enhanced dough strength were observed. The enriched bread samples showed less variation in texture over six days of storage, with the initial firmness attributed to the moisture absorption properties of carrot pomace powder. While the enriched dough exhibited changes in texture and sensory qualities compared to the control sample, the 3% enrichment level was found to be superior in terms of aroma and overall acceptability. The study emphasizes the importance of carefully balancing the incorporation of dietary fiber-rich ingredients in baked products to improve their nutritional profile without compromising sensory qualities. Overall, incorporating carrot pomace powder into Sangak bread at an optimal level of 3% is suggested as a promising approach to create a more nutritious and appealing product for consumers, addressing both health and sustainability concerns in the baking industry.

1.Introduction

Dietary fiber is a widely recognized term that has been defined by the Codex Alimentarius Commission as carbohydrate polymers that resist hydrolysis by endogenous enzymes in the small intestine of humans. In contrast to dietary fiber, antioxidant dietary fiber (ADF) is a novel concept introduced by Saura-Calixto (1998), with its key feature being the association of dietary fiber with natural antioxidants like phenolic compounds. ADF is predominantly found in plant-based foods such as vegetables (e.g., carrots, cabbage, cactus), fruits (e.g., apples, grapes, oranges, mangoes), cereals (e.g., wheat, rye, oats), and seeds (e.g., cocoa beans, coffee beans) (Xu et al., 2021). Utilizing high-fiber wastes such as tomato, beet, apple, and carrot pomaces from processing plants can not only reduce food waste but also enhance the nutritional value of food products, contributing to the development of healthier and more sustainable food options. Vegetable or fruit by-products are abundant sources of ADF and have traditionally been utilized as low-value commodities, primarily for animal feed (Xu et al., 2021; Nawirska and Kwaśniewska, 2005). Bread is a widely consumed staple food in many parts of the world. Unfortunately, most breads are crafted using refined flours that lack essential nutrients like fiber, vitamins, minerals, and antioxidants. This deficiency arises from the

extraction of wheat bran and germ during processing (Tebben et al., 2018). Hence, breads made from white wheat flour fail to fulfill the growing nutritional and health requirements of consumers. There are many ways to reduce bread staling today, such as the use of fiber in bread formulation. Sangak bread, because of its good flavor, is one of the most widely used kinds of flat bread in Iran (Khoshakhlagh et al., 2014). Sangak bread has the highest amount of insoluble fiber among traditional bread types in the country, and adding soluble fiber to it, due to differences in physiological responses of these fibers, seems desirable. Most studies have focused on reducing and delaying bread staling using bread improvers.

Carrot)Daucus carota(, is a widely used and valuable vegetable because of its nutritional properties, carotene, and carotenoids content, as well as vitamins B1, B2, B6, B12 and minerals. Carrot contains large amounts of fiber, protein, fat, and carbohydrates (Hernández-Ortega et al., 2013). Carrots have been used in foods such as bread, cakes, pickles, enriched wheat bread, and biscuit to enhance the fiber content of these products (Salehi et al., 2016).

In recent years, Iran has faced increasing bread waste. Approximately 23% of wheat, 7% of flour, and 22% of bread, equivalent to 4 million tons of imported wheat, are converted into waste in Iran each year. Due to the high volume of bread consumption in the country, this figure is very significant (Mohammadi, 2007). Bread shelf life is generally limited by two microbial and staling factors. Bread after baking, due to moisture, is a good environment for the growth of molds and yeasts that cause spoilage of bread (Avital et al., 1990).

Adding fiber sources to the bread formula is often accompanied by problems in the properties of the dough and the quality of the bread. Adding fiber reduces the volume, and increases the firmness and darkness of the bread (Wang et al., 2002). Besides, the resulting doughs have high water absorption which reduces the fermentation tolerance. The negative effects of fiber on bread structure are related to the decrease in gluten content and the increase in bran particles in bread texture (Katina et al., 2006). Turfani et al. (2017) studied the nutritional value of bread enriched with lentil flour. According to the results of this study, protein and fiber of the bread increased significantly. The rheological properties of the dough and the characteristics of the bread were also changed. According to these studies, there is no research on the use of carrot pomace powder fiber in Sangak bread production. This study aims to use carrot pomace waste and extract its fiber to increase the nutritional value of Sangak bread and to prevent bread waste by using new and cost-effective packaging.

2. Materials and methods

2.1. Materials

2.2.1. Material preparation

In this research, wheat flour with a 90% extraction rate, was supplied from the Marianaj-kar factory in Hamedan. Carrot pomace was collected as a juice waste from the local fruit juice shops. For the packaging of bread, silicon Nano-polymer film and lightweight polyethylene film were prepared from Tehran Aitak Nano-polymer and Tehran Plast companies, respectively. The Nano-bags (Zip-Kip) are made of silicone polymers, which allow the entry and exit of oxygen and carbon dioxide to be controlled and minimized. As a result, they prevent the spoilage of food inside these bags, and keep fresh the foods for a long time, and retain their properties until they are punctured or torn.

2.2.2. Extraction and maintenance of carrot pomace fiber

Carrot pomace fiber was prepared using the method developed by Chantaro et al. (2008). At first, the carrot pomace was blanched in hot water at 90 ± 2 °C (1 to 6 pomace ratio to water) for 1 minute. After rinsing, they were dried at 60 °C to 80 °C up to 6% moisture with an Avon from Fan Azemaghostar Company. It was then powdered with a home grind to obtain homogeneous particles. The resulting powder was passed through a 35-degree mesh sieve (500 μm) and stored in aluminum packaging at 4 °C.

2.2.3. Chemical analysis of carrot pomace powder

Moisture content was determined according to the AACC 44-19 standard method. Ash content was analyzed using the electric furnace method as per the AACC 08-01 standard. Crude protein content was determined by the Kjeldahl method using Soxhlet extraction, following the AACC 30-100 standard. Total nitrogen content for protein determination was measured by the Kjeldahl method and converted to protein content using a conversion factor of 6.38, in accordance with the AACC 46-10 standard.

2.2.4. Measuring the amount of total fiber

Fatless and dry samples were used to measure total fiber (AOAC, standard 1995). At first, 2 g of the sample was weighed accurately using model (AND FX-300GD) digital scale. After transferring to a beaker with a volume of 600 ml, the sample was boiled for 30 min in 200 ml of 0.25 normal sulfuric acid and the beaker contents were shaken once every 5 minutes. Subsequently, the content of the beaker was filtered using a filter paper, a Buchner funnel, and a vacuum pump. Then it was transferred to another beaker. Then 200 ml of 0.3 normal NaOH solution was added to the beaker and boiled for 30 min. At this step, the contents of the container were again filtered, and the residual material on the filter paper was transferred into a previously weighed porcelain crucible and placed in an oven at 105 °C for 24 h. Then, the porcelain crucible containing the sample was cooled in the desiccator and reweighed. For removing all organic materials, the porcelain crucible and its contents were placed in the oven for 3 h at a temperature of 550 °C. After this time, the crucible was cooled in the desiccator and reweighed. Finally, Equation (1) was used to calculate the percentage of fiber.

$$F = \frac{W_1 - W_2}{W} \times 100 \quad (1)$$

F: Total fiber content; W1: The weight of the porcelain crucible and sample before burning (g); W2: The weight of the porcelain crucible and sample after burning (g); W: The sample weight (g).

2.2.5. Preparation of dough samples

Wheat flour (1 kg), salt (45 g), and water were used to prepare the dough. Carrot pomace powder was added to the dough at different percentages of 3, 5, and 7 % (w/w based on flour) at the mixing stage. The dough was then rested at 25 ± 2 °C for 1 to 2 hours to complete fermentation (AACC-I, 2000).

2.2.6. Investigation of tissue parameters of dough

To obtain the textural features of the enriched and control dough, the back-extrusion test was carried out by a food texture analyzer (Zwick / Roell model BT1_FR0.5TH.D14) made in Germany with a load cell capacity of 500 N. In this test, the diameters of the retaining container and the probe were 45.92 mm and 40 mm, respectively. The test was defined in three cycles and in three repetitions so that in each cycle, loading and unloading last 30 and 10 seconds, respectively. The test speed was 40 mm / min, and the displacement rate after loading was 5 mm. Textural features such as elasticity, adhesion, chewiness, and cohesion were plotted in the form of a force-displacement curve using (Test Xpert) software (Fig. 1).

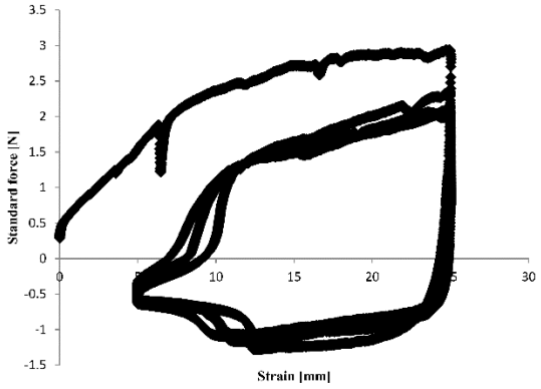


Figure 1. An example of a food texture analyzer graph

2.2.7. Sangak bread preparation method

Wheat flour (3 kg), salt (45 g), and water (as needed) were used to prepare the dough. Then, the sourdough (20%) was added, and the dough

stirred for half an hour with the stirrer. The fiber was added to the dough at various levels during the mixing process. The dough was then transferred to a large container and was rested for 1-2 hours, at 25 ± 2 °C to complete fermentation. It was then baked in the oven for 4 min at about 400 °C. To convert direct heat to indirect heat, a wall of fireproof brick at an approximate height of 50 cm was installed in front of the direct furnace flame.

2.2.8. Bread packaging

The bread was cooled to 20 °C and transferred to the laboratory for sensory and mechanical analysis. The bread was cut into 15 × 15 cm with a sterilized knife and packaged in two kinds of package form including silicon Nano-polymer film and polyethylene film with a thickness of 100 μm and dimensions of 25 × 35 cm using a special stitching machine (Dookht Plast). The samples were kept at 6 °C (refrigerator) and 20 °C (ambient) until the mold was observed.

2.2.9. Determination of mechanical properties of bread texture

Tensile test is a destructive test in which a sample is studied under uniaxial tension until failure. This test was determined using a food texture analyzer (Zwick/Roell model BT1_FR0.5TH.D14 load cell x force hp) made in Germany with a capacity of 500 N according to standard ASTM 882-02 (2002). (Fig. 2). For this test, two fixed and movable jaws with adjustable jaw opening were used. The distance between the two jaws was calibrated at 50 mm at a speed of 25 mm/min. First, bread was cut (3 x 10 cm). Then its thickness was measured by caliper and recorded in machine software. When the machine started working, the specimen was stretched and the textural features such as maximum force required to tear the bread, breaking force (Fbreak), the amount of rupture energy were plotted by machine software (Test Xpert), in the form of force (N) - displacement (mm) curve (Fig. 2).

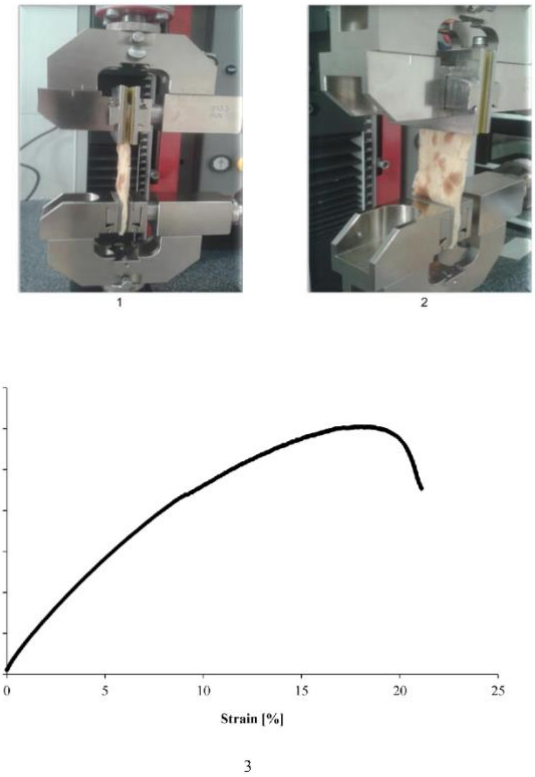


Figure 2. Material Texture Machine, tensile test. 1- Front view, 2- Side view. 3- An example of a food texture analyzer graph

2.2.10. Sensory evaluation of bread

The aroma, taste, color, chewiness, and acceptability of the produced bread sample and the control sample were determined by the sensory evaluator group, using the Consumer Desire Test (5-point hedonic test). The samples were evaluated by eight students of Bu Ali University who were familiar with the principles of sensory evaluation. Enriched and control bread was prepared and given to the evaluators with a special form. The evaluators completed the forms according to their tastes. For this purpose, the scores of 5 and 1 were assigned for the high-quality and low-quality types of bread, respectively.

2.2.11. Experimental design and statistical analysis

In this study, all stages and experiments were performed in three replications. The results of the experiments were analyzed by factorial test in a completely randomized design after normalization using SPSS 23 software. The mean treatments were compared with Duncan's

method at a 95% confidence level. EXCEL 2016 software was used to draw charts.

3.Results and discussions

3.1. Chemical analysis

Table 1 shows the amounts of protein, fat, ash, moisture, fiber, and carbohydrates of wheat flour and carrot pomace powder as follows. According to Table 1, the protein content of the flour is 78.8%. Protein content increased by 11.87% by adding carrot pomace powder at the level of 3% (w/w based on the flour). Scanlon et al. (2000) in their study on the textural features of bread made from weak and strong flour, showed that increasing the quantity and quality of protein in flours, made the dough stronger. The protein content of carrot pomace powder was 9.62%. Kohajdová et al. (2012) reported the protein content of carrot pomace powder 6.86%, and Chau et al. (2004) reported it 8.44%. According to the results, flour fat content was 0.83%, which was increased to 0.85% by the addition of carrot pomace powder, which was not a significant increase. According to National Standard No. 11136, the amount of flour fat was reported to be 1.2%. Flour ash content is significantly higher than carrot pomace powder. Flour ash was 5.55%, but carrot pomace powder was 1.15%, indicating higher mineral content in flour. This rate for enriched flour was 4.13%. Bread is high in carbohydrates, and carbohydrates are major contributors to obesity and overweight (Nandini et al., 2001). According to Table (1), carbohydrate content is 83.13% for flour and 86.73% for carrot pomace powder. But for enriched flour with carrot pomace powder, the carbohydrate content is 68.58%. Thus, the effect of carrot pomace powder is significant because it reduces the carbohydrate content, which is a major contributor to overweight in bread consumption. Kim et al. (2012) investigated the physical and chemical properties of cactus-enriched sponge cake. Their results showed that with the addition of cactus fiber, the amount of carbohydrates decreased, which was consistent with the results of this study. Reducing the moisture content of flour and carrot pomace powder means keeping them in the right place. According to the results, the moisture content of flour was 2.61%, which was reduced to 2.53% by the addition of carrot pomace powder. So, the carrot pomace powder has been able to reduce the moisture content of the flour.

When grinding wheat grains, a large percentage of the fiber in the wheat shell is removed. By decreasing extraction rate, the amount of minerals and fibers in flour decreases and subsequently, the nutritional value of flour is reduced. According to Table 1, with the addition of carrot pomace powder to wheat flour at a ratio of 1:3, the total fiber content also increased, so that the total fiber content of flour increased from 1.49% to 6.74%. Ateş and Elmacı (2018) showed that by adding coffee to one kind of cupcake formulation the fiber content increased, which was consistent with the results of this study. In a study conducted by Hussein et al. (2013), it was reported that the ash and crude fiber contents of the baked pan bread increased with higher levels of carrot powder (CP). The authors suggested that this increase could be linked to the elevated ash and fiber contents present in CP when compared to fine wheat flour. These results align with previous studies by Doweidar (2001), Gopulan et al. (1991), and Chantaro et al. (2008) (Body text TNR 12 normal, ident first line 0.66 cm, line spacing Single).

Table 1. Chemical analysis of wheat flour and carrot pomace powder

Treatment	Moisture (%)	Fiber (%)	Carbohydrate (%)	Ash (%)	Fat (%)	Protein (%)
Flour	2.61 ± 0.04	1.49 ± 0.03	83.18 ± 0.01	5.55 ± 0.06	0.83 ± 0.04	7.87 ± 0.09
CPP	2.47 ± 0.09	11.05 ± 0.04	86.73 ± 0.05	1.15 ± 0.07	0.24 ± 0.03	9.62 ± 0.07

3.2. The evaluation results of the dough texture

3.2.1. Gumminess

Gumminess is the amount of force needed to break down a semi-solid product into a form that is ready to be eaten (Mousavi et al., 2019). According to Table 2, the results for the gumminess of enriched dough show that by adding carrot pomace powder to the dough, the dough gumminess has increased from 1.87 N/mm2 to 5.57 N/mm2. Meanwhile, the dough with 3% carrot pomace powder has changed less than the control sample, but doughs with 5% and 7% carrot pomace powder have high gumminess which, has increased the volume of the dough. The Maillard reaction, which requires low activation energy, produces carbon dioxide, which can contribute to increasing the volume of the dough. Dietary fiber plays a significant role in influencing bread dough gumminess. Research indicates that incorporating dietary fiber into dough alters its rheological properties, impacting bread quality. Fiber can disrupt the gluten network, delay gluten hydration, and compete for water molecules in the dough (He, 2023; Lu et al., 2018), potentially leading to increased bread hardness and gumminess (Shiau et al., 2015). Moreover, dietary fibers, such as insoluble arabin oxylans, can increase water absorption during bread making, further affecting dough gumminess (Iraqui et al., 2013). The interaction between fiber and gluten influences dough rheology and bread quality (Liu et al., 2017), with fiber addition potentially causing bread hardening due to gluten dilution (Sivam et al., 2010). The results of this study is in agreement with those of Majzoobi et al. (2016) and Lebesi and Tzia (2011). Monthe et al. (2019), in a study on the rheological properties of gluten-free bread and its enrichment with sorghum and potatoes, showed that increased gumminess was a negative parameter in bread preparation, which was consistent with our results.

3.2.2. Elasticity

Elasticity refers to how quickly a material, like food, returns to its original shape after being deformed (Garrido et al., 2015). The results show that adding carrot pomace powder to Sangak dough reduces its elasticity. Specifically, the addition of carrot pulp powder decreased the elasticity from 0.66 N in the control sample to 0.60 N in the sample at the 7% level (Table 2). The incorporation of dietary fibers into bread formulations has been found to reduce the elasticity of bread while enhancing its mechanical properties (Culeţu et al., 2020). In a study by Wu and Shiau (2015), it was reported that adding pineapple peel fiber (PPF) at levels of 0%, 5%, 10%, and 15% with particle sizes of 250-420 micrometers resulted in a decrease in the elasticity of the dough, which was attributed to the increase in PPF particle size in the dough.

3.2.3. Cohesiveness

Cohesiveness refers to the internal strength of food structures and the capacity to bind together the components of a product (Nourmohammadi et al., 2021). The results show that the addition of carrot pomace powder decreases the cohesiveness of the dough compared to the control sample and a significant decrease is observed in the levels. The highest and lowest amount of cohesion were observed in the control dough (0.94 N) and in the enriched dough at the level of 7% (0.77 N), respectively. Among the levels, the level of 3% with the cohesion of 0.88 N has better cohesion in the dough structure than other levels (Table 2). In the study by Wu and Shiau (2015), the effect of pineapple peel fiber (PPF) on the cohesiveness of bread dough was also investigated. It was reported that increasing the level of PPF in the dough led to a decrease in cohesiveness. Specifically, the cohesiveness decreased from 0.75 N in the control sample to 0.68 N, 0.62 N, and 0.58 N in samples with PPF levels of 5%, 10%, and 15%, respectively. This decrease in cohesiveness was attributed to the presence of PPF particles in the dough, which may have disrupted the structure and cohesiveness of the dough.

3.2.4. Chewiness

The chewiness is a property of being chewy. Dietary fiber, particularly insoluble fiber, can increase the water-holding capacity of the

dough, leading to improved texture and chewiness. Additionally, dietary fiber can also strengthen the gluten network in the dough, resulting in a more elastic and chewier crumb. There was no significant difference between the level of 3% (2.73 N) and the control sample (1.87 N). But among the levels, the level of 7% (5.53 N) had the highest rate of chewiness (Table 2). Gómez et al. (2010) found that by replacing wheat bran percentages and increasing fiber, the dough flow behavior index decreased. The high fiber content and a large number of hydroxyl groups present in the fiber structure, react with water molecules through hydrogen bonds, and by absorbing the water present in the dough formulation, increases the chewiness and reduces the flow behavior of the dough (Horstmann et al., 2018). Studies have demonstrated that incorporating fiber-rich ingredients such as whole grains, bran, or seeds into bread formulations can enhance the chewiness of the final product. However, the type and amount of fiber added can also influence the overall texture and mouthfeel of the bread.

Regarding the texture parameters of the dough, the results showed that the level of 3% had the best performance compared to the other levels for baking Sangak bread. Thus, this level was used for baking Sangak bread and packing it with films.

Table 2. The results of the comparison table of average evaluation of dough texture

Variations sources	Cohesiveness	Chewiness	Gumminess	Springiness
Dough control	$0.94^{a} \pm 0.02$	$1.87^{b} \pm 0.02$	$1.87^{c} \pm 0.01$	$0.66^{a} \pm 0.06$
Enriched dough 3%	$0.88^{ab} \pm 0.03$	$2.73^{b} \pm 0.03$	$2.73^{b} \pm 0.04$	$0.63^{a} \pm 0.03$
Enriched dough 5%	$0.81^{ab} \pm 0.05$	$5.72^{a} \pm 0.07$	$5.53^{a} \pm 0.03$	$0.61^{a} \pm 0.09$
Enriched dough 7%	$0.77^{b} \pm 0.01$	$5.53^{a} \pm 0.08$	$5.57^{a} \pm 0.06$	$0.60^{a} \pm 0.08$

3.2.5. Investigation of textural changes in bread by tensile test during storage at 6°C and 20 °C

In spite of the common use of the compressive force and the penetration test to examine the texture of bread, there have been few studies on the use of the tensile test for this purpose. The most important reason for not using this method is the difficulty of obtaining a sample with a uniform geometric shape (Pavinee et al., 2018). After preparing the sample by geometric method, the parameters of the maximum tensile force (Fmax) and maximum breaking force (Fbreak) were measured. According to the results in (Table3), the interaction effect of bread × day × film × temperature is significant at the 0.05 level for maximum tensile force (Fmax), Fbreak, and tensile strength. Changes in Fmax, Fbreak, and tensile strength of enriched and control bread samples after 6 days showed an increasing trend, but enriched bread samples exhibited less variation compared to control samples. The reason for the increased firmness of enriched stone bread on the first day compared to control bread is the fiber property present in carrot pomace powder (Kumar et al., 2011). Moisture absorption by fiber prevented mold spoilage in bread but led to initial firmness compared to the control sample. These results are consistent with the study by Sharoba et al. (2013), where using carrot pomace powder in cake increased the firmness of the cake structure due to the water and oil absorption ability of the fibers in carrot pomace powder. Moreover, Majzoobi et al. (2016) reported an increase in cake structure firmness by using soy isolate in the cake. Nikzadeh et al. (1390) examined the effect of adding two different types of sourdough on the rheological properties and sensory evaluation of

bread. Tensile test results showed that the required force for stretching oat-containing bread dough decreased after 48 hours but increased after 72 hours.

Table 3. Variance analysis table of the effects of day, bread, film, atmosphere and temperature on the stretch ability parameters of Sangak bread

Variations sources	Degrees of freedom	F_{max} (N)	F_{Break} (N)
Day (A)	2	1.64^{**}	1.47^{**}
Bread (B)	1	5.64^{**}	4.07^{**}
Film (C)	1	0.00^{ns}	0.00^{ns}
Temperature (D)	1	4.00^{**}	2.92^{**}
A×B	2	1.01^{**}	0.80^{*}
A×C	2	2.21^{ns}	0.32^{*}
C×D	1	0.66^{ns}	0.33^{*}
A×B×D	2	0.77^{*}	0.45^{*}
A×C×D	2	1.24^{**}	0.76^{**}
A×B×C×D	2	0.64^{*}	0.49^{*}
Error	96	0.16	0.11

Notes: * Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$, and ns not significant.

3.2.6. Sensory analysis results

The results showed that the addition of carrot pomace powder decreased satisfaction with the taste of enriched bread. Enriched Sangak sample at a 3% level, was not significantly different from the control sample, and the addition of carrot pomace powder did not change bread taste. The worst taste was related to the enriched bread at the level of 7% with a score of 1.37, and the best taste was related to the enriched bread at the level of 3% with a score of 3.75 (Fig. 3).

According to the sensory test results, the enriched Sangak bread at the level of 3% had the highest score in terms of aroma, and the enriched bread at the level of 7% had the lowest score compared to the control sample.

Color is one of the most important sensory properties of the food, and the consumer first evaluates the quality of the food by this property. Color is one of the best quality attributes of food and at the same time, it conveys the texture and taste of the product. According to the sensory analysis of bread, it was found that adding carrot pomace powder had a significant effect on the color of bread. This was due to the presence of carotenoids in the bread. Enriched bread at the level of 7%, with a score of 4.61, had the highest score among the other levels compared to the control sample.

Chewing is a mechanical aspect of eating and refers to crushing the food between teeth. According to Figure 3, with the addition of carrot pomace powder, the chewiness of the bread was reduced, so that according to the reports of evaluators, the enriched bread had a stiffer texture than the control sample. The results also showed that the control sample with a score of 3.66 was the best one in terms of chewiness. The worst score was related to the enriched bread at the level of 7% with a score of 1.5. Also, according to the mean comparison, enriched bread at the level of 3% was not significantly different from the control sample.

In terms of overall acceptability, two kinds of enriched bread (levels of 3% and 7%) with the total scores of 4.32 and 2.89, respectively, were the best and the worst kinds of bread compared to the control sample. Kohajuvada et al. (2012), found that using carrot pulp powder (up to 10%), increased the acceptability of the product, but at higher levels, due to negative effects on color, aroma, texture, and taste, the overall acceptability of the product was reduced. Sharuba et al. (2013) also reported a decline in cake acceptability by adding carrot pomace at high levels. Turksoy and Ozkaya (2011) also reported that the use of carrot pomace powder by more than 15% in a kind of pancake decreased its acceptability by the evaluator group.

According to the rheology of the dough and the overall acceptability of the bread by the evaluators, the enriched bread at the level of 3%, was selected for packaging.

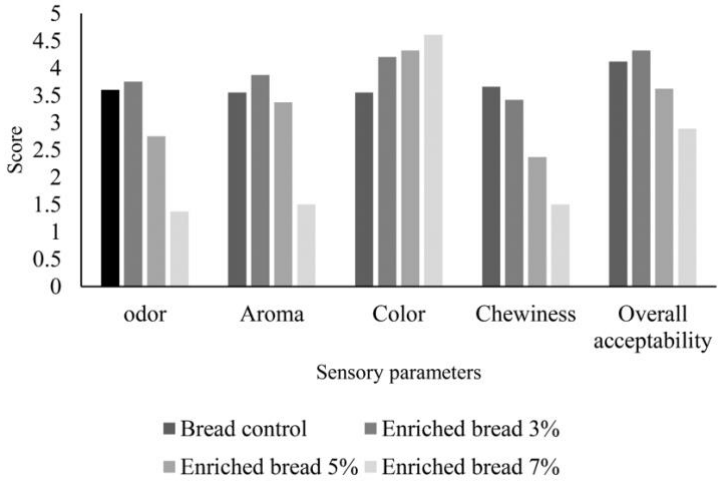


Figure 3. Sensory analysis chart

4. Conclusions

The overall results showed that with the addition of carrot pomace to flour, the protein, fat, and fiber contents increased but the carbohydrate content decreased. Thus, the carrot pomace powder had a significant effect on Sangak bread. The texture indices of the dough showed that the addition of carrot pomace powder decreased the cohesion, chewiness, and elasticity of the dough but increased its adhesion. The second step of this study was to investigate the sensory analysis of enriched bread at three levels of 3%, 5%, and 7% compared to the control sample. These results showed that the addition of carrot pomace decreased the chewiness of bread, but increased the color of enriched bread compared to the control sample. Evaluators found the taste, aroma, and overall acceptability of the enriched bread at the level of 3% better than the control sample. According to the results of texture and sensory analyses, the dough at the level of 3% had the best performance compared to the control sample and was therefore selected for baking and packaging Sangak bread. The third stage of the study was to determine the mechanical properties of enriched and control Sangak bread at 6 and 20 °C with two types of silicon Nano-polymer film and light polyethylene film. The texture results of the tensile test showed that the addition of carrot pomace to the bread reduced the staling process of bread, but the enriched bread had more stiff texture on the first day than the control sample. Investigation of two types of films shows that silicon Nano-polymer film acts better than polyethylene film in increasing the shelf life of the Sangak bread. Finally, it is worth noting that extracting carrot pomace fiber and its use in bread industry products, considering its economical and high nutritional value, can be a good option.

5.References

AACC. 2000. Approved methods of American Association of Cereal Chemists (10th Ed.). USA: The Association. St. Paul, MN.

Ateş, G., and Elmacı, Y. (2018). Physical, chemical and sensory characteristics of fiber-enriched cakes prepared with coffee silverskin as wheat flour substitution. Journal of Food Measurement and Characterization, 1-9.

Avital, Y., Mannheim, C. H., Miltz, J. (1990): Effect of carbon dioxide atmosphere on staling and water relations in bread. Journal of Food Science. 55(2), 413-416.

Chantaro, P., Devahastin, S., & Chiewchan, N. (2008). Production of antioxidant high dietary fiber powder from carrot peels.

LWT-Food Science and Technology, 41(10), 1987-1994.

Chau, C. F., Chen, C. H., & Lee, M. H. (2004). Comparison of the characteristics, functional properties, and in vitro hypoglycemic effects of various carrot insoluble fiber-rich fractions. LWT-Food Science and Technology, 37(2), 155-160.

Doweidar, M. M. (2001). Chemical and physical studies on some natural resources used in improving bakery products (Doctoral dissertation, Ph. D. Thesis, Fac. of Agric, Cairo Univ., 2001, Egypt).

Duţă, D. E., Culeţu, A., & Belc, N. (2021). Fortification of bread with wheat processing by-products. Trends in Wheat and Bread Making, 343-369.

Fu, J. T., Chang, Y. H., & Shiau, S. Y. (2015). Rheological, antioxidative and sensory properties of dough and Mantou (steamed bread) enriched with lemon fiber. LWT-Food Science and Technology, 61(1), 56-62.

Garrido, J. I., Lozano, J. E., & Genovese, D. B. (2015). Effect of formulation variables on rheology, texture, colour, and acceptability of apple jelly: Modelling and optimization. LWT-Food Science and Technology, 62(1), 325-332.

Gómez, M., Manchón, L., Oliete, B., Ruiz, E., and Caballero, P. A. (2010). Adequacy of wholegrain non-wheat flours for layer cake elaboration. LWT-Food Science and Technology, 43(3), 507-513

He, N., Pan, Z., Li, L., Zhang, X., Yuan, Y., Yang, Y., ... & Li, B. (2023). Improving the microstructural and rheological properties of frozen unfermented wheat dough with laccase and ferulic acid. Foods, 12(14), 2772.

He, X. (2023). Impact of dietary fiber on bread dough rheology. Journal of Food Science, 45(2), 123-135.

Hernández-Ortega, M., Kissangou, G., Necoechea-Mondragón, H., Sánchez-Pardo, M. E., and Ortiz-Moreno, A. (2013). Microwave dried carrot pomace as a source of fiber and carotenoids. Food and Nutrition Sciences, 4(10), 1037.

Horstmann, S. W., Axel, C., and Arendt, E. K. (2018). Water absorption as a prediction tool for the application of hydrocolloids in potato starch-based bread. Food Hydrocolloids, 81, 129-138.

Hussein, M. A., Yonis, A. A. M., & El-Mageed, A. (2013). Effect of adding carrot powder on the rheological and sensory properties of pan bread. Journal of Food and Dairy Sciences, 4(6), 281-289.

Iraqui, M. M., Ali, N. A., & Barakat, A. S. (2013). effect of particles size of some cereal brans on the quality of high fiber Balday bread. Egyptian Journal of Agricultural Sciences, 64(1), 30-36.

Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P., and Autio, K. (2006). Effects of sourdough and enzymes on staling of high-fibre wheat bread. LWT-Food Science and Technology, 39(5), 479-491.

Khoshakhlagh, K., Hamdami, N., Shahedi, M., and Le-bail, A. (2014). Heat and mass transfer modeling during storage of part-baked Sangak traditional flat bread in MAP. Journal of Food Engineering, 140, 52-59.

Kim, J. H., Lee, H. J., Lee, H. S., Lim, E. J., Imm, J. Y., & Suh, H. J. (2012). Physical and sensory characteristics of fibre-enriched sponge cakes made with Opuntia humifusa. LWT-Food Science and Technology, 47(2), 478-484.

Kohajdová, Z., Karovičová, J., and Jurasová, M. (2012). Influence of carrot pomace powder on the rheological characteristics of wheat flour dough and on wheat rolls quality. Acta Scientiarum Polonorum. Technologia Alimentaria, 11(4).

Kumar, N., Sarkar, B. C., and Sharma, H. K. (2011). Effect of air velocity on kinetics of thin layer carrot pomace drying. Food Science and Technology International, 17(5), 459-469.

Lebesi, D. M., and Tzia, C. (2011). Effect of the addition of different dietary fiber and edible cereal bran sources on the baking and sensory characteristics of cupcakes. Food and Bioprocess Technology, 4(5), 710-722.

Lu, X., Brennan, M. A., Serventi, L., & Brennan, C. S. (2018). Incorporation of mushroom powder into bread dough—effects on dough rheology and bread properties. Cereal Chemistry, 95(3), 418-427.

Majzoobi, M., Poor, Z. V., Jamalian, J., and Farahnaky, A. (2016). Improvement of the quality of gluten-free sponge cake using different levels and particle sizes of carrot pomace powder. International Journal of Food Science & Technology, 51(6), 1369-1377.

Mohammadi, I. M. (2007). Factors influencing wheat, flour, and bread waste in Iran. Journal of New Seeds, 8(4), 67-78.

Monthe, O. C., Grosmaire, L., Nguimbou, R. M., Dahdouh, L., Ricci, J., Tran, T., and Ndjouenkeu, R. (2019). Rheological and textural properties of gluten-free doughs and breads based on fermented cassava, sweet potato and sorghum mixed flours LWT-Food Science and Technology, 101, 575-582.

Mousavi, M., Heshmati, A., Garmakhany, A. D., Vahidinia, A., & Taheri, M. (2019). Optimization of the viability of Lactobacillus acidophilus and physico-chemical, textural and sensorial characteristics of flaxseed-enriched stirred probiotic yogurt by using response surface methodology. LWT-Food Science and Technology, 102, 80-88.

Nawirska, A., and Kwaśniewska, M. (2005). Dietary fibre fractions from fruit and vegetable processing waste. Food Chemistry, 91(2), 221-225.

Nourmohammadi, A., Ahmadi, E., & Heshmati, A. (2021). Optimization of physicochemical, textural, and rheological properties of sour cherry jam containing stevioside by using response surface methodology. Food Science & Nutrition, 9(5), 2483-2496.

Pavinee, C., Elena, V., Mooyeol, B., and Yael, V. (2018). Instrumental Techniques Used in Bread Staling Analysis. In Bread Staling (pp. 93-112). CRC Press.

Salehi, F., Kashaninejad, M., Akbari, E., Sobhani, S. M., and Asadi, F. (2016). Potential of sponge cakemaking using infrared–hot air dried carrot. Journal of Texture Studies, 47(1), 34-39.

Saura-Calixto, F. (1998). Antioxidant dietary fiber product: a new concept and a potential food ingredient. Journal of Agricultural and Food Chemistry, 46(10), 4303-4306.

Scanlon, M. G., Sapirstein, H. D., and Fahloul, D. (2000). Mechanical properties of bread crumb prepared from flours of different dough strength. Journal of Cereal Science, 32(3), 235-243.

Sharoba, A. M., Farrag, M. A., and Abd El-Salam, A. M. (2013). Utilization of some fruits and vegetables waste as a source of dietary fiber and its effect on the cake making and its quality attributes. Journal of Agroalimentary Processes and Technologies, 19(4), 429-444.

Sivam, A. S., Sun-Waterhouse, D., Quek, S., & Perera, C. O. (2010). Properties of bread dough with added fiber polysaccharides and phenolic antioxidants: A review. Journal of food science, 75(8), R163-R174.

Tebben, L., Shen, Y., & Li, Y. (2018). Improvers and functional ingredients in whole wheat bread: A review of their effects on dough properties and bread quality. Trends in Food Science & Technology, 81, 10-24.

Turfani, V., Narducci, V., Durazzo, A., Galli, V., and Carcea, M. (2017). Technological, nutritional and functional properties of wheat bread enriched with lentil or carob flours. LWT-Food Science and Technology, 78, 361-366.

Turksoy, S., and Özkaya, B. (2011). Pumpkin and carrot pomace powders as a source of dietary fiber and their effects on the mixing properties of wheat flour dough and cookie quality. Food Science and Technology Research, 17(6), 545-553

Wang, J., Rosell, C. M., etal. (2002), Effect of the addition of different fibres on wheat dough performance and bread quality. Food Chemistry, 79:221-226.

Wu, M. Y., & Shiau, S. Y. (2015). Effect of the amount and particle size of pineapple peel fiber on dough rheology and steamed bread quality. Journal of Food Processing and Preservation, 39(6), 549-558.

Xu, J., Li, Y., Zhao, Y., Wang, D., & Wang, W. (2021). Influence of antioxidant dietary fiber on dough properties and bread qualities: A review. Journal of Functional Foods, 80, 104434.

Acknowledgment

Bu-Ali Sina University is appreciated for supporting this research in terms of supplying laboratory materials and equipment.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

MATHEMATICAL MODEL STUDY TO OPTIMIZE THE FREEZE DRYING PROCESS FOR PRODUCTION OF DRIED YOGURT

Hoang Van Chuyen[1], Tam Le Minh[1], Quoi Phung Phu[1], Liviu Giurgiulescu[2], Dzung Tan Nguyen[1]✉

[1]Department of Food Technology, Faculty of Chemical and Food Technology, HCMC University of Technology and Education, No 01-Vo Van Ngan Street, Thu Duc City, Ho Chi Minh City, Viet Nam.
[2]Chemistry-Biology Department, Technical University of Cluj Napoca, North Universitary Center of Baia Mare, Romania,
✉tandzung072@hcmute.edu.vn

https://doi.org/10.34302/crpjfst/2024.16.4.12

Article history:
Received
February 7th, 2024
Accepted
October 28th, 2024

Keywords:
Freeze-drying;
Yogurt;
Optimization;
Quality;
Energy consumption.

ABSTRACT

The aim of this study was to build mathematical models for optimizing a technological process producing a freeze-dried yogurt product with good quality based on solving multi-objective optimization problems. The application of Utopia Point Method for the optimization process determined the optimal freeze-drying conditions including drying temperature of 36.6°C, drying pressure of 0.023 mmHg and drying time of 35.6 hours. The optimal drying process resulted in the freeze-dried yogurt product with a moisture content of 0.963%, a crispiness of 15.953 mN and 69.291% of viable beneficial microorganisms were preserved. In addition to the good quality criteria of the dried product, the drying process also consumed only 19.94 kWh of electrical energy to produce 1 kg of product, which suggests the high production applicability of the developed freeze-drying process.

1. Introduction

Yogurt is usually produced as semisolid food products, which derived from processed animal milks under appropriate fermentation conditions with the involvement of Streptococcus salivarius ssp thermophilus and Lactobacillus delbrueckii ssp bulgaricus bacteria (Bamforth C. W. and Cook D. J., 2019). The combination of nutritional components and live microorganisms forms the basis for a highly nutritious food that can easily complement a healthy dietary regimen (German J. B., 2014). Yogurt possesses a diverse and balanced chemical composition, including carbohydrates, proteins, lipids, minerals, and various vitamins. Lactose constitutes approximately 98% of the carbohydrates and 54% of the total solids in non-fat yogurt, along with small amounts of galactose, glucose, and oligosaccharides (Yildiz F., 2010). The protein content of yogurt is improved compared to raw milk, making yogurt a rich source of biologically active and plentiful protein, providing all essential amino acids and containing growth factors and precursors for bioactive peptides (Yildiz F., 2010). The fat content of yogurt primarily consists of triglycerides, accounting for about 98%, with the remainder comprising phospholipids, cholesterol, and β-carotene. Traditional whole-milk yogurt contains 3 to 4 g of lipids per 100 g, of which 65% are saturated fatty acids. The remaining portion consists of 31% monounsaturated fatty acids and 4% polyunsaturated fatty acids (Marette A., Picard-Deland É., and Fernandez M., 2017). The mineral composition of yogurt and dairy products includes both major elements (Ca, Mg, Na, K, P, and Cl) and trace elements (Fe, Cu, Zn, and Se) (Marette A., Picard-Deland É., and Fernandez M., 2017). Both fat-soluble and water-soluble vitamins are present in milk and yogurt. Full cream yogurt may contain significant amounts of vitamin A, B-complex vitamins, and vitamin D (Marette A., Picard-

Deland É., and Fernandez M., 2017). Additionally, yogurt is a rich source of vitamin B12 (Karmi O., Zayed A., Baraghethi S., Qadi M., and Ghanem R., 2011).

Freeze-drying is a process used to remove water from products by sublimation. The freeze-drying process involves three stages: 1) freezing the raw material, 2) primary drying, and 3) secondary drying (G.Wilhelm. Oetjen and Peter. Haseley., 2004; Bhushani A. and Anandharamakrishnan C., 2017; Dzung N.T, Chuyen H.V, Linh V.T.K, and et al., 2022). Freeze-drying is the most complex method of water removal and finds application primarily in the production of high-value food products. Currently, freeze-drying technology is being used to produce various food products such as instant coffee, tea, meat, herbs, and high quality fruits and vegetables (Dzung N.T, Chuyen H.V, Linh V.T.K, and et al., 2022; Anandharamakrishnan C., 2017). Freeze drying help preserving high quality of food products that are challenging to achieve with other drying methods. Another outstanding feature of this drying method is the structural stability of the product, preventing the collapse of the solid matrix after drying. As a result, a porous, non-caking product is obtained, facilitating rapid rehydration when water is added to the substance thereafter (Athanasios I. Liapis and Roberto Bruttini, 2020). Freeze drying can prevent the denaturation of whey proteins and the Maillard reaction between lactose and protein in milk. In the dairy industry, freeze-drying is mainly employed to preserve original strains of cultures and probiotic microorganisms for use as functional ingredients (Anandharamakrishnan C., 2017). The biological values of freeze-dried bio products endow them with robust survivability, providing an advantage in developing functional dairy components (Fellows P., 2000; Dzung N.T, Chuyen H.V, Linh V.T.K, and et al., 2022).

2. Materials and methods

2.1. Raw material

Yogurt was prepared using ingredients include full cream milk powder (210g), sugar (37g), starter culture (3g) and water (750g). Prior to fermentation, yogurt samples were standardized to achieve a consistent dry matter content of 25%.

2.2. Equipment

The main equipment used in this study is the DS-12 Freeze Drying System, which was designed, and fabricated by the research team of Associate Professor Dr. Dzung N.T from the Department of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education, Vietnam.

Figure 2. The DS-12 Freeze Drying System

2.3. Methods

2.3.1. Determination of factors affecting the freeze-drying process

In this study, the factors influencing the yogurt freeze-drying process including the drying temperature (Z_1, °C), the drying pressure (Z_2, mmHg), and the drying time (Z_3, hours) were investigated. These parameters were measured and controlled using temperature sensors, pressure gauges, and time counters integrated within the DS-12 freeze-drying system.

2.3.2. Determination of output responses

- Energy consumption: The energy consumption per unit mass (Y_1, kWh/kg dried product) was calculated using a wattmeter (Dzung N.T and Phuong V.D., 2016). The formula for calculating the energy consumption is as follows:

$$Y_1 = \frac{P.\tau}{G} = \frac{(U.I.\cos\varphi).\tau}{G}, \text{(kWh/kg)} \quad (1)$$

Where: U represents the Voltmeter reading (V); I represents the Ammeter reading (A); τ stands for time in seconds (h); cosφ denotes the power factor; P indicates the value reading on the Wattmeter (kW), G represents the weight of the material.

- Moisture content of the product: The moisture content of the product (Y_2) was determined using the convective drying method in a drying oven, as described in AOAC – 927.05. Accurately weighed 5g of finely ground sample was placed in a clean, dry, pre-weighed aluminum dish. The sample was then subjected to the drying cabinet at a temperature of 105 °C until a constant mass was achieved (AOAC International, 2000). The moisture content of the product was determined using the following formula:

$$Y_2 = 100 - \frac{G_i}{G_e}(100 - W_i), (\%) \quad (2)$$

Where: G_i represents the initial mass of yogurt before drying (g); G_e represents the mass of yogurt after drying (g); W_i represents the initial moisture content of yogurt (%).

- Texture analysis of the product: The texture of freeze-dried yogurt (Y_3) was measured based on the structural deformation obtained using the Brookfield CT3 Texture Analyzer equipped with a TA-SBS cylindrical probe. For all measurements, the sample thickness is set to 14 mm. The following parameters are configured: single sample test, probe speed of 1 mm/s, and target distance of 25 mm. The crispiness of a sample was defined as the maximum pressing force (mN) to cause the structural deformation of the sample. The lower pressing force represents better crispiness.

- Preservation of microorganisms: The preservation of microorganisms was determined by measuring the remaining proportion of lactic acid bacteria after each freeze-drying experiment. The total number of lactic acid bacteria in both yogurt samples (before and after drying) was determined using the method described in the ISO 15214:1998 and following the description by TCVN 7906:2008. The total number of lactic acid bacteria was determined using the following formula:

$$L = \frac{\sum C}{V.\left(n_1 + \frac{1}{10}n_2\right)d} \quad (3)$$

Where: $\sum C$ represents the total count of viable lactic acid bacteria counted on all plates with at least one plate containing a minimum of 15 lactic acid bacteria colonies; V denotes the volume of the diluted sample plated on each plate, measured in milliliters; n_1 is the number of plates retained in the first dilution step; n_2 is the number of plates retained in the second dilution step; d is the dilution factor corresponding to the plates retained in the first dilution step.

The survival rate of microorganisms, expressed as the percentage of viable lactic acid bacteria (Y_4 %), is determined using the following formula:

$$Y_4 = \frac{L_i}{L_e} \times 100, \ (\%) \quad (3)$$

Where: L_i represents the total number of lactic acid bacteria in 1g of yogurt before freeze drying; L_e represents the total number of lactic acid bacteria in the corresponding amount of 1g of yogurt before drying.

2.3.3. Experimental Design Method

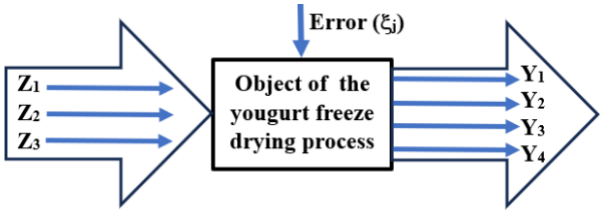


Figure 1. Black Box model for experimental design method

The objective functions of the freeze-dried yogurt product in this study are Y_1, Y_2, Y_3, and Y_4, which are closely related to the technological factors Z_1, Z_2, and Z_3.

A second-order orthogonal experimental design model was constructed with k = 3. The

variables x_1, x_2, x_3 represent the coded variables of Z_1, Z_2, and Z_3, respectively. The experimental mathematical model designed as a second-order orthogonal matrix is described as the following equation:

$$Y_j = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{u\neq i;u=1}^{k} b_{ui} x_u x_i + \sum_{i=1}^{k} b_{ii}\left(x_i^2 - \lambda\right) (5)$$

With i, u = 1 ÷ k; k =3; j = 1 ÷ 4

These variables x_1, x_2, x_3 were coded by variables of Z_1, Z_2, Z_3 presented as follow:

$$x_i = \frac{Z_i - Z_i^0}{\Delta Z_i};\ Z_i = x_i.\Delta Z_i + Z_i^0 \quad (6)$$

Where:

$Z_i^0 = (Z_i^{max} + Z_i^{min})/2;$

$\Delta Z_i = (Z_i^{max} - Z_i^{min})/2;$ (7)

$Z_i^{min} \le Z_i \le Z_i^{max};\ i = 1 \text{ to } 3$ (8)

The experimental design model consists of a number of experiments:

$$N = n_k + n_* + n_0 = 2^k + 2k + n_0 = 18 \quad (9)$$

Where: k = 3; n_0 = 4

α value is calculated as:

$$\alpha = \sqrt{\sqrt{N.2^{(k-2)}} - 2^{(k-1)}} = \sqrt{\sqrt{18.2^{(3-2)}} - 2^{(3-1)}} = 1.414 \quad (10)$$

Conditions for obtaining an orthogonal matrix:

$$\lambda = \frac{1}{N}\left(2^k + 2\alpha^2\right) = \frac{1}{18}\left(2^3 + 2.\sqrt{2}^2\right) = \frac{2}{3} \quad (11)$$

2.3.4. Optimization method

- Single-objective optimization problem: Considering the yogurt freeze drying process as the technological object, the objective functions of interest are $Y_j = f_j(Z) = f_j(x)$, which depend on the technological factors Z_1, Z_2, and Z_3 those were coded as x_1, x_2 and x_3. These factors form a vector of influencing variables, also known as the variable vector $Z = \{Z_i\} = (Z_1, Z_2, Z_3)$, corresponding to $x = \{x_i\} = (x_1, x_2, x_3)$, where i = 1 ÷ 3. These variables vary within the defined domain Ω_x, and the values of the objective function $f_j(x)$ constitute the value domain Ω_f (Dzung N.T and Hai D.T. H., 2016). Hence, the single-objective optimization problem can be established as follows:

Find the optimal solution $x^{jopt} = (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) \in \Omega_x$:

$$\begin{cases} Y_j = f_{jmin}\left(x_1^{jopt}, x_2^{jopt}, x_3^{jopt}\right) = Min\left\{f_j\left(x_1, x_2, x_3\right)\right\} \\ j = 1 \div 4; \\ \forall x \in \Omega_x = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\}; \end{cases} \quad (12)$$

- Multi-objective optimization problem: For the yogurt freeze drying process as the technological object, the technological factors $Z = (Z_1, Z_2, Z_3) \in \Omega_z$, those were coded as $x = (x_1, x_2, x_3)$ simultaneously influence multiple objective functions: $f_1(x)$, $f_2(x)$, $f_3(x)$, $f_4(x)$. Therefore, it is necessary to concurrently investigate the objective functions $f_j(x)$ within the same variable space Ω_x, varying within the domain Ω_x. Hence, a multi-objective optimization problem arises (Dzung N.T and Hai D.T.H., 2016). In the case all single-objective optimization problems seek to find the minimal, the multi-objective optimization problem can be established as follows:

Find the optimal solution $x^{opt} = (x_1^{opt}, x_2^{opt}, x_3^{opt}) \in \Omega_x$:

$$\begin{cases} Y_j = f_{jmin}\left(x_1^{opt}, x_2^{opt}, x_3^{opt}\right) = Min\left\{f_j\left(x_1, x_2, x_3\right)\right\} \\ j = 1 \div 4; \\ \forall x \in \Omega_x = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\}; \end{cases} \quad (13)$$

The multi-objective optimization problems were solved using the Utopia Point Method:

In the situation that a common solution existing when solving single-objective optimization problems (12) or the solutions to all single-objective optimization problems coincide, which means when $Y_1 = f_1(x_1, x_2, x_3)$, $Y_2 = f_2(x_1, x_2, x_3)$ and $Y_3 = f_3(x_1, x_2, x_3)$; $Y_4 = f_4(x_1, x_2, x_3)$ reach the minimum values (Y_{1min}, Y_{2min}, Y_{3min}, Y_{4min}), all optimal solutions (x_1^{jopt},

x_2^{jopt}, x_3^{jopt}) ≡ (x_1^{opt}, x_2^{opt}, x_3^{opt}) were achieved with all j = 1 ÷ 4. Therefore, the utopia optimal method is existent, and (x_1^{jopt}, x_2^{jopt}, x_3^{jopt}) ≡ (x_1^{opt}, x_2^{opt}, Z_3^{opt}) is referred to as the utopia optimal solution of the utopia optimal method. This solution also serves as the solution for the multi-objective optimization problem (13). In this case Y^{UT} = (Y_{1min}, Y_{2min}, Y_{3min}, Y_{4min}) is called utopia point.

In cases where solving single-objective optimization problems (12) doesn't result a common solution, meaning a utopia solution and a utopia optimal method don't exist, the task now changes to solving the multi-objective problem (13) to search for a set of compromise solutions called optimal solutions (x_1^{opt}, x_2^{opt}, x_3^{opt}) which satisfy all the objective functions Y_j (j = 1 ÷ 4) simultaneously converge to their minimum values.

To find the optimal solution set (x_1^{opt}, x_2^{opt}, x_3^{opt}), this study employed the utopia point method with the combination norm S(x).

Although a utopia solution does not exist, a utopia point still exists as Y^{UT} = (Y_{1min}, Y_{2min}, Y_{3min}, Y_{4min}). As a result, the combination norm S(x) is established as follows:

$$S(x) = \sqrt{\sum_{j=1}^{m}\left(Y_j - Y_{jmin}\right)^2} \qquad (14)$$

With all $x = (x_1, x_2, x_3) \in \Omega_x$. Thus, the multi-objective optimization problem is restated as follows: Find $x^{opt} = (x_1^{opt}, x_2^{opt}, x_3^{opt}) \in \Omega_x$ to satisfy the following requirements:

$$\begin{cases} S_{min} = S(x_1^{opt}, x_2^{opt}, x_3^{opt}) = Min\left\{\sqrt{\sum_{j=1}^{m}\left(Y_j - Y_{jmin}\right)^2}\right\} \\ \forall x \in \Omega_x = \left\{-1.414 \le x_1, x_2, x_3 \le 1.414\right\} \end{cases} \qquad (15)$$

Solving problem (15) will yield the solution $x^{opt} = (x_1^{opt}, x_2^{opt}, x_3^{opt}) \in \Omega_x$. In that case: $Y_j^S = f_j(x_1^{opt}, x_2^{opt}, x_3^{opt})$, with m = 4.

3. Results and discussions

3.1. Determination of chemical composition of the raw material

Table 1. Chemical composition of yogurt material

N_o	Composition	Percentage (%)
1	Water	83.0 ± 2.0
2	Protein	2.9 ± 0.1
3	Carbohydrate	10.2 ± 0.2
4	Lipid	3.5 ± 0.1
5	Mineral	0.2 ± 0.0

As shown in Table 1, the water content in yogurt was 83%. Therefore, the requirement to reduce water activity to the desired moisture content would be significant. Low water activity helps inhibit the growth of most bacteria, yeasts, and molds, as well as oxidative reactions and enzymatic activities. Moreover, the removal of water from the product facilitates preservation and transportation (Mawilai P., Chaloeichitratham N., and Pornchaloempong P., 2019; Sogi D. S., Siddiq M., and Dolan K. D., 2015).

3.2. Determination of the appropriate ranges of the input technological factors

The single-factor experiments were conducted to determine the suitable ranges of temperature, pressure, and time to be used in the optimization model. The results are illustrated in Fig. 2, 3 and 4.

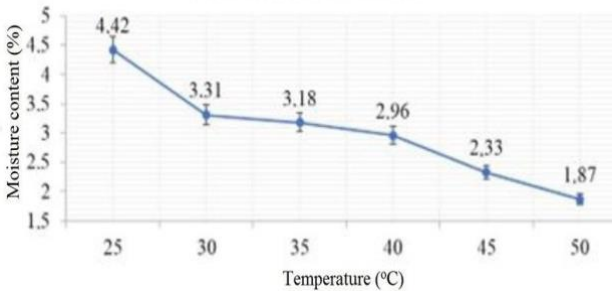


Figure 2. Relationship between product's moisture content and drying environment temperature

Based on the graph in Figure 2, it is observed that when the drying environment temperature is below 30°C, the product moisture is high. Choosing such a low temperature for drying would prolong the drying time and increase energy costs. Therefore, a reasonable temperature range for drying is between 30°C and 40°C. Within this range, the product moisture remains stable and meets the structural requirements of the final product.

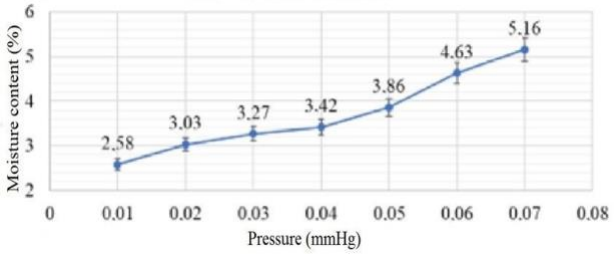


Figure 3. Relationship between product's moisture content and drying environment pressure

Referring to the graph in Figure 3, the data shows that the drying environment pressure has an impact on the product moisture after drying. As the drying environment pressure increased, the product moisture also increased. Thus, it is necessary to select an appropriate pressure range to save energy costs and minimize product losses during the drying process. Accordingly, the chosen pressure range is from 0.02 to 0.04 mmHg.

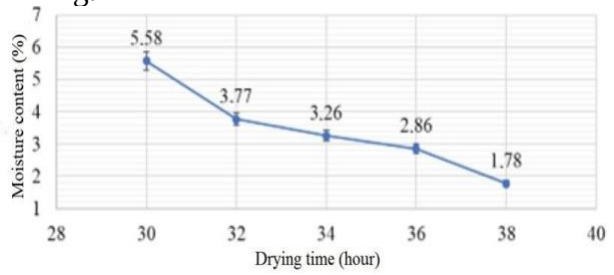


Figure 4. Relationship between product's moisture content and drying time

Based on the data in Figure 4, it is observed that within the time range from 32 to 36 hours, the product moisture exhibits minimal variation, remaining stable and meeting the required specifications. Therefore, we can select a drying time between 32 and 36 hours for experimental purposes.

3.3.Construction of experimental models describing the yogurt freeze drying process

After conducting single-factor experiments to identify appropriate experimental ranges for each technological factor, a central composite design model was constructed and presented in Table 2.

Table 2. Data for the levels of influencing factors

Input factors		Z_1 (°C)	Z_2 (mmHg)	Z_3 (h)
Coded experimental levels	$-\alpha$	32.17	0.016	31.17
	-1	33	0.02	32
	0	35	0.03	34
	+1	37	0.04	36
	$+\alpha$	37.83	0.044	36.83
Variance range ΔZ_i		2	0.01	2

From Table 2, a design of a second-order orthogonal experimental matrix was proceeded with a total of 18 experiments based on the combinations of Z_1, Z_2, Z_3 (Experimental variables) coded as x_1, x_2, x_3 (Coded variables) in Table 3.

The experiments for freeze drying of yogurt were carried out at the different combinations of Z_1, Z_2 and Z_3 as shown in Table 3. After each experiment, the products are collected and subjected to analysis for determining the values of Y_1 (kWh/kg), Y_2 (%), Y_3 (mN) and Y_4 (%). The results of the objective functions (Y_1, Y_2, Y_3 and Y_4) were recorded and presented in Table 3a and Table 3b.

Table 3a. Results for the objective functions in the experimental model

N_o		**Experimental variables**			**Coded variables**		
N		Z_1	Z_2	Z_3	x_1	x_2	x_3
		°C	mmHg	h			
2^k	1	37	0.04	36	1	1	1
	2	33	0.04	36	-1	1	1
	3	37	0.02	36	1	-1	1
	4	33	0.02	36	-1	-1	1
	5	37	0.04	32	1	1	-1
	6	33	0.04	32	-1	1	-1
	7	37	0.02	32	1	-1	-1
	8	33	0.02	32	-1	-1	-1

2k	9	37.8	0.03	34	1.414	0	0
	10	32.2	0.03	34	-1.414	0	0
	11	35	0.044	34	0	1.414	0
	12	35	0.156	34	0	-1.414	0
	13	35	0.03	36.8	0	0	1.414
	14	35	0.03	31.2	0	0	-1.414
n_0	15	35	0.03	34	0	0	0
	16	35	0.03	34	0	0	0
	17	35	0.03	34	0	0	0
	18	35	0.03	34	0	0	0

Table 3b. Results for the objective functions in the experimental model

N_o		**Output responses**			
N		Y_1	Y_2	Y_3	Y_4
		(kWh/kg)	(%)	(mN)	(%)
2^k	1	19.64	1.28	27.73	69.34
	2	19.04	1.53	31.49	63.78
	3	20.44	1.26	24.48	77.21
	4	20.04	2.21	30.61	68.66
	5	16.03	2.80	44.95	65.07
	6	15.9	3.51	51.04	67.25
	7	15.92	2.36	35.77	71.41
	8	15.73	2.80	50.11	65.9
2k	9	17.96	0.94	18.31	72.58
	10	16.96	3.50	39.31	66.4
	11	16.76	2.85	41.66	69.38
	12	19.19	0.84	28.13	74.01
	13	22.99	0.86	13.05	72.67
	14	14.94	3.81	41.99	69.07
n_0	15	17.51	1.65	24.69	65.18
	16	17.62	1.33	21.92	68.56
	17	18.04	1.42	22.83	65.96
	18	17.66	1.21	25.96	74.8

After processing the experimental data, calculating coefficients (b_i, b_{ui} and b_{ii}) in the regression equation (5), testing the significance of the regression equation coefficients using the Student's t-test, and checking the compatibility of the regression equation with the experimental results using the Fisher test, we obtained the following regression equations describing the low-temperature vacuum drying process of yogurt material:

- Regression equation describing energy cost:

$$Y_1 = 17.81 + 0.23x_1 - 0.413x_2 + 2.25x_3 - 0.26x_2x_3 - 0.307x_1^2 + 0.45x_3^2 \quad (16)$$

- Regression equation describing product moisture:

$$Y_2 = 1.550 - 0.497x_1 + 0.278x_2 - 0.780x_3 - 0.226x_2x_3 + 0.315x_1^2 + 0.373x_3^2 \quad (17)$$

- Regression equation describing product crispness:

$$Y_3 = 20.042 - 5.001x_1 + 2.781x_2 - 9.04x_3 + 3.586x_1^2 + 6.643x_2^2 + 2.937x_3^2 \quad (18)$$

- Regression equation describing preservation of microorganisms

$$Y_4 = 69.291 \quad (19)$$

The results of the mathematical models indicate that the experimental regression equations Y_1 (kWh/kg), Y_2 (%), Y_3 (mN) and Y_4 (%) describing the energy cost per 1 kg of the product, product moisture content, crispiness and the survival rate of microorganisms in the dried yogurt product, respectively, consistently align with experimental data through testing using Fisher's standard. The mathematical models for Y_1, Y_2 and Y_3 depended on the temperature of drying environment x_1 (Z_1, ^{0}C), pressure of drying environment x_2 (Z_2, mmHg) and drying time x_3 (Z_3, h). Meanwhile, the Y_4 objective function, which describes the beneficial microorganism survival rate for gut health, did not significantly depend on any investigated factors (Y_4 = 69.291% = const.). Ideally, Y_4 should be 100% because in a low-pressure and low-temperature environment, microorganisms can theorically survive. However, this loss occurred due to the fact that during the sublimation process, microorganism cells were carried away by steam.

To provide evidence for this, after completing the drying process, an analysis performingwith the condensed water sample in the freeze-condensation equipment of the melting system revealed the presence of microorganism cells. This demonstrates that microorganism cells were carried away with the sublimating water vapor.

3.4. Solving the optimization problems to determine the technological conditions

3.4.1. Solving the single-objective optimization problem

The experimental results have demonstrated that the objective function Y_4 is independent of x_1 (Z_1, ^{0}C), x_2 (Z_2, mmHg) and x_3 (Z_3, h). As a result, it can be excluded from the objective function space or the value domain of the objective function. Consequently, only three objectives Y_1, Y_2, Y_3 remain in the multi-objective optimization problem (13). However, to solve the multi-objective optimization problem (13) and find the optimal drying technological conditions, the first step is to determine whether the utopia method and utopia solution are existing. Therefore, the single-objective optimization problem (12) needs to be solved.

Table 4. Optimal coded variable values and predicted values for the objective functions, j = 1 ÷ 3

Optimal values	Y_{1min}	Y_{2min}	Y_{3min}
x_1^{jopt}	-1.414	0.789	0.697
x_2^{jopt}	1.414	-1.414	-0.209
x_3^{jopt}	-1.414	0.617	1.414
Y_{jmin}	14.54	0.82	14.17

The single-objective optimization problem (12) was solved using the Add – in – Solver function in Microsoft Excel 2022. The results of the single-objective optimization are presented in Table 4.

The optimal values for each single-objective optimization problem (Y_{1min}, Y_{2min}, Y_{3min}) are as follows:

The optimal value for energy cost (Y_{1min}) is as follows: Y_{1min} = 14.54 kWh/kg with the corresponding technological conditions: Temperature of drying chamber x_1^{1opt} = -1.414 (coded value), which is equivalent to 32.17°C; Pressure of drying chamber x_2^{1opt} = 1.414 (coded value), which is equivalent to 0.044 mmHg; Drying time x_3^{1opt} = -1.414 (coded value), which is equivalent to 31.17 hours.

The optimal value for product moisture (Y_{2min}) is as follows: Y_{2min} = 0.82% with the corresponding technological conditions: Temperature of drying chamber x_1^{2opt} = 0.79 (coded value), which is equivalent to 36.58°C; Pressure of drying chamber x_2^{2opt} = -1.414 (coded value), which is equivalent to 0.0156 mmHg; Drying time x_3^{2opt} = 0.62 (coded value), which is equivalent to 35.26 hours.

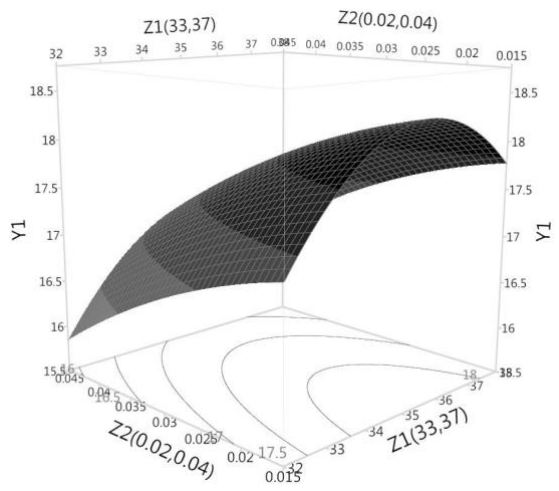
The optimal value for product crispiness (Y_{3min}) is as follows: Y_{3min} = 14.17 mN with the corresponding technological conditions: Temperature of drying chamber x_1^{3opt} = 0.70 (coded value), which is equivalent to 36.4°C; Pressure of drying chamber x_2^{3opt} = -0.21 (coded value), which is equivalent to 0.028 mmHg; Drying time x_3^{3opt} = 1.414 (coded value), which is equivalent to 36.8 hours.

Thus, the single-objective optimization problems do not have a common solution for the entire system (x_1^{iopt}, x_2^{iopt}, x_3^{iopt}) ≠ (x_1^{kopt}, x_2^{kopt}, x_3^{kopt}) with i, k = 1 ÷ 3 and i ≠ k to simultaneously satisfy the minimum values of all three objectives Y_j (j = 1 ÷ 3).

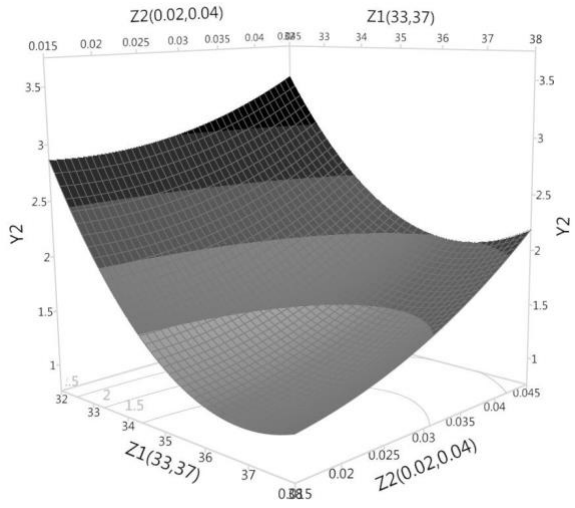
Table 4 shows that the single-objective optimization problems (12) do not have common solutions so there are no utopia optimal methods and no utopia solutions existing.

Figure 5 "a), b), c), d)" illustrates the interactive effects of input technological factors on the output objectives including energy consumption, product moisture content, crispiness, and the survival rate of microorganisms in the dried products.

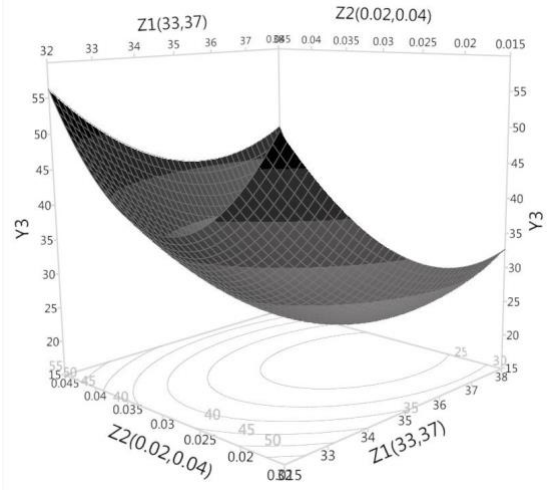
The 3D graphs indicate that energy consumption increased as the drying environment pressure was reduced. Additionally, raising the drying temperature also demanded higher energy input to achieve the desired product moisture content. Therefore, selecting an appropriate combination of pressure and drying temperature plays a crucial role in minimizing the energy requirements for the drying process, thereby contributing to cost reduction in product manufacturing.



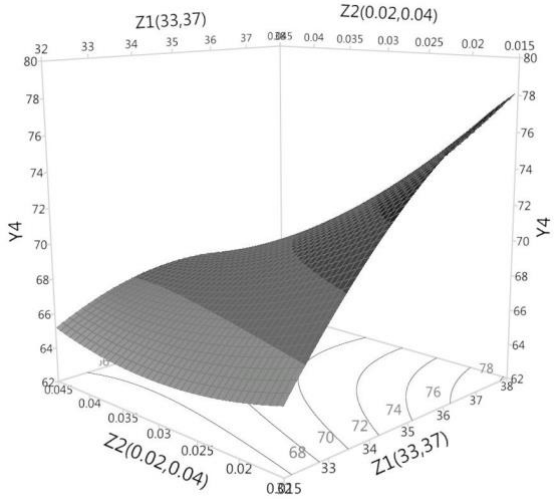
a) $Y_1 = f_1(Z_1, Z_2, Z_3 = 34.00)$, regression equation (16)



b) $Y_2 = f_2(Z_1, Z_2, Z_3 = 34.00)$, regression equation (17)



c) $Y_3 = f_3(Z_1, Z_2, Z_3 = 34.00)$, regression equation (18)



d) $Y_4 = f_4(Z_1, Z_2, Z_3 = 34.00)$, regression equation (19)

Figure 5. 3D plots presenting the interactive effects of input technological factors on the output objectives.

Z_1: temperature of drying chamber; Z_2: pressure of drying chamber; Z_3: drying time

Y_1: energy consumption (kWh/kg); Y_2: moisture content (%); Y_3: crispiness (mN); Y_4: microbial survival rate(%).

3.4.2. The multi-objective optimization problem

Because single-objective optimization problems do not share a common solution so the utopian solution does not exist to satisfy all single-objective optimization problems. As a result, the selection of a solution to achieve the optimal value of one objective often leads to the deterioration of the other objectives, which is a common and inherent issue in the field of engineering. The major purpose of this study is to find a compromise solution that satisfies all objectives and meets the technological requirements. Hence, at this point, the research problem has evolved into a multi-objective optimization problem (Dzung N.T, Chuyen H.V, Linh V.T.K, and et al., 2022).

Although utopia optimal solutions do not exist, there is still the presence of a utopia point $Y^{UT} = (Y_{1min}, Y_{2min}, Y_{3min}) = (14.54, 0.82, 14.17)$. This serves as the basis for establishing the composite standard S(x) (14). The multi-objective optimization problem with the composite standard S(x) (15) was solved using the Add – in – Solver function in Microsoft

Excel 2022. The results of the multi-objective optimization problem are presented in Table 5.

Table 5. Coded values, actual values after multi-objective optimization, and predicted results for the optimal sample.

Coded values			**Paréto experimental values**	
x_1^{opt}	x_2^{opt}	x_3^{opt}	j	Y^S_{jmin}
0.8113	-0.0681	0.8034	1	19.935
			2	0.963
			3	15.953
			4	69.291

After conducting the experiments and solving the multi-objective optimization problem using utopian point method, the optimal technological parameters for the production of freeze-dried yogurt were found as follows:

- Temperature of drying chamber x_1^{opt} = 0.8113, which is equivalent to Z_1^{opt} = 36.60°C
- Pressure of drying chamber x_2^{opt} = -0.0681, which is equivalent to Z_2^{opt} = 0.023 mmHg
- Time of drying process x_3^{opt} = 0.8034, which is equivalent to Z_3^{opt} = 35.6 hours

The predicted values for the corresponding objective functions are as follows:

- Energy cost Y^S_{1min} = 19.935 kWh/kg
- Product moisture content Y^S_{2min} = 0.963%
- Crispiness Y^S_{3min} = 15.953 mN

These optimized parameters and predicted results will contribute to the efficient production of dried yogurt with the desired characteristics.

3.5. Validation of the predicted values

To validate whether the optimal technological conditions in Table 5, derived from the multi-objective optimization problem (15), are suitable for practical use and production, an experiments for freeze drying of yogurt were conducted under the optimal conditions (Z_1^{opt} = 36.60°C; Z_2^{opt} = 0.023 mmHg; Z_3^{opt} = 35.6 hours) using a freeze drying system (DS-12). The results of the drying process are presented in Table 6.

Table 6. Predicted results, actual results, and the differences.

Outputs	Y_1	Y_2	Y_3
Unit	kWh/kg	%	mN
Experimental results, Y^E_j	21.15	0.874	17.23
Predicted results, Y^S_{jmin}	19.935	0.963	15.953
Difference (%)	5.75	10.18	7.41

The calculated optimal energy cost during the drying process (Y^S_1) was 19.935 kWh/kg, which is 5.75% lower than the actual experimental result of 21.15 kWh/kg. Regarding the product's moisture content (Y^S_2), the optimized calculation yielded 0.963%, which is 10.18% higher than the actual experimental result of 0.874%. For the crispiness of the dried yogurt (Y^S_3), the optimal calculation resulted in 15.953mN, which is 7.41% lower than the actual experimental result of 17.23mN.

Figure 6. Freeze drying yogurt product of the optimal technological conditions.

Based on the experimental verification of the optimal technological conditions, it can be concluded that while there are some deviations between the predicted values in Table 5 and the experimental results, these discrepancies are at the acceptable levels. Therefore, the optimal technological conditions in Table 5 can be applied in practical yogurt freeze drying processes. In fact, these optimized conditions have been successfully implemented in some dried yogurt production companies in Vietnam. Freeze drying yogurt product of the optimal technological conditions can be seen in Figure 6.

3.6. Evaluation of product quality

According to the report by Duan et al. (2016), the energy consumption during the freeze-drying process is significant, especially for high-value materials with high moisture content (Gallardo-Rivera C. *et al.*, 2021). Therefore, it can be concluded that improving the freeze-drying method by setting up optimization problems to find the process parameters will enhance heat transfer efficiency, reduce drying time, and consequently lower energy consumption during the freeze-drying process. The experimental results show that the energy consumption of 21.15 kWh/kg is an effective energy cost within the realistic combination condition of Y_1 less than 26.04 kWh/kg. As per the optimal parameters for the freeze-dried yogurt, the crispiness value is 17.23 mN, which is in accordance with the set condition of 15.953 mN. Thus, the crispiness of the freeze-dried yogurt in the optimal sample fully meets the structural requirements. Several reports have shown that the preservation of lactic acid bacteria in yogurt through freeze-drying is highly effective, with survival rates reaching up to 88.23% as reported by Gallardo-Rivera et al. (2021), and up to 87.2% as reported by Lim Y., Hong S., Shin,. et al. (2015). In this study, the survival rate of lactic acid bacteria reached 69.29%, which, although not as expected, was achieved without using additional components to protect the bacteria, only using plain yogurt. The results also demonstrate the potential for application and improvement in preserving live microbial resources through freeze-drying methods.

4. Conclusions

This study has successfully addressed the issues regarding the chemical composition analysis of yogurt, established optimization problems, and consequently determined the optimal freeze-drying regime for yogurt. Additionally, a complete and feasible technological process for production has been proposed. The optimal freeze-drying regime was determined to be at an ambient drying temperature of 36.6°C, an environmental pressure of 0.023 mmHg, and a drying time of 35.6 hours.

This optimized drying regime resulted in a dried yogurt product with a moisture content of 0.874%, a crispness of 17.23 mN, and the capability to retain 69.29% of the initial population of lactic acid bacteria. Furthermore, it has been found to consume 21.15 kWh per 1 kg of the product. These findings demonstrate the successful application of the proposed freeze-drying process for yogurt production. The results of this research provide valuable insights into optimizing the yogurt drying process and offer potential benefits in terms of preserving the product's quality and microbial content.

The established technological process can be a valuable reference for industrial yogurt production. Further studies and improvements in freeze-drying technology may lead to even more efficient and sustainable yogurt production in the future.

5. References

Anandharamakrishnan, C., (2017). Handbook of drying for dairy products. John Wiley & Sons, Ltd, 2017. doi: 10.1002/9781118930526.

AOAC International, AOAC-927.05 Moisture in Dried Milk. 2000.

Athanasios, I. Liapis and Roberto Bruttini, (2020). “Freeze drying,” in Handbook of industrial drying, 4th ed.CRC press, pp. 309–343, 2020.

Bamforth, C. W. and Cook, D. J., (2019). "Yoghurt and Other Fermented Milk Products," in Food, Fermentation, and Micro-organisms, 2nd Edition.Wiley-Blackwell, pp. 183–186, 2019.

Bhushani, A. and Anandharamakrishnan, C., (2017). "Freeze Drying," in Handbook of Drying for Dairy Products, John Wiley & Sons, pp. 95–121, 2017.

Dzung, N.T and Phuong, V.D., (2016). "The Multi-objective Optimization by the Restricted Area Method to Determine the Technological Mode of Cold Drying Process of Carrot Product," Research *Journal of Applied Sciences, Engineering and Technology*, vol. 13, no. 1, pp. 64–74, 2016, doi: 10.19026/rjaset.13.2891.

Dzung, N.T and Hai, D.T.H, (2016). "The Multi-objective Optimization by the Utopian Point Method to Determine the Technological Mode of Infrared Radiation Drying Process of Jackfruit Product in Viet Nam" Research *Journal of Applied Sciences, Engineering and Technology*, vol. 13, no. 1, pp.75-84, 2016.

Dzung, N. T. (Ed)., Chuyen, H. Van, Linh, V. T. K, Nhiem, L.T., Hoan, P. T., Linh, D.T.K, Quang, N.D., Duyen N.D.M, Tung, P.T., (2022) Freeze drying. VNU-HCM Press, pp. 187-192, 2022.

Duan, X., Yang, X., Ren, G., Pang, Y., Liu, L., and Liu, Y., (2016). "Technical aspects in freeze-drying of foods," *Drying Technology*, vol. 34, no. 11. 2016. doi: 10.1080/07373937.2015.1099545.

Fellows, P., (2000). Food Proccesing Technology, 2nd ed. Woodhead Publishing Limited and CRC Press LLC, 2000.

German, J. B., (2014). "The future of yogurt: Scientific and regulatory needs," *American Journal of Clinical Nutrition*, vol. 99, no. 5, pp. 1271–1278, May 2014, doi: 10.3945/AJCN.113.076844.

Gallardo-Rivera, C. et al., (2021). "Effect of three types of drying on the viability of lactic acid bacteria in foam-mat dried yogurt," *Processes*, vol. 9, no. 12, 2021, doi: 10.3390/pr9122123.

G.Wilhelm, Oetjen, and Peter Haseley, Freeze-drying. Wiley-VCH, 2004.

Karmi, O., Zayed, A., Baraghethi, S., Qadi, M., and Ghanem, R., (2011). "Measurement of Vitamin B 12 concentration: a review on available methods," *IIOAB Journal*, vol. 2, no. 2, pp. 23–32, 2011.

Lim, Y., Hong, S., Shin, Y. K. Kang, S. H., and Center D., (2015). "Changes in the viability of lactic acid bacteria during storage of freeze-dried yogurt snacks," *Journal of Dairy Science and Biotechnology*, vol. 33, no. 3, p. 203, 2015.

Mawilai, P., Chaloeichitratham, N., and Pornchaloempong, P., (2019). "Processing feasibility and qualities of freeze dried mango powder for SME scale," in IOP Conference Series: Earth and Environmental Science, 2019. doi: 10.1088/1755-1315/301/1/012059.

Marette, A., Picard-Deland, É., and Fernandez, M., (2017). Yogurt: roles in nutrition and impacts on health. 2017.

Sogi, D. S., Siddiq, M., and Dolan, K. D., (2015). "Total phenolics, carotenoids and antioxidant properties of Tommy Atkin mango cubes as affected by drying techniques," *LWT*, vol. 62, no. 1, 2015, doi: 10.1016/j.lwt.2014.04.015.

TCVN 7906:2008, "Microorganisms in food and animal feeding stuffs - Quantitative determination of mesophilic lactic acid bacteria - Colony-count technique at 30°C," *TCVN*, 7906:2008, 2008.

Yildiz, F., (2010). Development and manufacture of yogurt and other functional dairy products. CRC Press/Taylor & Francis, 2010.

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

This research is supported by Ho Chi Minh City University of Technology and Education (HCMUTE), Vietnam.

The authors thank the Head of Food Engineering and Technology Lab, Department

of Food Technology, Faculty of Chemical and Food Technology, HCMC University of Technical Education, Viet Nam, for their support and help to carry out the experiments.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

APPLICATION OF FTIR SPECTROSCOPY AND DIFFERENT METHODS TO DETECT ADULTERATION IN MANGO DRINKS

Lobna Haridy[1]✉, Aziza Gamal[1], Marwa Hussein[1] and Woroud A. Alsanei [2]

[1] Department of Horticulture Crops Research, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

[2] Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah 21589, Saudi Arabia.

✉*hamadajojo788@gmail.com*

https://doi.org/10.34302/crpjfst/2024.16.4.13

Article history:
Received
July 7th, 2024
Accepted
October 28th, 2024

Keywords:
Mango drink;
Drink adulteration;
Adulterant detection;
FTIR spectroscopy;
HPLC.

ABSTRACT

The increasing popularity of fruit drinks in meals, diets, school lunchboxes, and restaurants has raised concerns about the authenticity and quality of these products due to potential adulteration. Efficient and reliable analytical techniques are crucial for the detection of such adulteration. In this study, random samples of mango drink brands accepted by school students sold in Egyptian markets were evaluated for adulteration using Fourier transform infrared (FTIR) spectroscopy, fruit juice percentage, sugars, and preservatives. Findings revealed that brands C, D, E, and F exhibit significant levels of adulteration, as evidenced by fruit percentages that are lower than the assigned value. Brands C and D contained higher concentrations of preservatives and sucrose levels, respectively, than the standard specification, as indicated by high-performance liquid chromatography (HPLC). FTIR spectroscopy of drinks free from adulteration showed that the main functional groups detected were in the molecular structure of brand A, which contained abundant hydroxyl groups, polysaccharide, and phenols at 1330–1340 and 3449–3620cm^{-1}, followed by brand B. Brands D and F had an amide 111-band aromatic ester at 1253–1255cm^{-1} with transmittance percentages of 50.38 and 21.11, respectively, potentially indicating the addition of water, polymer, and plasticizer. Accurate labeling of fruit drinks is essential for protecting consumers from potential health risks associated with adulterated fruit drinks.

1.Introduction

The fruit drink industry constantly introduces new drinks to attract consumers, making them a popular component of meals, diets, school lunchboxes, and restaurants. However, the adulteration of fruit drinks remains a serious health risk, with risks ranging from poisoning, hypertension, cancer, paralysis, and mental retardation (Tomar & Alka, 2022). To increase profits, some fruit drink manufacturers adulterate their products and deceive customers by adding low-cost or inappropriate materials, such as water and cheap fruit juices, and removing valuable nutrients from their products. These products are often advertised as 100% fruit juice but frequently include chemical substances such as colorants, synthetic flavor enhancers, preservatives, or texture improvers, which cause health hazards such as tremors, headaches, and allergies (Maireva et al., 2013; Uddin et al., 2017). Mango, orange, and apple drinks are among the top seven fruit drinks susceptible to adulteration, requiring the use of traditional and contemporary anti-adulteration methods to effectively combat fraudulent activity (Pithava & Pandey, 2018). Therefore, fruit drink manufacturers must strictly adhere to quality

control measures to guarantee pure and safe fruit drinks, while ensuring authenticity and preventing adulteration, for healthy consumption.

Various methods have been developed for detecting adulteration of fruit drinks, and research studies have revealed that fruit content, dilution with water, mineral content, and sugar content are important parameters in the detection of adulteration. For example, Maireva et al. (2013) found that the levels of calcium, magnesium, and potassium in fruit drinks can be used to identify adulteration, with higher levels suggesting the presence of added water or other non-fruit additives. Detecting adulteration requires the consideration of other parameters in addition to fruit drink composition, such as fractionated sugars, preservatives content by high performance liquid chromatography (HPLC), ash, and minerals content. In particular, high levels of added sugar can mask the taste of fruit, making it more difficult to identify adulteration (Richardson et al., 2019). It is crucial to highlight the adulteration of certain fruit drinks to increase awareness about the issue of fruit drink adulteration and guide industry initiatives aimed at addressing this problem.

Mango juice is a beloved and nutrition-packed drink that is a favorite during the summer months due to its numerous health advantages. A study by Reddy et al. (2020) indicates that mango juice contains carotene, which has anti-cancer properties, and it is rich in various nutrients such as vitamins A, C, B1, B2, and B3, calcium, iron, phosphorus, and potassium. However, commercial mango drinks are frequently considered adulterated. The process of detecting adulteration of mango products may include chemical, sensory, microscopic, DNA-based, HPLC, and Fourier transform infrared (FTIR) spectroscopy analysis (Jha & Gunasekaran, 2010; Uddin et al., 2017). Based on the detection of adulteration, utilizing these methods can verify the integrity of mango juice, preserving its nutrient density and potential health advantages.

Research conducted in Egypt has identified several points for an investigation into the adulteration of fruit drinks, including mango drink (Maireva et al., 2013). An effective method for detecting adulteration in food products are FTIR and HPLC. These methods have been widely employed in quality control and process control applications due to their rapidity, noninvasiveness, and minimal preparation required for detection. However, to date, no FTIR spectroscopic or chemometric studies have been carried out to determine whether Egyptian mango drinks are adulterated or safe with an excess of simple sugars (Uddin et al., 2017). Additionally, FTIR technology has emerged as a promising technique for the detection of food adulterants and their legitimacy since it is a less time-consuming method that is more effective at eradicating the problems experienced by industrial members.

To combat the deficiencies in adulteration detection, this study was conducted to assess the likelihood of adulteration in six popular commercial mango drink brands frequently consumed by school students, purchased from Egyptian markets via traditional anti-adulteration methods (physicochemical tests) and contemporary anti-adulteration techniques (HPLC and FTIR spectroscopy techniques).

2. Materials and methods

2.1. Materials and chemicals

Commercial mango drinks coded as A, B, C, D, E, and F were purchased from local Egyptian markets (cardboard containers).

Food Technology Research Institute in Egypt and Sigma-Aldrich Company, cat. no. (AA8887) provided sodium hydroxide, phenol, potassium sorbate, sodium benzoate, hydrochloric acid, methanol, potassium dihydrogen phosphate, and sulphoric acid phosphoric acid.

2.2. Physicochemical tests

The total ash, Brix value (TSS%), were determined using the methods prescribed by the Association of Official Analytical Chemists (AOAC) (AOAC, 2019). The total carbohydrate content was determined using the phenol-sulfuric acid method (DuBois et al., 1956).

2.3. Determination of fruit content in the drinks

In total, 10ml of each fruit drink sample were diluted with 0.25N sodium hydroxide solution at pH 8.1, as described by Pithava and Pandey (2018). Equal amounts of formaldehyde solution were then added. After one minute, the solution was potentiometrically titrated at pH 8.1 with 0.25N NaOH. The percentage of fruit content was calculated according to the following Equation 1.

$$\%\ \boldsymbol{fruit\ content} = \frac{\mathbf{1.05\ F}}{\mathbf{1.4}} \quad (1)$$

*where F refers to the formol number (formol index)

2.4. Viscosity

A Brookfield AMETEK RV viscometer was used to directly measure the flow properties (shear rate, shear stress, and apparent viscosity) of all tested mango drinks. Samples were placed in small sample adapters, and the SC4-18 spindle was utilized to measure each sample. The viscosity of the mango drinks was measured at room temperature with shear rates ranging from 13.2 to 79.2s^{-1}, and the results were presented as centipoise (cP).

2.5. Turbidity

Turbidity measurements were carried out using a PC Compact Turbidimeter (Aqualitic Germany) (Turb 430T, serial no. 19430784) as a Nephelometric Turbidity Unit (NTU). Each mango drink was placed in a 15ml cell, capped, and gently inverted twice to ensure even mixing.

2.6. Preparation of standards and samples for sugar profile analysis and determination of preservatives by HPLC

The HPLC analysis was conducted using an Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a refractive index detector (RID). A Shim-pack SCR-101N analytical column was utilized. The mobile phase consisted of ultrapure water, and the flow rate was kept at 0.7ml/min for a total run time of 20 minutes with isocratic elution.

A sugar profile analysis of mango drinks was conducted using an official method described by the AOAC (AOAC, 1995), with minor modifications. A 10µL portion of each prepared sample was injected into an HPLC equipped with RI detection (Shimadzu refractive index, RID-10A). A ShimpackSCR-101N separation column (250mm L × 4.6mm I.D., 10µm) was used, and the column temperature was maintained at 30°C. The mobile phase was a mixture of water/acetonitrile (80:20v/v), and the flow rate was 1.3ml/min. Sugars were identified by comparing their retention times with appropriate sugar standards. Quantitation was done using the external standard method on peak areas or peak heights (Al-Mahasneh et al., 2021).

HPLC was used to determine sodium benzoate and potassium sorbate preservatives in all tested mango drinks. To prepare a stock solution of 1000ppm, 0.01g of each standard (sodium benzoate and potassium sorbate) was weighed and dissolved in 10ml of deionized water. A series of dilutions was prepared, and the peak area was plotted against each concentration. As part of the HPLC method, samples were prepared after concentration, and the mobile phase was diluted prior to analysis. These preservatives were determined according to Burana-osot et al. (2014). The results were expressed as benzoic and sorbic acid equivalents by equations 2 and 3.

$$\boldsymbol{Benzoic\ acid} \times \mathbf{1.18} = \boldsymbol{sodium\ benzoate} \quad (2)$$

$$\boldsymbol{Sorbic\ acid} \times \mathbf{1.2} = \boldsymbol{potassium\ sorbate} \quad (3)$$

2.7. Detecting adulteration by FTIR spectroscopy

FTIR spectroscopy was used to detect adulteration by Thermo Nicolet Nexus 670 instrument equipped with a mercury cadmium telluride A (MCTA) detector, XT-KBr beam-splitter, and OMNIC software. Drink samples were analyzed by placing a 0.5ml aliquot of each on a multi-bounce ZnSe crystal. The spectra

were measured by taking 128 scans at a resolution of 4cm-1, which were then averaged over the 4000–650cm-1 region. Prior to each analysis, a background was collected and then automatically subtracted from the sample spectra (Vardin et al., 2008).

2.8. Statistical analysis

For statistical analysis, a one-way analysis of variance (ANOVA) assessment was conducted at a significant rate of 0.05 for the entire results using Co-Stat (Ver. 6.400) according to (Steel et al., 1997). Prediction performance was quantified using a correlation coefficient (R). The least significant difference (LSD) test was used to assess the significance of the results among the drinks. All experiments were conducted in triplicate.

3. Results and discussions

The six commercial brands of mango drinks A to F were tested for adulteration using traditional and modern methods, including physicochemical tests, qualitative and quantitative fractionated sugars and preservative materials of the drink were determined using the HPLC method, while functional groups were analyzed using FTIR spectroscopy.

3.1. Physicochemical analysis

Table 1 shows the results of the physicochemical analysis, including ash, TSS, total carbohydrates, fruits percent of mango drinks (denoted as A, B, C, D, E, and F).

3.1.1. Ash

The total ash content is an important traditional indicator for detecting adulteration in fruit drink products. In Table 1, commercial mango drink brands C and F exhibited significantly higher total ash content (0.154 and 0.124%, respectively) than brand A (0.007%), indicating the presence of adulterants. The findings are consistent with previous studies by Usman et al. (2018). Total ash content in adulterated fruit drinks often arise from several sources, including the addition of non-fruit ingredients like fillers and sweeteners, the use of low-quality raw materials or cheaper fruit, and the deliberate incorporation of mineral salts for flavor enhancement. Impurities introduced during manufacturing (Ammari et al., 2015; Pasha et al., 1994).

3.1.2. Total soluble solids

TSS% is the simplest and most economical way to determine fruit drink adulteration. Table 1 shows that the TSS values varied between 11 and 15% for all tested mango drinks. The highest TSS values were obtained for brands C and D, 15 and 14.7%, respectively, which may be attributable to the addition of sucrose during processing (adulteration). The increase in TSS is related to the greater degree of tissue breakdown, where more compounds such as sugars are released (Nath et al., 2015). Brands E and F had slightly lower values (11 and 12%) than brands A and B (13.5%), suggesting water dilution (adulteration). These values are comparable with those of (Dżugan et al., 2018).

Table 1 also shows that the TSS recorded on the drinks' label descriptions as "no less than 8, average between 8 and 9, and similar or different numbers than actual" differed for all tested commercial mango drinks, which is considered adulteration and misleading to consumers (Jha et al., 2016). The TSS label was lower than the obtained results for brands C, D, and E. This finding indicates that adulteration in fruit drinks occurs by manipulating the TSS levels from those stated on drink labels.

3.1.3. Total carbohydrates

Detection of adulteration in fruit drinks, where the inclusion of sugars is a frequent occurrence, is often accomplished by detecting the presence of carbohydrates. In examining correlations between fruit content and total carbohydrates within various mango drinks, a strong correlation between these metrics was observed (r=0.982), as shown in Table 1, because mangoes are known to possess a high level of carbohydrates throughout the process of maturation. Analysis of the carbohydrates presented in Table 1 indicated that brands D and E possessed the highest carbohydrate levels of all the tested brands (18.14 and 18.13%, respectively), which exceeded the values stated

on their packaging label (17.12 and 14%, respectively).

With respect to the other tested drinks, brands A and B showed the lowest levels of carbohydrates (14.59 and 13.55%, respectively), although the value of brand B was closer to that stated on its label. Incorrectly stated carbohydrate levels on drink labels may be indicative of adulteration (Martínez Montero et al., 2004).

Table 1. Physicochemical properties of mango drink brands.

Mango drinks	Ash (%)	Laboratory test	Label claim	Fruit content (%)	Sucrose (%)	Sucrose (%) Quantitative HPLC	Laboratory test	Label claim
		TSS (°Brix)			Label claim	Laboratory test	Total carbohydrates (%)	
A	0.007^{b}±0.00	13.5^{b}±0	14.0	14.68^{a}±0.27	13.0	10.03	14.59^{c}±0.37	14
B	0.062^{ab}±0.05	13.5^{b}±0	14.5	11.53^{b}±0.33	15.00	9.39	13.55^{d}±0.29	14
C	0.154^{a}±0.14	15.0^{a}±0	8	7.84^{e}±00	13.90	12.33	16.89^{b}±0.98	13.4
D	0.091^{ab}±0.017	14.7^{a}±0	Not less than 8	5.31^{f}±0.27	13.45	12.62	18.14^{a}±0.32	17.12
E	0.070^{ab}±0.035	11.0^{d}±0	Average 8-9	7.09^{e}±00	14.00	11.37	18.13^{a}±0.33	14
F	0.124^{a}±0.02	12.0^{d}±0	15	7.03^{e}±00	15.00	11.31	16.89^{b}±0.11	16

Means in the same column with different superscripts (a, b, c …) are significantly different ($p \leq 0.05$).

3.1.4. Fruit and sucrose content in drinks

Fruit content is a valuable indicator

of adulteration. As shown in Table 1, the fruit content of mango drink brands A and B were 14.68 and 11.53%, respectively which are slightly lower than the general standards (15-25%) (CODEX-STAN247, 2005; EOSQ, 2017). In contrast, brands C, D, E, and F were significantly below standards 7.84, 5.31, 7.09, and 7.03%, respectively. The low fruit content indicates that minimal mango was added, contributing to economic gains in the mango concentrate and, therefore, indicating adulteration and misleading consumers (Jha et al., 2016).

The results in Table 1 shows a negative correlation between the fruit constituents in drink and the sucrose content in brand A and B. Specifically, the mango-based A demonstrated a strong and negative correlation, denoted by a correlation coefficient of -0.98. This observation indicates that an increase in fruit composition results in a concomitant decrease in sucrose quantity, which conforms to the predominate fruit-derived sugar, according to the extant literature (Duarte et al., 2002). Brands A and B mango drinks used fruit as a natural sweetener rather than heavily relying on added sugars (Kumar et al., 2020). Conversely, the brand D mango drink displayed a comparatively weaker negative correlation between the fruit constituents in drink (5.31%) and the sucrose content (12.62%). This finding suggests that although there is still a tendency for the fruit content to be a natural sweetener in this product, it is not as pronounced as in brands A and B mango drink. It is possible that the brands C and D mango drink may rely more on added sugars or may use a different method for sweetening the product that is not as closely related to the fruit content (Richardson et al., 2019).

According to current classification conventions, fruit drinks with less than 10% fruit content are typified as artificial fruit drinks (Rajauria & Tiwari, 2017). Overall, the present study offers valuable insight into the correlation between the fruit constituents and the sucrose content in mango drinks, underscoring the importance of understanding fruit drink product attributes for those seeking healthier dietary options.

3.1.5. Viscosity

Viscosity has been employed as an indicator of the quality of fruit drinks and, particularly, to determine the degree of their adulteration. In Table 2, viscosity of mango drink brands C, D, E and F increased with adulteration by 48.4, 45.8, 44.8 and 46.0cP, respectively. The low viscosity of brands A and B (22.4 and 25cP, respectively) may be attributed to its degree of methylation, pH imbalances, and TSS of the drink (Giap, 2010). Conversely, the highest viscosity in all tested brands may be attributable to the presence of pectin, pulp juice, stabilizer, and gum which enhance the consistency of drinks and create highly viscous media. All tested brands showed higher viscosity than brands A and B as expected due to adulteration.

3.1.6. Turbidity

Turbidity is the level of cloudiness or haziness caused by the dispersed matter in fruit juices, primarily composed of comminuted cellular tissues. In Table 2, turbidity can be a desirable attribute (cloudy), as shown in all tested mango drinks. The turbidity value of brands A and B were significantly lower (493 and 442NTU) than the values of all tested drinks C, D, E, and F, which were gradually increased by 897, 680, 636, and 657NTU, respectively providing brand drinks with higher turbidity. The rise in turbidity levels of drink brands compared to brands A and B may be a result of mature mango usage, insoluble pulp tissue fragments, or undissolved components such as pectin, cellulose, hemicellulose, starch, protein, and lignin released during enzymatic prepress maceration (Rai et al., 2022). Brands A and B however, exhibited a negative attribute (lack of apparent turbidity) may be due to the filtration process used to reduce turbidity and eliminate impurities, which was preferred by consumers (Calle et al., 2022). It might be concluded that brands C, D, E, and F have markedly higher adulteration levels.

Table 2. Viscosity and turbidity in brands of the mango drinks.

Mango drinks	Viscosity	Turbidity
	cP	NTU
A	6.4	493
B	35.0	742
C	38.4	897
D	44.8	680
E	44.8	636
F	32.0	657

3.2. HPLC analysis of sugars in mango drinks

The most abundant sugars in fruit drinks, particularly mango, are sucrose, glucose, and fructose (Duarte et al., 2002). However, sugars are commonly added to neutralize acidity or to provide sweetness (Hammond, 2016). Consequently, an analysis of sugar in tested mango drinks was conducted using HPLC analysis to detect adulterants, quantify and qualify added sugars, and provide valuable insight into a drink's purity. Three sugars were analyzed using HPLC, namely, sucrose, glucose, and fructose (Table 3 and Figure 1). The results indicated that all tested mango drinks containing sucrose levels ranged from 10.03 to 12.62%. For all drink brands, there are

noticeable changes in glucose concentrations (1.69 – 3.6%) and fructose concentrations (1.49 – 5.60%) in comparison with brands A and B which shows a slight decrease in sucrose (10.03 and 9.39%, respectively) and an increase in both glucose (3.6 and 2.66%) and fructose (5.6 and 4.27%) compared with other drinks.

As shown in Table 3 and Figure 1, the recorded sucrose levels on the label packages of brands A, B, C, D, E, and F were 13, 15, 13.9, 13.45, 14, and 15%, respectively. The labeled values indicate poor agreement with laboratory values. Due to mango maturity, processing, and storage conditions, sugar degradation may occur in mango drinks. In the case of brands A and B, on the label packages, sucrose levels were high, when compared with the same brands laboratory test which may be attributable to the addition of sucrose during processing. It is possible that the brands C and D mango drink may rely more on added sugars or may use a different method for sweetening the product that is not as closely related to the fruit content (Richardson et al., 2019). The low or high sugars content of mango drinks are likely caused by the addition of fruit juice (peach or grape) or the use of cane sugar as a sugar substitute.

Table 3. Quantitative sugars of mango drink brands, as determined by HPLC.

Mango drinks	Sucrose		Glucose	Fructose
	%			
	Label claim	Laboratory test		
A	13	10.03	3.6	5.6
B	15	9.39	2.66	4.27
C	13.9	12.33	1.69	1.49
D	13.45	12.62	1.82	1.68
E	14	11.37	2.14	1.76
F	15	11.31	2.49	2.25

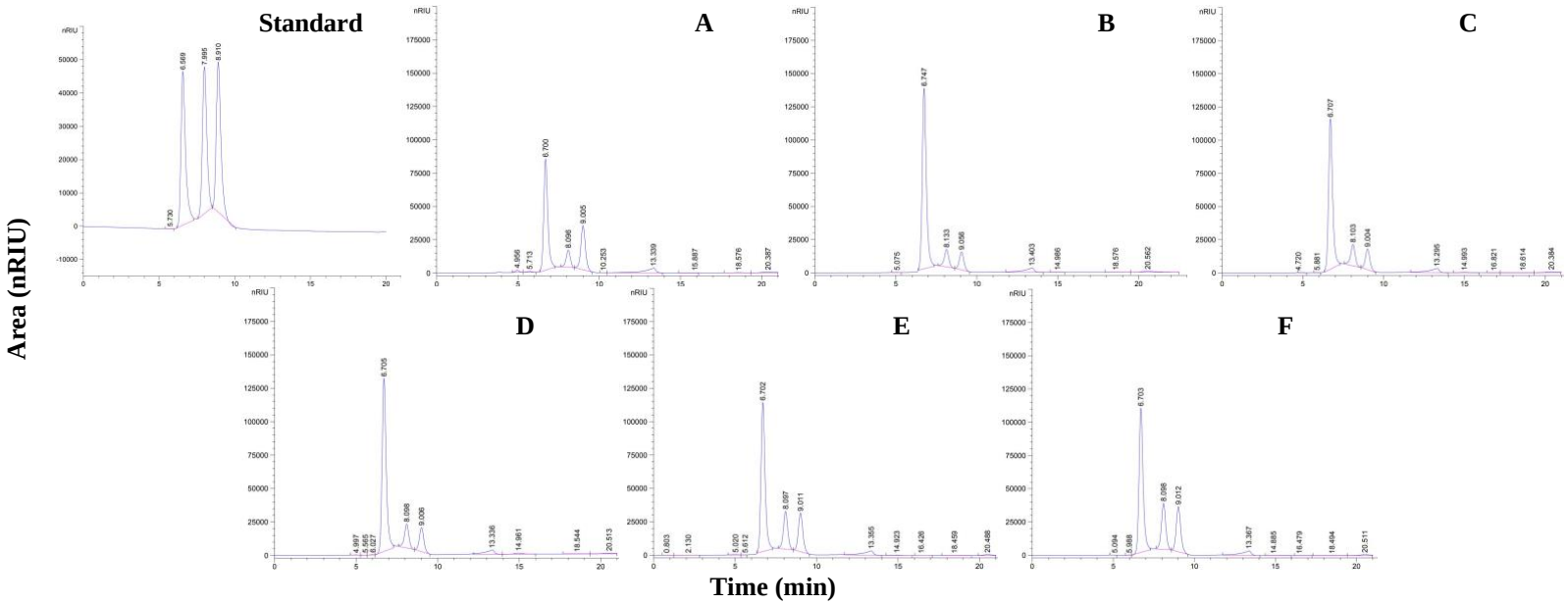


Figure 1. Qualitative HPLC analysis of standard sugar solutions, mango drink brands (A to F).

3.3. HPLC analysis of preservative determination in mango drinks

The use of preservatives such as sodium benzoate (E211) and potassium sorbate (E202) in fruit drinks is vital in modern food and drink technology for shelf-life extension and maintaining quality. However, excessive consumption and adulteration of these preservatives pose significant health risks (Ahmed et al., 2013; Aslam et al., 2020). Using the HPLC technique, as standards g/100g (%), this study analyzed six mango drink brands to detect the presence and amounts of sodium benzoate and potassium sorbate (Table 3 and Figure 2). The results revealed that sodium benzoate was the prevailing preservative utilized, while potassium sorbate was absent in all tested drinks. A similar indication has been reported in the literature (Ahmed et al., 2013). According to the U.S. Food and Drug Administration (U.S. FDA) (FDA, 1999), brands C and D contained high levels of sodium benzoate (0.804 and 1.442%, respectively), and the values differ from those on their package labels, indicating significant adulteration. The remaining mango drink brands were free of these preservatives. Accordingly, sodium benzoate should not exceed 0.1%, whereas potassium sorbate is permitted at 0.1 to 0.2% (100mg/100ml), respectively. As allowable food additives, both analyzed preservatives must not exceed recognized limits to ensure consumer safety (Aslam et al., 2020). Additionally, the results indicate that commercial mango drinks (A and B) with a shelf life comparable to that of brands E and F, were produced without preservatives by using appropriate processing and storage techniques, with the exception of brand C and D. According to the package label, during processing, for example, citric acid and ascorbic acid were added to brand C, while ascorbic acid and stabilizers E415 were added to brand D. However, where required, potassium sorbate and sodium benzoate or their combination at prescribed concentrations can be effectively used to preserve mango drinks' quality (Ahmed et al., 2013).

To ensure consumer safety, it is crucial to verify both the names and specific quantities of preservatives used in fruit drinks, which can now be more easily identified with the latest advancements in analytical chemistry and instrumentation (Calle et al., 2022).

Table 3. HPLC-based quantitative preservatives according to standards g/100g (%) in the mango drinks brands.

Mango drinks	Label Claim	Sodium Benzoate	Potassium Sorbate
A	No preservatives	ND	ND
B	No preservatives	ND	ND
C	Permitted preservative (citric acid+ascorbic acid)	0.804	ND
D	Permitted preservative (ascorbic acid+E415)	1.442	ND
E	No preservatives	ND	ND
F	Permitted preservative (citric acid+potassium citrate)	ND	ND

ND: not detected.

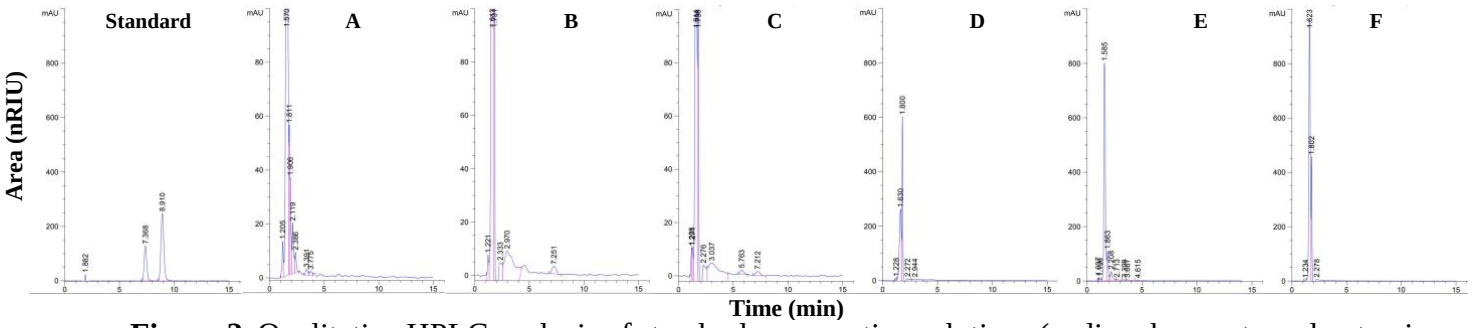


Figure 2. Qualitative HPLC analysis of standard preservative solutions (sodium benzoate and potassium sorbate), mango drinks brands (A to F).

3.4. FTIR spectroscopy analysis of functional groups in mango drinks

Adulteration of fruit drinks such as mango with cheaper ingredients has become a serious concern in the food and drink industry. FTIR spectroscopy has been identified as a reliable method for identifying the presence of adulterants by detecting changes in the functional groups of the drink. The study investigated the functional groups present in commercial mango drink brands (A to F) using FTIR spectroscopy. The spectra of all types of drink brands were found to be nearly identical, with absorption bands at 3449, 2387, 2059, 1636, 1416, 1330, 1253, 1106, 1054, 996, 485, and 550cm^{-1}, respectively (Table 4). The hydroxyl groups (O-H) at 1330–1340 and 3449–3620cm^{-1} were the most prominent functional groups detected in mango drinks brand A and B, followed by other commercial brands. The bands at 1045–1340cm^{-1} (polysaccharide and phenols) were observed, with the asymmetric CH stretching vibration causing these bands.

Table 4 shows the bands of the major functional groups, wavenumbers, and transmittance percentages of mango drinks. The bands at 1045, 1106, and 1253cm^{-1} were characterized by (C-O) stretching vibrations in the furanose ring. The strong bands at 996, 1636, and 2378cm^{-1} were allocated to an α-D-glcopyranosyl deposit in the carbohydrate conjugated chain (C\\C). These findings are consistent with previous reports of the FTIR spectra of mango juice (Uddin et al., 2017). The band at 1106–1107cm^{-1} was not specific for mango drink brands C, D, E, and F. Additionally, the band at 1253–1255cm^{-1} (amide 111 band and aromatic ester) for brand C (zero transmittance percentage) could be attributable to dilution with water, which is an adulteration method, indicating the hygroscopic properties of this homo polysaccharide.

Additionally, the same table shows that the bands at 1416 and 1417cm^{-1} in mango drinks spectra confirmed C-H ring vibration in the presence of 2-ketofuranose. Polyphenols, carbohydrates (intermolecular bonded O-H), and hydroxyl bonds were detected at 3449–3620cm^{-1} in brand A drink. The mango drink spectra confirmed strong N=C=S rings between 2059 and 2068cm^{-1}, suggesting that the functional group isothiocyanate is present where the drink brands A and B have a high transmittance. The percentage of transmission was higher in brand A followed by brand B, which could be attributed to the absence of adulteration. The bands at 2387cm^{-1} in the mango brand's F spectra confirmed C=C conjugated ring vibration in the presence of amino perfluoro alkyl sulfonate (polymer additives and plasticizers), as shown in Table 4 and Figure 3.

Amide 111-band aromatic ester was observed at 1253–1255cm^{-1} and 2387cm^{-1} for brands D and F with a transmittance percentage of 50.38 and 21.11, respectively. This finding could be attributed to the addition of polymer and plasticizer. The study found that brands A and B were free of adulterants and contained the main functional hydroxyl groups (O-H) and

polysaccharides, as well as phenols. These results demonstrate the potential of FTIR spectrometry, coupled with chemometrics, in detecting the adulteration of mango drinks (Jha & Gunasekaran, 2010). Thus, the advances in analytical chemistry and instrumentation have simplified the detection of fruit drink adulteration (Calle et al., 2022)

Table 4. Summary of the band assignments used for the FTIR spectra of the major functional groups, wavenumbers, and % transmittances for mango drink brands.

Functional groups	**Wavenumber** cm^{-1}	**Transmittance** %					
		A	**B**	**C**	**D**	**E**	**F**
CH out of plane aromatic band	485-550	27.115	3.754	-0.773	10.637	0.800	10.012
C=C, strong-bending-alkene-monosubstituted	996-997	68.245	42.434	-	39.26	-	24.022
C-O, strong-stretching-carbohydrate-polysaccharide	1045-1057	66.210	34.893	2.097	32.906	4.168	21.110
C-O, strong-stretching-polysaccharide	1106-1107	79.700	50.448	-	-	-	-
C-O, strong-stretching-aromatic ester, C-N, Amide 111band	1253-1255	10.371	12.032	-	50.380	9.982	21.110
OH, medium, bending, phenol	1330-1340	92.246	70.155	18.258	50.986	9.133	28.326
C-H, bending, alkane-methyl group, stretching C=O, inorganic carbonate	1416-1417	92.748	70.941	18.302	50.728	8.997	28.258
C=C, medium, stretching, alkene (disubstituted Cis), C=O amide 1 band	1636-1651	34.536	5.909	-0.256	9.747	0.611	9.953
N=C=S, strong, stretching, isothiocyanate	2059-2068	115.147	101.019	50.932	66.961	14.706	32.502
C=C, conjugated	2387	-	-	-	-	-	37.202
O-H, stretching, carbohydrate, polyphenols (intermolecular bonded OH), N-H, medium stretching, primary amine	3449-3620	6.912	-1.020	-1.440	1.586	-0.248	1.400

* Mango drink brands D and F contained polymer additives and plasticizers (amine perfluorealkylesulfonate) only. Mango drink brands C and E contained polymer additives and plasticizers, and dyes, nitro, and azo compounds such as polyetherinide and N,N-BIS(salicylidene)-1,3-propanediamine.

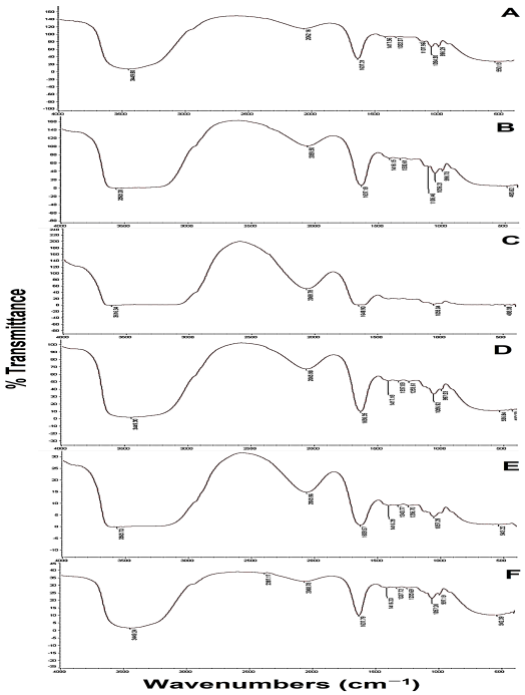


Figure 3. Qualitative FTIR spectra characterization of brands (A to F) of mango drinks.

4. Conclusions

The extensive use of fruit drinks in various settings highlights the significant health hazards associated with the adulteration of this industry. As a result, there is an urgent need for accurate and prompt analytical techniques to guarantee the authenticity and quality of these drinks. This study has effectively demonstrated that conventional and contemporary anti-adulteration methods, namely, physicochemical tests, HPLC, and FTIR spectroscopy, can efficiently identify adulteration in commercial mango drink brands. According to HPLC analysis, brands C and D have been adulterated with sucrose, and preservative materials. The potential of FTIR spectrometry, coupled with chemometrics, in detecting the adulteration of mango drinks. Brands D and F had an amide 111-band aromatic ester at 1253–1255cm^{-1} with transmittance percentages of 50.38 and 21.11, respectively, potentially indicating the addition of water, polymer, and plasticizer. Furthermore, the findings of this study reveal that brands C, D, E, and F have markedly higher adulteration levels and lower fruit content, suggesting water dilution. Therefore, fruit drink manufacturers must ensure accurate product labeling to mitigate health hazards related to adulterated fruit drinks. In addition, it is possible to eliminate the need for preservatives in commercial mango drinks or to use acceptable levels of preservatives by employing appropriate processing and storage techniques to provide safer and healthier drinks. Regulatory limits on food additives must also be enforced, accompanied by accurate food product labeling to ensure consumer safety.

5. References

Ahmed, M., Shahzadi, S. K., Waseem, R., Shahzad, S., & Ahmad, W. (2013). Sodium benzoate and potassium sorbate in Pakistani retail foodstuffs. *International Journal of Chemical Sciences and Research*, *3*(2), 1-6.

Al-Mahasneh, M., Al-U'datt, M., Rababah, T., Al-Widyan, M., Abu Kaeed, A., Al-Mahasneh, A. J., & Abu-Khalaf, N. (2021). Classification and Prediction of Bee Honey Indirect Adulteration Using Physiochemical Properties Coupled with K-Means Clustering and Simulated Annealing-Artificial Neural Networks (SA-ANNs). *Journal of Food Quality*, *2021*(1), 6634598.

Ammari, F., Redjdal, L., & Rutledge, D. N. (2015). Detection of orange juice frauds using front-face fluorescence spectroscopy and Independent Components Analysis. *Food chemistry*, *168*, 211-217.

AOAC. (1995). Sugar products, Official Method 991.41: C-4 plant sugars in Honey. *AOAC Official Methods of Analysis*.

AOAC. (2019). *Official methods of analysis* (21st ed ed.).

Aslam, M., Hamid, S., Khalid, S., Kamran, H., & Azhar, S. (2020). Determination of preservative sodium benzoate in selected samples of fruit juices and squashes. *Asian Journal of Allied Health Sciences (AJAHS)*, 27-32.

Burana-osot, J., Arunsingkharat, L., Naksuk, M., Naungnamjai, S., & Saetun, T. (2014). Validation of a HPLC method for the determination of benzoic acid and sorbic acid in noodles. *Chiang Mai Journal of Science*, *41*(2), 370-382.

Calle, J. L. P., Ferreiro-González, M., Ruiz-Rodríguez, A., Fernández, D., & Palma, M. (2022). Detection of adulterations in fruit juices using machine learning methods over FT-IR spectroscopic data. *Agronomy*, *12*(3), 683.

CODEX-STAN247. (2005). *General standard for fruit juices and nectars Codex Alimentarius Commission.* Retrieved 5 November from

https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%2

Duarte, I. F., Barros, A., Delgadillo, I., Almeida, C., & Gil, A. M. (2002). Application of FTIR spectroscopy for the quantification of sugars in mango juice as a function of ripening. *Journal of agricultural and food chemistry*, *50*(11), 3104-3111.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. t., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356.

Dżugan, M., Wesołowska, M., Zaguła, G., & Puchalski, C. (2018). The comparison of the physicochemical parameters and antioxidant activity of homemade and commercial pomegranate juices. *Acta Scientiarum Polonorum Technologia Alimentaria*, *17*(1), 59-68.

EOSQ. (2017). *Standard for Fruit Juices and Nectars 1602*. Retrieved 15 July from https://www.eos.org.eg/en

FDA, U. S. (1999). *Code of Federal Regulations 21: 184.1733, Sodium Benzoate and 182.3640, Potassium Sorbate*. Food and Drug Administration. Retrieved 09 December from http://www.access.gpo.gov/nara/cfr/cfr-table-search.html

Giap, S. G. E. (2010). The hidden property of Arrhenius-type relationship: viscosity as a function of temperature. *Journal of Physical Science*, *21*(1), 29-39.

Hammond, D. A. (2016). Analysis of soft drinks and fruit juices. *Chemistry and Technology of Soft Drinks and Fruit Juices*, 231-289.

Jha, & Gunasekaran, S. (2010). Authentication of sweetness of mango juice using Fourier transform infrared-attenuated total reflection spectroscopy. *Journal of Food Engineering*, *101*(3), 337-342.

Jha, Jaiswal, P., Grewal, M. K., Gupta, M., & Bhardwaj, R. (2016). Detection of adulterants and contaminants in liquid foods—a review. *Critical reviews in food science and nutrition*, *56*(10), 1662-1684.

Kumar, C. S., Ali, A., & Manickavasagan, A. (2020). Health benefits of substituting added sugars with fruits in developing value-added food products: a review. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *10*(3), 75-90.

Maireva, S., Usai, T., & Manhokwe, S. (2013). The determination of adulteration in orange based fruit juices. *International Journal of science and Technology*, *2*(5), 365-372.

Martínez Montero, C., Rodríguez Dodero, M., Guillén Sánchez, D., & Barroso, C. (2004). Analysis of low molecular weight carbohydrates in food and beverages: a review. *Chromatographia*, *59*(1), 15-30.

Nath, P., Varghese, E., & Kaur, C. (2015). Optimization of enzymatic maceration for extraction of carotenoids and total phenolics from sweet pepper using response surface methodology. *Indian Journal of Horticulture*, *72*(4), 547-552.

Pasha, A., Butt, M., & Mohyuddin, G. (1994). Quality evaluation of some commercially manufactured fruit beverages. *Pakistan Journal of Agricultural Sciences*, *31*(3).

Pithava, V., & Pandey, A. (2018). Quality assessment of different brands of mango juice available in Indian market for carbohydrates and acids (ascorbic acid) by conventional titration method. *International Journal of Pharmaceutical Sciences and Research*, *9*(11), 4826-4831.

Rai, P., Mehrotra, S., & Sharma, S. K. (2022). Challenges in assessing the quality of

fruit juices: Intervening role of biosensors. *Food chemistry*, *386*, 132825.

Rajauria, G., & Tiwari, B. K. (2017). *Fruit juices: Extraction, composition, quality and analysis*. Academic press.

Reddy, L. V., Kim, Y.-M., & Wee, Y.-J. (2020). Rapid and enhanced liquefaction of pulp from mango (Mangifera indica L.) cv. Totapuri using ultrasound-assisted enzyme pretreatment. *Processes*, *8*(6), 718.

Richardson, P. I., Muhamadali, H., Ellis, D. I., & Goodacre, R. (2019). Rapid quantification of the adulteration of fresh coconut water by dilution and sugars using Raman spectroscopy and chemometrics. *Food chemistry*, *272*, 157-164.

Steel, R. G., Torrie, J. H., & Dickey, D. A. (1997). *Principles and procedures of statistics: a biometrical approach*.

Tomar, P., & Alka, G. (2022). Food adulteration and its impact on health. *International Journal of Home Science*, *8*(2), 164-168.

Uddin, M., Majumder, A., Ahamed, S., Saha, B., & Mumtaz, B. (2017). Chemometrics assisted method for classification of mango juice by FTIR spectroscopic data. *Bangladesh Journal of Scientific and Industrial Research*, *52*(2), 73-80.

Usman, A., Bala, N., & Bashiru, M. (2018). Effect of plantain peel ash (PPA) on the mechanical properties of concrete. *Noble International Journal of Scientific Research*, *2*(03), 11-18.

Vardin, H., Tay, A., Ozen, B., & Mauer, L. (2008). Authentication of pomegranate juice concentrate using FTIR spectroscopy and chemometrics. *Food chemistry*, *108*(2), 742-748.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

DETERMINATION OF TOTAL PHENOLIC CONTENT, QUERCETIN, AND RUTIN OF COSMOS CAUDATUS LEAF EXTRACTS AND THEIR CONTRIBUTION TOWARD SCAVENGING DPPH RADICALS

Mustofa Ahda[1,2,3]✉, Amalya Nurul Khairi[2,4], Kholil Fahri[1*], Mohd Salleh Rofiee[5,6]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta
[2]Ahmad Dahlan Halal Center, Universitas Ahmad Dahlan, Yogyakarta
[3]Department of Pharmaceutical Chemistry, International Islamic University Malaysia, Kuantan Malaysia
[4]Department of Food Technology, Faculty of Engineering, Universitas Ahmad Dahlan, Yogyakarta
[5]Integrative Pharmacogenomics Institute (iPROMISE), Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, Bandar Puncak Alam, Selangor.
[6]Faculty of Health Sciences, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, Bandar Puncak Alam, Selangor.
✉mustofa_ahda@yahoo.com

https://doi.org/10.34302/crpjfst/2024.16.4.14

Article history:
Received
July 7th, 2024
Accepted
October 28th, 2024

Keywords:
C. caudatus leaves;
The scavenging activity of radicals;
Quercetin concentrations;
Rutin concentrations.

ABSTRACT

C. caudatus leaves are traditionally served as a salad. The total phenolic content (TPC), rutin and quercetin levels, and inhibition against DPPH radicals were all determined in this study. This plant has a TPC of 35.891-91.321 µg gallic acid equivalent/mg dried extract. Rutin and quercetin levels in this plant are approximately 17.97-18.59 µg/mg and 0.73-0.79 µg/mg, respectively. The extract with the highest TPC and rutin levels is 40% ethanolic extract, whereas the extract with the highest quercetin levels and DPPH radicals inhibition is 80% ethanolic extract. Furthermore, both 80% ethanolic extract and 60% ethanolic extract were classified as being similar in this investigation. Meanwhile, the good solid-to-solvent ratio employed in the extraction is 1:6 (w/v). As the outcome of this research, it was recommended that this herb be extracted using 60-80% ethanolic extract with a solid solvent ratio of 1:6.

1.Introduction

Cosmos caudatus Kunth (*C. caudatus*) leaves have numerous beneficial effects on human health. The main strength of this herb has antioxidant activity (Cheng et al., 2015[a]). It was traditionally eaten as a salad in Asian countries such as Indonesia and Malaysia. Furthermore, this plant can be extracted and used in a variety of pharmaceutical, cosmetic, and food ingredients. The active compound content of herb extracts determines their antioxidant activity. *C. caudatus* leaves have potential bioactive compounds such as ascorbic acid, flavonoids, and their derivates, and chlorogenic acid, (Cheng et al., 2015[a], Mediani et al., 2013; Nurhayati et al., 2018; Seyedreihani, et al., 2016). Research from Chan et al., (2016) reported that its leaves also contain major compounds such as flavonoids, phenolic acids, and diterpenoids. Besides, other active compounds such as α-tocopherol, cyclohexen-1-carboxylic acid, stigmasterol, benzoic acid, lycopene, and myo-inositol are contained in the fresh leaves (Javadi et al., 2014; Javadi et al., 2015). Its leaves also contained α-cadinene as one of the major volatiles among the 13 types of volatiles. In general, these herb leaves contain three types of active compounds such as flavonoids and their derivatives, non-flavonoid

groups, and volatile compound groups (Ahda et al., 2023).

The active compounds in its leaves are primarily responsible for its potency. However, the solvents used can affect the active compounds extracted, resulting in different activities. Therefore, selecting a solvent system is a key technique for producing the desired bioactive compounds, with the polarity of the active compounds being affected by the solvent used (Sasidharan et al., 2011; Altemimi et al., 2017). The hydrophilic compounds can be extracted using polar solvents such as methanol, ethanol, or ethyl-acetate and more lipophilic compounds use dichloromethane, or its mixtures (Sasidharan et al., 2011). Altemimi et al. (2017) reported the sequencing of the solvent polarity, from non-polar to polar is hexane < chloroform < ethylacetate < acetone < methanol < water. Furthermore, the solvent used, such as ethanol, is safer for human consumption, has lower toxicological effects, and is suitable for food systems (Suhaimi et al., 2019).

The effect of solvent was explained by Mediani et al. (2013), who found that an 80% methanol extract of *C. caudatus* had higher antioxidant activity than an 80% ethanol extract. Similarly, Cheng et al. (2016b) found that 100% methanol and 50% ethanol extracts of *C. caudatus* leaves had the highest antioxidant activity and were a powerful solvent for extracting its active compounds when compared to other 100% ethanol, 95% ethanol, and 100% water extracts. Besides the solvent concentration, the solid-to-solvent ratio also affected the extracted active compound from the herbs. Therefore, to evaluate the contribution of quercetin and rutin in the *C. caudatus* leaves, this study looks for the equilibrium state between solid and solvent because the increasing solvent volume will cause an increase in the extracted active compound (Norshazila et al., 2017). Aside from the solvent concentration, the solid-to-solvent ratio influenced the extracted active compound from the herbs. To evaluate the contribution of quercetin and rutin in *C. caudatus* leaves, this study looks for the equilibrium state between solid and solvent in the extraction process.

2. Materials and methods

2.1. Materials

2.1.1. Samples

C. caudatus leaves were collected from West Sembuh, Sidomulyo, Godean, and Yogyakarta.

2.2. Methods

2.2.1. Samples Preparation

This plant was identified by the Department of Biology, Universitas Gadjah Mada. The dried *C. caudatus* leaves were washed using water and dried in Oven at 45 ^{0}C for 4 days. The dried leaves were ground to become a powder in 60 mesh.

2.2.2. Effect of Solvent Concentrations

The extraction procedure using the Soxhlet method was reported by Sharif et al., (2016) with slight modification. A 10 g sample was dissolved in ethanol (1:10, b/v) and then heated at 50 ^{0}C for 3 hours. the sample was filtered and then evaporated at 50 ^{0}C to produce the dried extract. To evaluate solvent concentration, the different concentration of Ethanol were used (100%, 80%, 60%, 40%, and 20%). All dried extracts were determined for the total phenolic content, Flavonoid content, and inhibition activity of DPPH.

2.2.3. Effect of Solid/Solvent Ratio

To evaluate the solid/solvent ratio, leaf extract powder of *C. caudatus* was weighted in variation ratio 1:12.5; 1:10: 1:8; and 1:6 b/v. The extraction procedure follows previous work. A 10 g sample was dissolved in 125 mL ethanol (solid/solvent ratio: 1:12.5, b/v) and then heated at 50 ^{0}C for 3 hours. the sample was filtered and then evaporated at 50 ^{0}C to produce the dried extract. For 1:10; 1:8; 1:6 b/v, 10 g sample was dissolved in 100 ml; 80 ml; 60 mL ethanol, respectively. All dried extracts were determined for the total phenolic content, Flavonoid content, and inhibition activity of DPPH.

2.2.4. Determination of Total Phenolic Content

The determination of total phenolic content (TPC) in the extract of *C. caudatus* leaves following the study reported by Ahda et al.,

(2019) with slight modification. To determine the TPC, 10 mg extract of *C. caudatus* leaves was dissolved using 10 mL water and then reacted with 1.5 mL Folin Ciocalteu (1:10 in water) and mixed for 3 minutes. After that, it was mixed with 1.2 mL of 7.5% sodium carbonate (b/v) and awaited for 60 minutes. The TPC was measured by a UV-Vis spectrophotometer at a maximum lambda of 743 nm. The standard solution used is a gallic acid solution in ranging concentrations from 30-80 µg/mL. The TPC is expressed in µg/mg equivalent of gallic acid.

2.2.5. Determination of Total Flavonoid Content

The determination of flavonoid compound types including rutin and quercetin. A procedure for rutin and quercetin in *C. caudatus* extract using HPLC as reported by Sharifuldin et al., (2016) with slight modification. RP-HPLC Instrument contains a stationary phase using the C18 column (Lichrospher@ 100 (5 µm)) and the mobile phase consists of 2% acetic acid in water and acetonitrile mixtures 70:30 v/v by the isocratic system and the detector used is a UV detector. The flow rate of the mobile phase is controlled at 1 ml/min and detected at 254 nm. The standard solution mixtures of rutin and quercetin were created in ranging of 2.5-80 µg/mL.

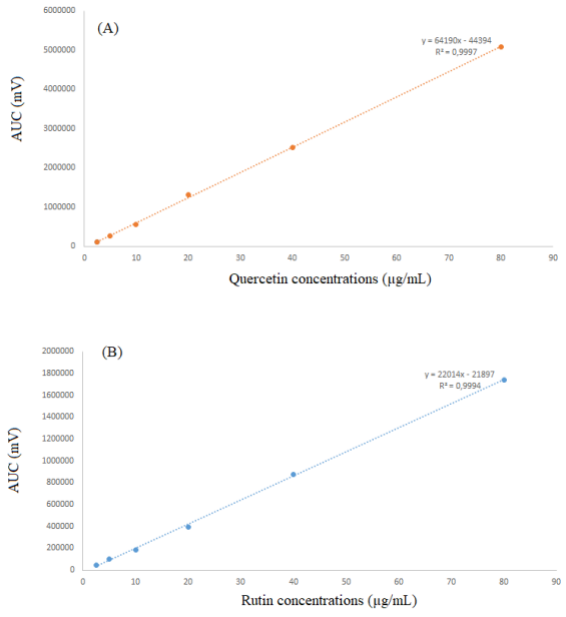


Figure 1. Standard Curve from HPLC: (A) Quercetin Standard; (B) Rutin Standard

Before injection, the sample or extract and standard solutions were filtered using 0.45 syringe filters. The flavonoid and Rutin standard curves (figure 1).

2.2.6. Antioxidant Evaluation of DPPH (2,2-diphenyl-1-picrylhydrazyl) Inhibition

The determination of the antioxidant activity of DPPH was illustrated by Ahda et al., (2019) with slight modification. The extract of *C. caudatus* leaves was dissolved using ethanol in a concentration range of 0-500 µg/mL. 1 mL extract solution was added to 1 mL of 0.05 mM DPPH solution and mixed for 1 minute and then kept for 1 hour. Finally, the solution was read the absorbance by a UV-Vis spectrophotometer at 516 nm and then determine the inhibition concentration 50 (IC_{50}) following the equation:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

Where A_0 is the absorbance of control; A_1 is the absorbance of the samples

2.2.6. Statistic Anlaysis

The data was calculated using Tukey's test to show significant differences ($p<0.05$) and the Coefficient (r) of Person correlation to determine the best correlation between two variables.

3.Results and discussions

3.1. Determination of Quercetin and Rutin Concentrations of *C. caudatus* Leaves extracted by various ethanol concentrations and solid-to-solvent Ratio

Several active compounds in *C. caudatus* leaf extract have high antioxidant activity. This extract contains flavonoids such as quercetin and kaempferol, according to previous research by Andarwulan et al. (2010). In another study, three active compounds were discovered in *C. caudatus* leaf extract: quercitrin, rutin, and quercetin (Sharifuldin, et al., 2016). These flavonoid classes have a strong inhibitory effect on DPPH radicals. However, this study only

examined the levels of rutin and quercetin in the extract.

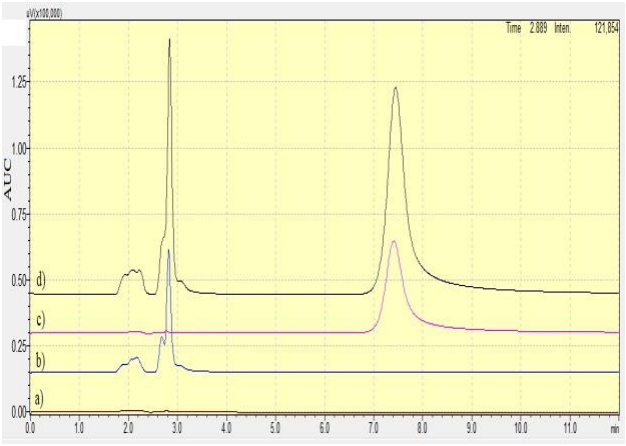


Figure 2. Chromatogram from HPLC (mobile phase: 0.3% formic acid in 0.3% formic acid and acetonitrile mixtures 30:70 v/v): a) Solvent; b) Rutin standard; c) Quercetin standard; d) Mixing of both rutin and quercetin standards

In a previous study, the mobile phase used to detect quercetin and rutin was a mixture of eluent A (water), eluent B (methanol), and eluent C (acetic acid) with gradient technique following initial conditions B 15% and C 5%, then B eluent is increased up to 25% in 15 min, 85% in 5 min, then is kept isocratic for 10 min, and B is decreased up to 15% in 5 min (Iacopini, et al., 2008). This study was carried out for 12 minutes using a mobile phase of 0.3% formic acid: acetonitrile: 30:70 (v/v) with isocratic. Rutin and quercetin were clearly separated, with rutin having a retension time of around 2.8 min and quercetin having a time retention of around 7.3 min, respectively (Figure 2). Figure 3 depicts the presence of four dominant active compounds in *C. caudatus* leaf extract, two of which are rutin and quercetin. Previous research indicates that quercitrin and kaempferol are two active compounds that may be the dominant peak (II) and peak (III). However, in order to clarify this statement, it should be proven by material standards. Based on this result, the mixtures of 0.3% formic acid: acetonitrile: 30:70 (v/v) can separate each component in the leaf extract of *C. caudatus*.

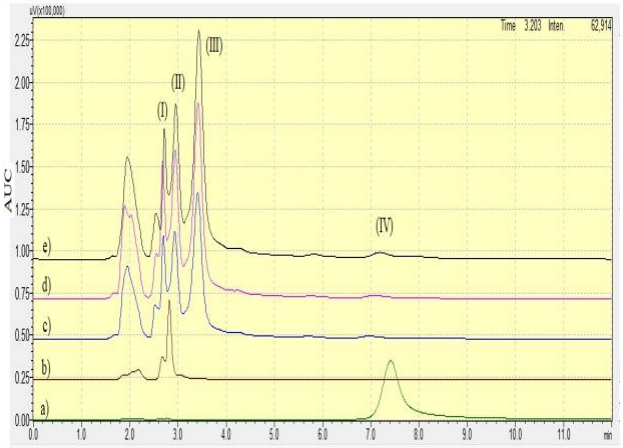


Figure 3. Chromatogram from HPLC (mobile phase: 0.3% formic acid in water and acetonitrile mixtures 30:70 v/v): a) Quercetin standard; b) Rutin standard; c) 40% ethanolic extract of *C. caudatus*; d) 60% ethanolic extract of *C. caudatus*; e) 80% ethanolic extract of *C. caudatus*. (I) rutin; (II) quercetin; (III) and (IV) unknown compounds.

The quercetin and rutin extracted from *C. caudatus* leaves were influenced by solvent concentrations and the solid-to-solvent ratio. According to Ghasemzadeh et al. (2011), increasing the solvent polarity from chloroform to methanol increases the concentrations of quercetin, catechin, and rutin in young Ginger extract. In general, the mechanism of organic solvent with flavonoid and rutin occurs through several steps such as solvation, intermolecular force, and hydrogen bonding interaction (Tamayo-Ramos et al., 2022). As a result, the extracted active compounds are strongly influenced by the extraction process, including solvent concentration, solvent type, temperature, and so on. Furthermore, they are directly associated with biological activities such as antioxidant activity. According to Felhi et al. (2017), solvent extraction affected yield, total phenolic, total flavonoid, and other active compounds, as well as their antioxidant and antimicrobial activities. Based on the current study, the 40% ethanolic extract of *C. caudatus* leaves has the highest rutin concentrations, while the 80% ethanolic extract has the highest quercetin concentrations (Figure 4 A). Besides, the solid-to-solvent ratio affected significantly

the rutin content of this extract but quercetin content was affected slightly. Therefore, in this study, the best solid-to-solvent used is 1:6 or 1:8 based on the rutin content. However, the highest quercetin was produced from the solid-to-solvent ratio of 1:12.5 (Figure 4 B). A previous study reported that both bioactive compounds (rutin and quercetin) play an important role in reducing oxidation damage, with quercetin being more effective than rutin, with DPPH inhibition rates of around 93.8% and 65.3% in Hydroxypropyl -Cyclodextrin formulations, respectively (Basaran et al., 2022). Therefore, the differences in quercetin and rutin contents of *C. caudatus* leaf extract will induce its biological activity including the inhibition activity of DPPH.

3.2. Total Phenolic Content and Inhibition Activity of DPPH Radicals

Total phenolic content (TPC) is a broad parameter that is linked to biological activities such as antioxidant properties. The solvent concentrations used also had an effect on the TPC of the herb extract. According to previous research, 80% methanol and 80% ethanol produced higher TPC and antioxidant activities (Sultana et al., 2009). There is no noticeable difference between TPC extracted with 100% ethanol and TPC extracted with 80% ethanol (Wan-Nadilah et al., 2019). Other studies discovered that extracts containing 50% ethanol, 70% ethanol, and acetone contained the most phenolic and flavonoid compounds, which will contribute to their biological activity as DPPH radical inhibition (Dirar et al., 2018; Ngo et al., 2017). According to Do et al., (2014), 100% ethanol can produce a good extract from *Limnophila aromatica* with TPC value, flavonoid concentrations, and DPPH antioxidant activity of 40.50±0.88 mg gallic acid equivalent/g of defatted extract, 31.11±0.433 mg quercetin equivalent/g of defatted extract, and IC_{50} value of 70.06±1.0 0 mg/mL, respectively.

According to this study, 40% ethanol is a good solvent for producing C. The highest TPC value and Rutin concentrations are found in *C. caudatus* extract, which has 91.32±0.92 µg gallic acid equivalent/mg dried extract and 11.35±0.19 µg/mg, respectively (Figure 5 A and Figure 4 A). Meanwhile, 80% ethanolic extract has the highest quercetin concentrations and DPPH radical inhibition activity (Figure 4 A and Figure 5 B). This study found that rutin content contributed to TPC, whereas quercetin is an active compound that inhibits DPPH radicals more effectively than rutin. The highest antioxidant activity (smaller IC_{50}: 116.66 µg/mL) was demonstrated by the 80% ethanolic extract. A similar result from Iacopini et al., (2008) reported that quercetin has better inhibition activity than rutin where inhibition activities are 22.37±0.3% and 17.17±0.3%, respectively.

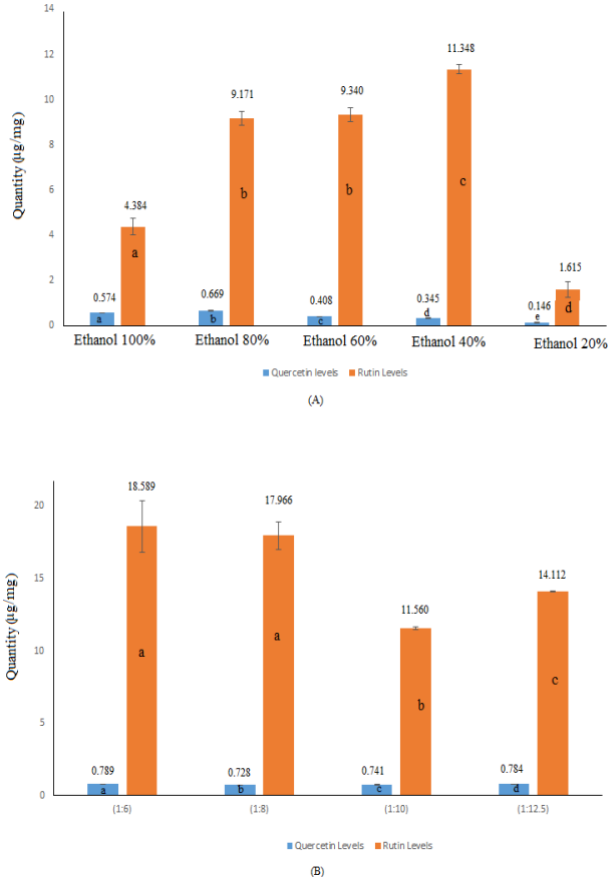


Figure 4. Quercetin and Rutin concentrations of *C. caudatus* leaves extracted by various ethanol concentrations (A) and raw material to ethanol ratio (B). Tukey's tests: a, b, c, d, and e have $p < 0.05$

Aside from solvent concentration, the solid-to-solvent ratio influences the extracted active compound from herbs. Wong et al. (2013) found that a solid-to-solvent ratio of 1:20 had a significant effect on TPC, TFC, and both antioxidant activities of DPPH and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\cdot}$). Despite this, a higher solid-to-solvent ratio increases the active compound's interaction with the solvent, and thus the extracted active compounds also increase. According to another study, increasing the solid-to-solvent ratio affected the extracted carotenoids from herbs (Norshaliza et al., 2017; Dianursanti et al., 2020).

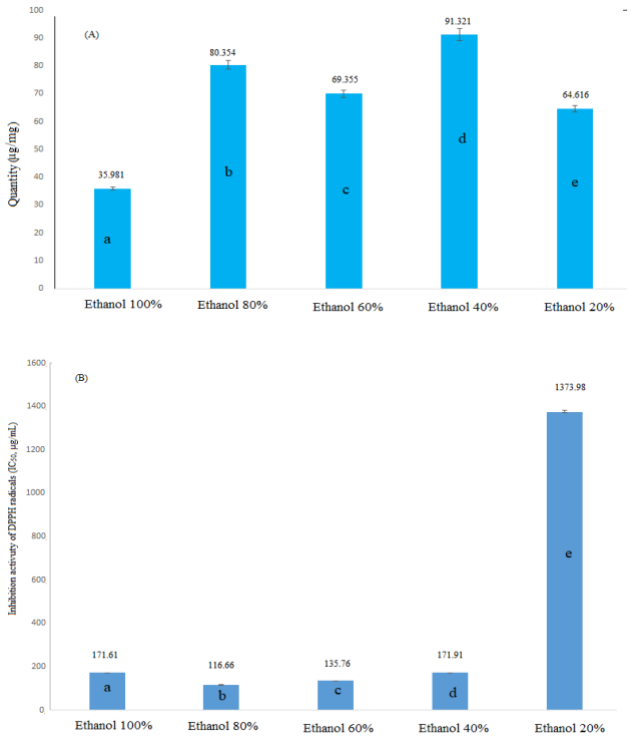


Figure 5. TPC of *C. caudatus* leaves (A) and Inhibition Activity of DPPH (B). n:3; Tukey's tests: a, b, c, d, and e have $p< 0.05$

Figure 6 illustrated that the increase in the solid-to-solvent ratio caused the TPC is decreasing. The optimum solid-to-solvent ratio providing the higher TPC is at 1:6 and 1:8 (Figure 5 A). Besides, the extract of *C. caudatus* (1:6) contains quercetin content is higher than the extract of *C. caudatus* (1:8) but the rutin content is the opposite. Rutin and quercetin concentrations of this herb are about 17.97-18.59 µg/mg (or 1.79-1.86%) and 0.73-0.79 µg/mg (or 0.073-0.079%), respectively. Previous research has reported that rutin and quercetin concentrations in *C. caudatus* are about 0.38-0.94% and 0.26-0.92%, respectively (Sharifuldin et al., 2016; Seyedreihani et al., 2016). However, the solid-to-solvent ratio of 1:6 is the better scavenging activity of DPPH radicals than 1:8 with IC_{50} values are 108.28±0.01 µg/mL and 128.81±0.18 µg/mL, respectively (Figure 5 B). In this case, quercetin content also acts significantly on inhibiting DPPH radicals.

According to this finding, the solid-to-solvent ratio is not always positively correlated with TPC, rutin, and quercetin concentrations, or their antioxidant activity, but is highly dependent on the nature of the active compound taken up by the solvent. According to this study, a smaller solid-to-solvent ratio produces the best extract, which contains more active compounds extracted and its antioxidant activity of DPPH because the kinetic rate of diffusion cannot be controlled for the extraction process at 50 ^{0}C. However, as the solvent used is reduced, the extraction temperature rises, and the extracted active compound rises as well.

In the discriminant analysis, both the 80% and 60% ethanol extracts of *C. caudatus* leaves were significant neighbors (Figure 7). This study discovered that ethanolic extracts of *C. caudatus* leave at 80% and 60% concentrations were not significantly different. This was supported by rutin levels in both the 80% ethanolic extract and the 60% ethanolic extract of this herb. Both extracts effectively inhibit DPPH radicals. It is further supported by Wan-Nadilah et al. (2019), who properly categorised the various extracts of this plant using H-NMR spectroscopy combined with Principal component analysis (PCA), with 60% extract and 80% extract being the closest. This study also produced a classification clarified by PC1 and PC2 of all *C. caudatus* leaf extracts with an Eigenvalue cumulative is 100%. (Figure 7). Therefore, the similarity of *C. caudatus* leaves 80% ethanol extract and 60% ethanol extract is

more accurate. To ensure this study, the determination of all active compounds in *C. caudatus* leaf extracts should be performed using an advanced analytical method such as LC-MS/MS

3.3. The Correlation of TPC, Rutin and Quercetin Levels, and Scavenging Activity of DPPH Radicals

The correlation of active compounds from *C. caudatus* leaves ethanol extract is usually related to TPC because it greatly contributed to its activity, particularly the DPPH radical scavenging activity (Muflihah et al., 2021). The best correlation between TPC and its antioxidant activity will provide useful information for product quality control. Several active compounds from Allium extract, such as catechin, epigallocatechin, and epicatechin gallate, were strongly influenced by antioxidant activity (Beretta et al., 2017). As a result, the concentrations of rutin and quercetin, TPC, and the DPPH radical scavenging activity will be correlated in this study.

This study explained the relationship between the active compounds in *C. caudatus* leaf extract and its antioxidant activity. Quercetin and rutin have a weak correlation with DPPH inhibition (Figure 8 in supplementary data). However, the antioxidant activity of an active compound is significantly affected by its configuration, total number of hydroxyl groups, and substitution of functional groups within the structure (Kumar and Pandey, 2013). Previous research found that curcumin is the most active compound in *Zingiberaceae* species and is highly correlated with antioxidant activity, while quercetin is the inverse (Muflihah et al., 2021). According to Souza et al. (2021), several active compounds in tomatoes, such as anthocyanins and carotenoids, have a moderate correlation with FRAP and a weak correlation with DPPH inhibition. Table 1 shows a Pearson's correlation that supported this finding. According to Table 1. Rutin concentrations correlate better with TPC than quercetin, while quercetin correlates better with DPPH inhibition than rutin. As a consequence, the mechanism of action takes place in this study to further clarify this finding, as illustrated in Figure 9.

The inhibition of DPPH radicals is heavily dependent on the ease of proton radical movement, so the DPPH radical becomes a non-radical product. Shamsudin et al., (2022) reported that the hydroxyl groups in the ring of C5, C7, C3', and C4' will cause antibacterial activity increase. According to its chemical structure, quercetin has 5 hydroxyl groups that are likely to inhibit DPPH radicals, whereas rutin only has 4 hydroxy groups involved in this reaction. The glucoside component of rutin has no effect on DPPH inhibition, but it is highly reactive with the Folin ciocalteu reagent. As a result, quercetin levels correlate with DPPH radical inhibition, while rutin levels correlate with TPC (Table 1). However, the antioxidant activity of quercetin is better than rutin (Kessler et al., 2003; Hernández-Barreto et al., 2023).

Table 1. Coefficient (r) of Pearson's correlation between TPC, Rutin and Quercetin concentrations and scavenging activity of DPPH

Variables	**TPC**	**Scavenging activity of DPPH (IC_{50}, µg/mL)**
TPC	1	-0.428
Rutin concentrations	0.880	-0.632
Quercetin concentrations	0.797	-0.713
Scavenging activity of DPPH	-0.428	1

* Medium Pearson's Correlation ($0.4 < r < 0.59$); high Pearson's Correlation ($0.6 < r < 0.79$); and very high Pearson's Correlation ($0.8 < r < 1.0$)

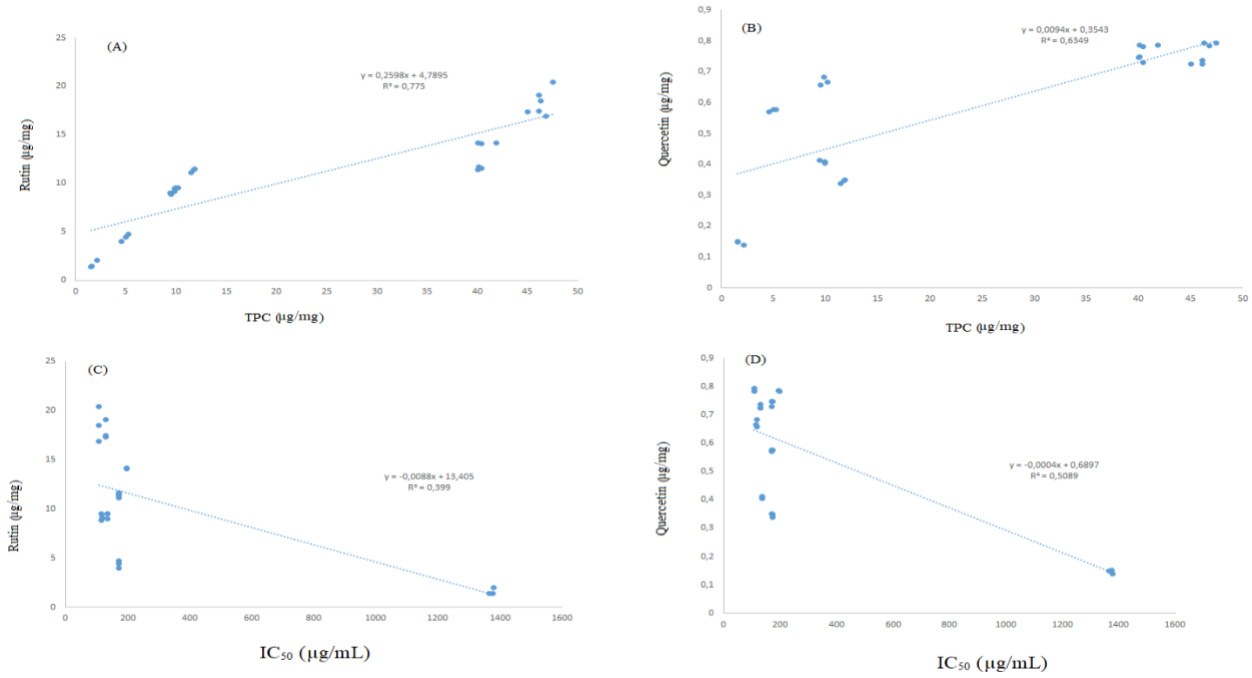


Figure 8. Correlation of the contribution of active compounds: A) Rutin Vs TPC; B) Quercetin vs TPC; C) Rutin Vs Inhibition activity of DPPH (IC_{50}); D) Quercetin Vs Inhibition activity of DPPH (IC_{50})

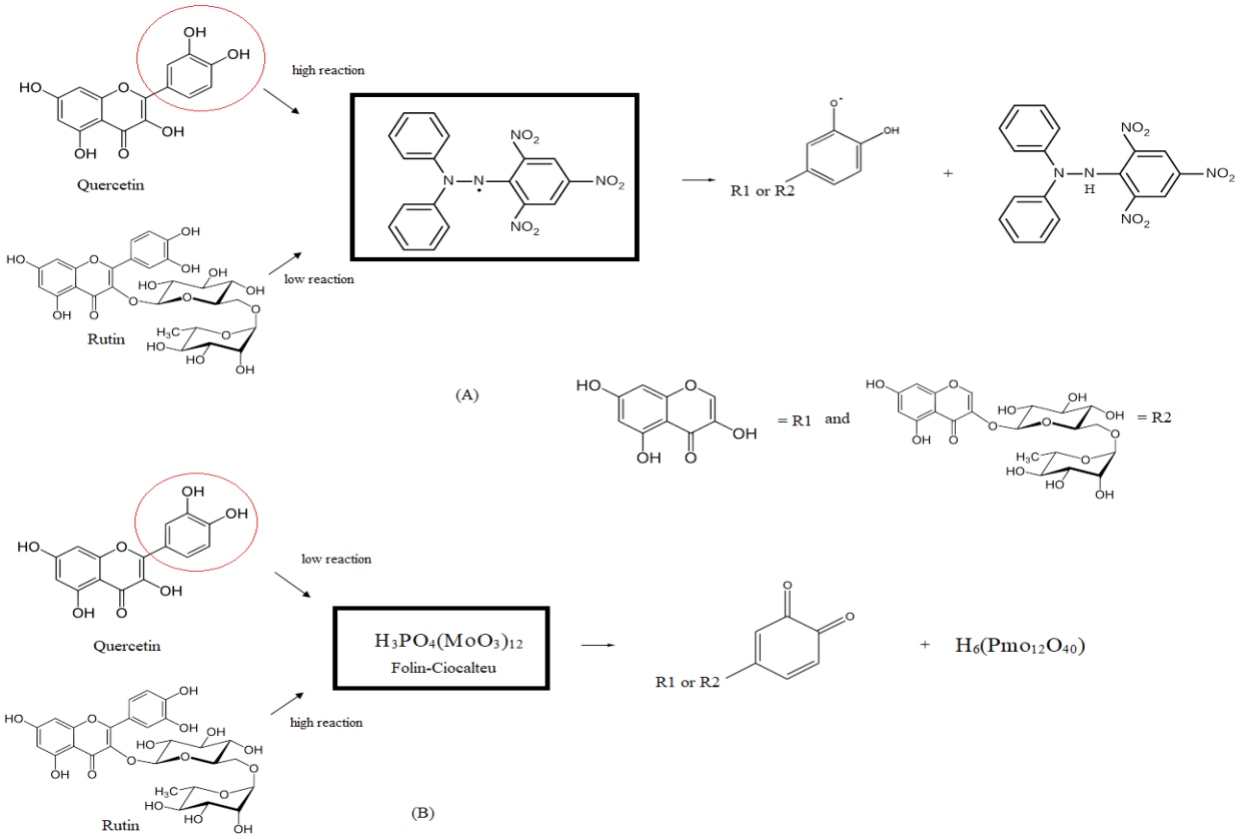


Figure 9. The oxidation reaction of DPPH radicals (A) and Folin Ciocalteu reagent (B)

4. Conclusions

This study showed that 60% ethanolic extract and 80% ethanolic extract of *C. caudatus* leaves had better antioxidant activity than other extracts. The optimum raw material to solvent ratio is 1:6 (b/v) containing rutin and quercetin concentrations around 18.59±1.77 µg/mg and 0.728±0.006 µg/mg, respectively. This study also concluded that quercetin is one of the active compounds from *C. caudatus* that plays a significant role in the DPPH radical scavenging, with better inhibition activity than rutin. Furthermore, TPC has a moderate correlation with DPPH inhibitory activity, as evidenced by Pearson's Correlation (r) < 0.6.

5. References

Ahda, M., Lestari, W, T., Rahayu, R. L., S. 2019. Total Phenol Content and Antioxidant activity of Various Concentrations of Ethanol Extract of Psidium Guajava L, *International Journal of Pharmaceutical Research*, 11(3), 1077-1082. https://doi.org/10.31838/ijpr/2019.11.03.078

Ahda, M., Jaswir, I., Khatib, A., Ahmed, Q. U., Syed Mohamad, S. N. A. 2023. A review on Cosmos caudatus as A potential medicinal plant based on pharmacognosy, phytochemistry, and pharmacological activities. *International Journal of Food Properties*, 26(1), 344-358. https://doi.org/10.1080/10942912.2022.2158862

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., and Lightfoot, D. 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6(4), 42. doi:10.3390/plants6040042

Basaran, E., OZTÜRK, A. A., Senel, B., Demirel, M.., and Sarica, S. 2022. Quercetin, Rutin And Quercetin-Rutin Incorporated Hydroxypropyl β-Cyclodextrin Inclusion Complexes, *European Journal of Pharmaceutical Sciences*, 173 (1), 106153, 1-11. https://doi.org/10.1016/j.ejps.2022.106153

Beretta, H. V., Bannoud, F., Insani, M., Berli, F., Galmarini, C. R., Cavagnaro, P. F., and Hirschegger, P. (2017). Relationships among Bioactive Compounds Content and the Antiplatelet and Antioxidant Activities of Six Allium Vegetable Species. *Food Technology and Biotechnology*, 55(2). doi:10.17113/ftb.55.02.17.4722

Chan, E. W. C., Wong, S. K., and Chan, H. T. 2016. Ulam Herbs of Oenanthe javanica and Cosmos caudatus: An Overview on their Medicinal Properties. Journal of Natural Remedies, 16(4), 137-147. doi: 10.18311/jnr/2016/8370

Cheng, S. H., Barakatun-Nisak, M.Y., Anthony, J., Ismail, A. 2015[a]. Potential medicinal benefits of *Cosmos caudatus* (Ulam Raja): A scoping review. *Journal of Research in Medical Sciences*, 20, 1000-6.

Cheng, S.H., Khoo, H. E., Ismail, A., Abdul-Hamid, A., and Barakatun-Nisak, M. Y. 2016[b]. Influence of Extraction Solvents on *Cosmos caudatus* Leaf Antioxidant Properties. Iranian *Journal of Science and Technology, Transactions A: Science*, 40(1), 51–58. doi:10.1007/s40995-016-0007-x

Dianursanti, Siregar, A.R., Maeda, Y., Yoshino, T., Tanaka, T., 2020. The Effects of Solvents and Raw material-to-Solvent Ratios on Ultrasound-Assisted Extraction of Carotenoids from Chlorella vulgaris. *International Journal of Technology*. 11(5), 941-950. doi **:** https://doi.org/10.14716/ijtech.v11i5.4331

Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., & Devkota, H. P. (2018). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*. doi:10.1016/j.sajb.2018.07.003

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food*

and Drug Analysis, 22(3), 296–302. doi:10.1016/j.jfda.2013.11.001

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., & Kadri, A. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. *Food Science and Technology*, 37(3), 483–492. doi:10.1590/1678-457x.23516

Ghasemzadeh, A., Jaafar, H. Z. E., and Rahmat, A. 2011. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (Zingiber officinale Roscoe) extracts. *Journal of Medicinal Plants Research*. 5(7), 1147-1154

Hernández-Barreto, E., Ruz-Sanjuan, V., Ribalta-Ribalta, V., Torres-Gómez, L. A. 2023. Optimization of the molybdenum blue method for estimating the antioxidant activity of natural products. *Journal of Pharmacy & Pharmacognosy Research*, 11 (6), 953-960. DOI: https://doi.org/10.56499/jppres23.1646_11.6.953

Iacopini, P., Baldi, M., Storchi, P., and Sebastiani, L. 2008. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. *Journal of Food Composition and Analysis*, 21(8), 589–598. doi:10.1016/j.jfca.2008.03.011

Javadi, N., Abas, F., Hamid, A. A., Simoh, S., Shaari, K., Ismail, I. S., Mediani, A., and Khatib, A. 2014. GC-MS-Based Metabolite Profiling of *Cosmos caudatus* Leaves Possessing Alpha-Glucosidase Inhibitory Activity. *Journal of Food Science*, 79(6), C1130–C1136. doi:10.1111/1750-3841.12491

Javadi, N., Abas, F., Mediani, A., Hamid, A. A., Khatib, A., Simoh, S., and Shaari, K., 2015. Effect of storage time on metabolite profile and alpha-glucosidase inhibitory activity of Cosmos caudatus leaves-GCMS based metabolomics approach. *Journal of Food and Drugs Analysis*, 23 (3), 433-441. doi:10.1016/j.jfda.2015.01.005

Kessler, M., Ubeaud, G., and Jung, L. 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *Journal of Pharmacy and Pharmacology*, 55(1), 131–142. doi:10.1211/002235702559

Kumar, S., and Pandey, A. K. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*, 1–16. doi:10.1155/2013/162750

Mediani, A., Abas, F., Khatib, A., and Tan, C.P. 2013. Cosmos Caudatus as a Potential Source of Polyphenolic Compounds: Optimisation of Oven Drying Conditions and Characterisation of Its Functional Properties. *Molecules*, 18, 10452-10464. doi:10.3390/molecules180910452

Muflihah, Y. M., Gollavelli, G., Ling, Y. C. 2021. Correlation Study of Antioxidant Activity with Phenolic and Flavonoid Compounds in 12 Indonesian Indigenous Herbs. *Antioxidants*, 10, 1530. https:// doi.org/10.3390/antiox10101530

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P. D., & Vuong, Q. V. 2017. Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of Salacia chinensis L. *Journal of Food Quality*, 1–8. doi:10.1155/2017/9305047

Norshazila, S., Koy, C. N., Rashidi, O., Ho, L. H., Azrina, I., Nurul Z. R. A., Zarinah, Z. 2017. The Effect of Time, Temperature and Solid-to-solvent Ratio on Pumpkin Carotenoids Extracted Using Food Grade Solvents, *Sains Malaysiana* 46(2), 231–237 http://dx.doi.org/10.17576/jsm-2017-4602-07

Nurhayati, B., Rahayu, I. G., Rinaldi, S. F., Zaini, W. S., Afifah, E., Arumwardana, S., Kusuma, H. S. W., Rizal, and Widowati, W. 2018. The Antioxidant and Cytotoxic Effects of Cosmos caudatus Ethanolic Extract on Cervical Cancer. The Indonesian Biomedical Journal, 10(3): 243-249.

Shamsudin, N.F., Ahmed, Q.U., Mahmood, S., Ali Shah, S.A., Khatib, A., Mukhtar, S.,

Alsharif, M.A., Parveen, H., Zakaria, Z.A. 2022. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules*, 27, 1149. https://doi.org/10.3390/molecules27041149

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., and Yoga Latha, L. 2011. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts, *African Journal* of *Traditional*, *Complementary* and *Alternative* Medicines, 8(1), 1-10

Seyedreihani, S. F., Tan, T.-C., Alkarkhi, A. F. M., and Easa, A. M. 2016. Total phenolic content and antioxidant activity of Ulam raja (Cosmos caudatus) and quantification of its selected marker compounds: Effect of extraction. *International Journal of Food Properties*, 20(2), 260–270. doi:10.1080/10942912.2016.1155055

Sharifuldin, M. M. A., Ismail, Z., Aisha, A. F. A., Seow, E. K., and Beh, H. K. 2016. Quantification of rutin, quercitrin and quercetin in Cosmos caudatus Kunth by reverse phase high performance liquid chromatography. *Quality Assurance and Safety of Crops & Foods*, 8(4), 617–622. doi:10.3920/qas2015.0839

Sharif, M. F., and Bennett, M. T. 2016. The Effect of Different Methods and Solvent on the Extraction of Polyphenol in Ginger (Zingiber officinale. *Jurnal Teknologi*, 78, 49-54. doi:10.11113/jt.v78.9943

Souza, A. V., Mello, J. M., Silva Favaro, V. F., Silva, V. F., Santos, T. G. F., Sartori, D., and Putti, F. F. 2021. Antioxidant activity, bioactive compounds, and agro-industrial quality: Correlations between parameters in fresh and processed tomatoes. *Journal of Food Processing and Preservation*, 45(9), 1-28. doi:10.1111/jfpp.15696

Suhaimi, S. H., Hasham, R., Hafiz Idris, M. K., Ismail, H. F., Mohd Ariffin, N. H., and Abdul Majid, F. A. 2019. Optimization of Ultrasound-Assisted Extraction Conditions Followed by Solid Phase Extraction Fractionation from Orthosiphon stamineus Benth (Lamiace) Leaves for Antiproliferative Effect on Prostate Cancer Cells. *Molecules*, 24(22), 4183. doi:10.3390/molecules24224183

Sultana, B., Anwar, F., and Ashraf, M. 2009. Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules*, 14(6), 2167–2180. doi:10.3390/molecules14062167

Tamayo-Ramos, J. A., Martel, S., Barros, R., Bol, A., Atilhan, M., and Aparicio, S. 2022. On the behavior of quercetin + organic solvent solutions and their role for C60 fullerene solubilization. *Journal of Molecular Liquids*, 345(117714), 1-10. https://doi.org/10.1016/j.molliq.2021.117714

Wan-Nadilah, W. A., Akhtar, M. T., Shaari, K., Khatib, A., Hamid, A. A., & Hamid, M. (2019). Variation in the metabolites and α-glucosidase inhibitory activity of Cosmos caudatus at different growth stages. *BMC Complementary and Alternative Medicine*, 19(1), 1-15. doi:10.1186/s12906-019-2655-9

Wong, B. Y., Tan, C. P., and Ho, C. W. 2013. Effect of solid-to-solvent ratio on phenolic content and antioxidant capacities of "Dukung Anak" (Phyllanthus niruri). *International Food Research Journal*, 20(1), 325-330.

Acknowledgment

The authors are thankful to LPPM Universitas Ahmad Dahlan due to research funding in 2021 with contract numbers: PD-091/SP3/LPPM-UAD/VI/2021.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

NEUROPROTECTIVE EFFECTS OF HESPERIDIN: IN-VITRO AND IN-SILICO EVALUATION OF ITS ANTIOXIDANT AND ENZYME INHIBITORY ACTIVITIES

Mimansa Kandhwal[1], Amarjot Kaur Grewal[1]✉, Manjinder Singh[1]✉, Varinder Singh[2], Thakur Gurjeet Singh[1]

[1]*Chitkara College of Pharmacy, Chitkara University, Punjab, India.*
[2]*Department of Pharmaceutical sciences and Technology, Maharaja Ranjit Singh Punjab Technical University, Bathinda, Punjab, India.*
✉*amarjot.kaur@chitkara.edu.in;* ✉*manjinder.singh@chitkara.edu.in;*

https://doi.org/10.34302/crpjfst/2024.16.4.15

Article history:
Received
October 23th, 2024
Accepted
November 30th, 2024

Keywords:
Acetylcholinesterase;
Hesperidin;
Monoamine oxidase;
Neuroprotection;
Flavonoid.

Abstract
Brain disorders are persistent medical conditions characterized by a gradual deterioration in neurological functioning. There is a worldwide increase in interest in phytomedicines for their beneficial health benefits and low adverse effects. Hesperidin (Hsp), a flavanone glycoside in citrus fruit peels, has many pharmacological characteristics. Nevertheless, there is a need for more comprehensive investigations that elucidate the underlying mechanism of action. The objective of this work is to assess the neuroprotective impact of Hsp using in-vitro tests for the inhibition of acetylcholinesterase (AChE), monoamine oxidase (MAO), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (H2O2), followed by in-silico techniques such as molecular docking and molecular dynamics. The outcomes of the current investigation demonstrate significant inhibitory effects on AChE, MAO, DPPH, and H2O2, which may be attributed to the intended pharmacological actions of Hsp. In-silico studies showed strong interactions of Hsp with targeted proteins. Thus, Hsp has the potential to be formulated as a neuroprotective medication.

1. Introduction

Neurons have a vital role in promoting communication, hence ensuring the efficient functioning of the human brain (Morrison 1997). The finite lifespan of neurons is a significant factor in the pathogenesis of various brain disorders, including Alzheimer's Disease, Parkinson's disease, Epilepsy, Huntington's disease, and major Depression. This is primarily attributed to the progressive reduction in neuronal population, alterations in structural integrity, and compromised functional capacities, collectively known as neurodegeneration. In addition to this, the modified functioning of metabolic enzymes, which subsequently impact the release of neurotransmitters, may also play a role in the pathophysiology of many affective disorders like anxiety and phobias. A complete understanding of the exact causes of brain disorders is not comprehensively elucidated; however, they are commonly correlated with abnormal production of reactive oxygen species, and disturbed activity of enzymes involved in the metabolism of Neurotransmitters.

The development and progression of brain diseases are associated with oxidative stress (Hayashi et al., 2012). The aforementioned situation occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the human body to mitigate the resulting damage. Reactive

oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are naturally occurring byproducts (Hoidal, 2001) from several biological processes, including metabolic activities. Usually, the human body has natural mechanisms to combat reactive oxygen species (ROS) and reduce damage. However, when the level of oxidative stress exceeds the ability of these protective systems, it may damage the structure and function of neurons (Rao et al. 2011; Popa et al. 2013). Reactive oxygen species (ROS) have been found to harm the brain, leading to the incorrect folding and buildup of specific proteins namely alpha-synuclein in Parkinson's disease, amyloid-beta in Alzheimer's disease, and huntingtin in Huntington's disease (Sun and Chen 1998; Abdelhamid et al. 2023; Liu et al. 2023). The formation of these misfolded proteins produces harmful clusters, disrupting cellular functions and facilitate the death of neuronal cells. ROS have the potential to cause adverse impacts on mitochondrial DNA and proteins, leading to compromised energy generation and an augmented ROS production (Li et al., 2022, Rehman et al., 2022). Antioxidant drugs have the potential to act as adjuvant drugs to benefit in brain disorders caused by ROS.

In addition to oxidative stress, the dysregulation of enzymes implicated in neurotransmitter metabolism also leads to disruptions in brain functionality, primarily linked to conditions such as Alzheimer's disease (AD) (Bai et al., 2022), major depression, and mood disorders (Bhatt et al., 2020). Alzheimer's disease (AD) is distinguished by the deterioration of cholinergic neurons, which are a specific kind of neuronal cell that plays a crucial role in facilitating communication within the central nervous system via the use of the neurotransmitter acetylcholine (ACh) (Bekdash, 2021). Acetylcholine (ACh) is mainly found in the hippocampal regions of the brain and is closely linked to memory and cognitive processes inside the human body. The enzyme acetylcholinesterase (AChE) is responsible for breaking down acetylcholine (ACh) in the synaptic cleft, which stops the passage of signals and impacts memory (Benzi and Moretti 1998). AD is characterized by increased activity of AChE, leading to the use of potent acetylcholinesterase inhibitors (tacrine and donepezil) as the primary therapy for AD (Marucci et al., 2021).

The course of depression symptoms is also influenced by changes in the levels of MAO-B in the brain (Klimek et al., 2003). The enzymatic catalyst known as monoamine oxidase (MAO) plays a crucial role in the modulation of neurotransmitters such as serotonin, norepinephrine, and dopamine in the cerebral region, (Shih et al., 1999). The primary role of MAO-B is the enzymatic breakdown of the monoamines which play a crucial role in regulating mood and motivation (Feinberg et al., 2016). Any change in the levels of monoamines contribute to manifestation of symptoms associated with depression and mood disorders. From extensive analysis of the literature, it has been determined that there is a need to develop drugs with neuroprotective properties to treat the brain disorders effectively. The significance of flavonoids in treating neurological diseases is increasing due to their diverse pharmacological actions (Ayaz et al., 2019, Kim et al., 2019). Hesperidin is a bioflavonoid predominantly found in Citrus fruits such as oranges, lemons, and grapefruits (Hajialyani et al., 2019). Its antioxidant and anti-inflammatory properties help protecting neural cells from oxidative stress and inflammation, which are often implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's (Kim et al., 2019). Research suggests that hesperidin may improve cognitive function and memory, offering a promising complementary approach to traditional treatments for brain health (Roohbakhsh et al., 2014). Despite of diverse pharmacological actions of Hsp, studies revealing the mechanism of action associated with Hsp's neuroprotective action are scanty.

In the present investigation, in-vitro assays were conducted to assess the antioxidant characteristics and AChE and MAO-B inhibitory ability of Hsp. To confirm the

efficacy of the pharmacological investigations on the specific enzymes in achieving the intended therapeutic outcome, molecular docking experiments were conducted, followed by molecular dynamics studies. The primary objective of this study is to assess the neuroprotective properties of Hsp through the utilization of in-vitro assays measuring the inhibitory effects on acetylcholinesterase (AChE), monoamine oxidase (MAO), and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Additionally, in-silico techniques such as molecular docking and molecular dynamics studies were employed to investigate the potential of Hsp as an adjuvant therapy for various brain disorders.

2. Material and Methods

Hesperidin was procured from Sigma Aldrich. All chemicals were of analytical grade.

2.1. *In vitro* AChE inhibitory activity

The inhibitory effect of the test extract on AChE activity was evaluated by the spectrophotometric method of Ellman et al. (Classics Ellman et al. 1961). Donepezil (10–80 µg/ml) was used as standard AChE inhibitor. The control, standard, and test samples contained the following: • Control = Phosphate buffer (0.1 M, 8 pH, 2.6 ml) + DTNB (0.01 M, 0.1 ml) + distilled water (0.1 ml) + AChE (0.1 U ml−1, 0.1 ml) + ATI (0.075 M, 0.1 ml). • Standard = Phosphate buffer (0.1 M, 8 pH, 2.6 ml) + DTNB (0.01 M, 0.1 ml) + Donepezil (0.1 ml) + AChE (0.1 U ml−1, 0.1 ml) + ATI (0.075 M, 0.1 ml). • Test = Phosphate buffer (0.1 M, 8 pH, 2.6 ml) + DTNB (0.01 M, 0.1 ml) + extract (0.l ml) + AChE (0.1 U ml−1, 0.1 ml) + ATI (0.075 M, 0.1 ml). All the readings were taken in triplicate. The percentage inhibition was calculated in comparison to control (test sample absent).

The percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = 1 - (\text{absorbance of test sample} / \text{absorbance of control}) \times 100. \quad (1)$$

2.3. *In vitro* MAO-B inhibitor activity

The fluorometric technique was used to conduct the in-vitro MAO-B inhibition test, whereby the percentages and IC50 values of Hsp was determined. This inhibition assay is based on the principle of revealing the hydrogen peroxide ions via a reaction called as horseradish peroxide coupled reaction by utilizing amplex red (10-acetyl-3,7-dihydroxyphenoxazine) reagent by the method of Can et al., 2017 (Can et al. 2017).

2.4. *In vitro* antioxidant property

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Blois 1958; Kaur et al. 2017) was used to evaluate the antioxidant activity of Hsp. The expression of DPPH radical scavenging activity was determined by calculating the percentage inhibition using the following equation:

$$\% \text{ Inhibition} = \{(\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance of control}\} \times 100 \quad (2)$$

2.5.Statistical Analysis

Acetylcholinesterase, MAO-B inhibitory, DPPH and H_2O_2 inhibitory assays were presented as mean±SD and statistically analysed by one-way ANOVA followed by Tukey's multiple comparison test.

2.6. *In silico* Studies

2.6.1. Molecular docking studies

The affinities of Hsp with acetylcholinesterase and MAO-B were investigated by molecular docking tests using the Biovia Discovery Studio application. The molecular docking of Hsp was performed using the crystalline structures of the acetylcholinesterase (AChE) and MAO-B (2V52 (1.6) proteins, which were obtained from the RCSB-protein database library (PDB) (https://www.rcsb.org/) (Sharma et al. 2023; Wu et al. 2003). The structures underwent pre-processing, preparation, and optimization using the "Macromolecule" module for later analysis.

The first phase in protein pre-processing was the removal of water molecules and heteroatoms, followed by the protonation technique using the 'Add Polar' function. Following this, the "Define and Edit Binding Site" tool was used to construct and generate a binding site encompassing the co-crystallized ligands. MarvinSketch and ChemDraw 16.0 software were used to construct Hesperidin's SMILES notations and molecular structure, respectively. The ligands were synthesized and underwent comprehensive minimizing utilization of the Small Molecules module. The technique used for the docking study was the "Dock Ligands (CDOCKER)" approach. Visual examination was used to analyze the molecular interactions between docked complexes of Hsp with AChE and MAO-B. The formation and establishment of a binding site encompassing the co-crystallized ligands depended on the docking scores of the amino acids found in AChE and MAO-B.

2.6.2. Molecular dynamic Simulations

The molecular dynamics (MD) studies were performed (with simulation for 20 nanoseconds) using the molecular dynamics component of Schrodinger's Desmond software. Additionally, the thermal equilibrium of hesperidin concerning time was assessed (Furlan et al., 2021; Bhatia et al., 2023; Li et al., 2023; Sharma et al., 2023; Mahajan et al., 2020; Nagu et al., 2021). The protein-ligand connection was strengthened by reducing or minimizing energy simulations, which allowed for the attainment of hesperidin's optimal structure via interactions over time. The study investigated the connection between the complex ligand and the target macromolecule during the whole 100 nanosecond simulations, using simulation-interaction analysis (Sharma et al. 2023). This methodology offers a valuable understanding of essential ligand-interacting relationships that may be used to ascertain a ligand's affinities with enhanced accuracy. The first construction of the component used the orthorhombic box-shaped TIP3P solvable method, whereas the ionic potential of the input system was altered by applying a 0.15 M salt solution. The modeling operation was conducted using the NPT consortium and a time interval of 1.0 fs. The Nose-Hoover Chain methodology provided a constant temperature of 310 K, while the Martyn-Tobias-Klein method was utilized to fix the barometric pressure at 1.01325 bar.

2.6.3. Drug likeliness and ADME studies

It has been demonstrated in periclinal studies of drug moieties discovered earlier that early estimation of ADME (absorption, distribution, metabolism, and excretion profile) in the discovery phase reduces drastically the fraction of pharmacokinetics-related failure in the clinical phases (Han et al., 2019). To predict the pharmacokinetic study Hsp the SWISS ADME, a web server tool was used. It provides the access to a pool of quick yet reliable predictive models for physicochemical characteristics, pharmacokinetics, and drug-likeness properties (Singh et al., 2017).

Using the swiss ADME programme, the pharmacokinetic characteristics of the Hsp was assessed (http://www.swissadme.ch). A wide molecular profile, including physicochemical characteristics, pharmacokinetics, solubility, lipophilicity, and drug likeliness were examined.

3. Results and Discusions

3.1. Pharmacological studies

3.1.1. In-vitro acetylcholinesterase and MAO-B inhibitory activity

Hsp was found to be strong AChE and MAO-B inhibitor (Table 1). The present study used donepezil and selegiline as the typical inhibitor of acetylcholinesterase and MAO-B, respectively.

3.1.2. In vitro Antioxidant Properties

Hsp showed strong inhibition of DPPH and H_2O_2 with comparable IC50 value to ascorbic acid (Table 1).

Table 1. The AChE inhibition, MAO-B inhibition and radical scavenging activities of Hesperidin and reference compounds.

Compounds	AChE Inhibitory activity IC_{50} values (Mean^n±SD, µg/ml)	MAO-B inhibitory activity IC_{50} values (Mean±SD, µg/ml)	Radical scavenging activity IC_{50} values (Mean±SD, µg/ml)	H_2O_2 Assay IC_{50} values (Mean±SD, µg/ml)
Hesperidin	17.18 ±0.93	28.49 ±1.72	8.92 ±0.91	11.28 ±0.61
Donepezil	19.27 ±1.41	-	-	-
Selegiline	-	26.29 ±1.72	-	-
Ascorbic Acid	-	-	7.63 ±0.83	9.11 ± 0.52

n=3. No statistical difference was observed in Hesperidin and test drugs in all activities.

Table 2. Docking score and key interactions of Hesperidin with AChE & MAO-B enzymes

Target Proteins	-CDOCKER Interaction Energy (kcal/mol)	Types of Interaction	Key Residues
AChE	-71.03	Conventional H-bond	Tyr121, Asp285, Ser286, Glu199, His440, Tyr70.
		Pi-Pi stacking	Trp84, Leu282
		Pi-donor H-bond	Tyr334
		Pi-alkyl	Trp84
		Van der Waals	Leu358, Phe290, Leu127,
MAO-B	-68.32	Conventional H-bond	Cys397, Arg42, Tyr435, Leu171
		Carbon H- bond	Gly57, Gln206, Ile264, Ala263, Thr426
		Pi-Cation	Arg42
		Pi-sigma	Gly13
		Pi-alkyl	Phe343, Tyr60

3.2. *In Silico* Studies

3.2.1. Molecular Docking

Figure 1 and 2 depicts hesperidin's molecular docking and protein interaction studies with AChE and MAO-B. Hsp exhibited interactions with both the peripheral anionic site (PAS, Trp279, Asp285, Ser286, Glu199, Tyr334) and catalytic anionic site (CAS, Trp84, Tyr70, His440) of AChE. The binding energies of hesperidin were -71.03 Kcal/mol, much higher than the binding energies of the standard medication donepezil, which were -51.89 Kcal/mol. Hesperidin exhibited interactions with the essential amino acid residues of the active site of MAO-B (Cys397, Arg42, Tyr435, Leu171, Gly57, Gln206, Ile264, Ala263, Thr426, Arg42, Gly13, Phe343, Tyr60) by several mechanisms such as hydrogen bonding, Pi-Pi stacking, Pi-Cation interactions, and Pi-alkyl interactions. Compared to the conventional medication selegiline, Hsp exhibited superior binding energies of -68.32 Kcal/mol, surpassing the latter's binding energy of -60.12 Kcal/mol. Table 2 presents

hesperidin's docking score and critical contacts with AChE and MAO-B enzymes. The interactions of potent test compound Hesperidin in the active gorge of AChE and MAO-B with the crucial residues are displayed in **Figure 1** and **Figure 2**.

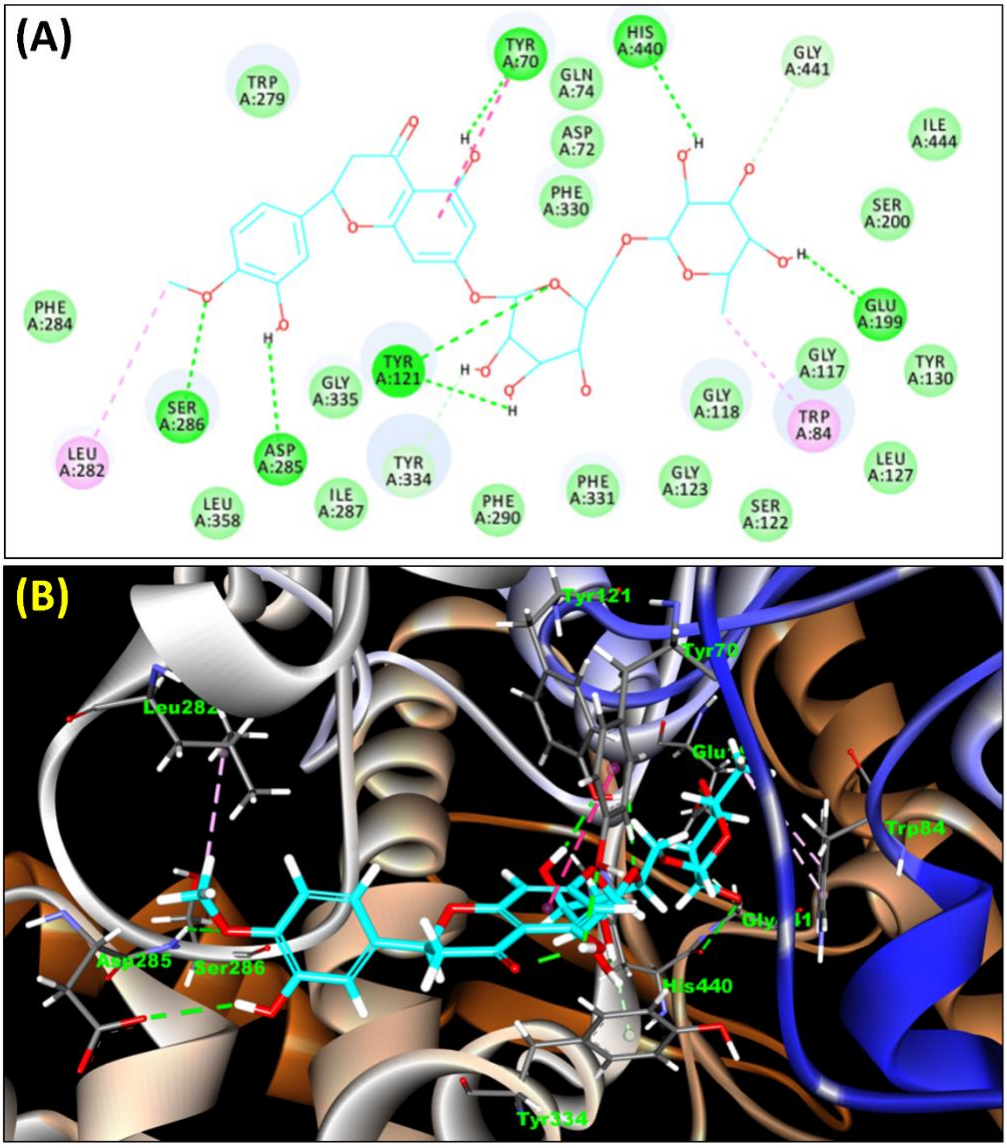


Figure 1. Interactions of Hesperidin within the binding site of AChE **(A)** 2D profile for different interactions of Hesperidin with AChE, and **(B)** 3D profile for binding of Hesperidin with TcAChE.

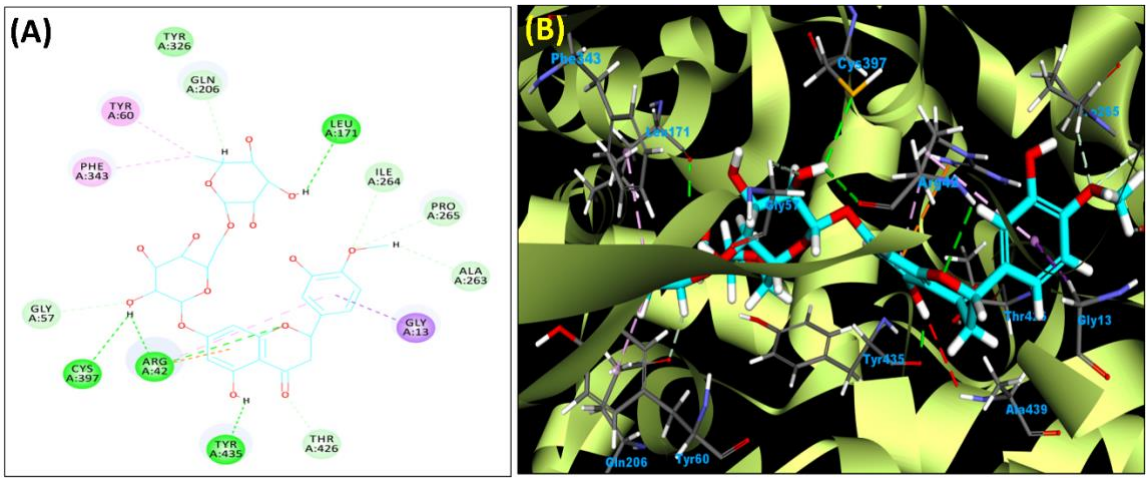


Figure 2. Interactions of Hesperidin within the binding site of MAO-B **(A)** 2D profile for different interactions of Hesperidin with MAO-B, and **(B)** 3D profile for binding of Hesperidin with MAO-B

3.2.2. Molecular dynamics simulations.

A molecular dynamics (MD) simulation was conducted on Hsp to investigate its thermodynamic stability, the dynamic behavior of the ligand-protein complex, and the influence of ligand association with the activated domain of Hsp on conformational changes (Furlan et al., 2021; Bhatia et al., 2023). The MD analysis maintained significant relationships while also revealing new linkages. The examination of the protein-ligand complex formed by AChE and Hsp demonstrated the presence of hydrophobic contacts with Trp279 and Tyr121, as well as polar and charged bonding interactions with His440 and Glu199, respectively (Figure 3A). The plot depicting the protein-ligand relationship (Figure 3B) comprehensively depicts these interactions. After conducting molecular dynamics (MD) simulations, the root mean square deviation (RMSD) of Hsp concerning the protein was calculated and is shown in Figure 3C.

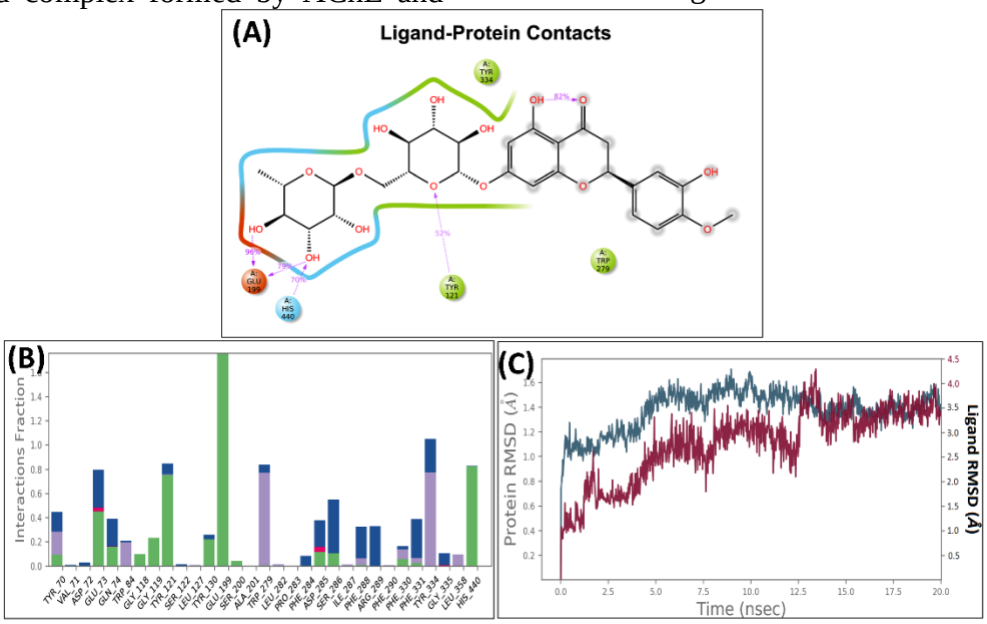


Figure 3. Post-MD interactions of Hesperidin with AChE; **(A)** Protein interactions fractions with the ligand (Hesperidin) plot throughout the simulation; **(B)** Protein-ligand contacts plot of compound Hesperidin with AChE; (C) RMSD trajectory plot for compound Hesperidin.

The similar pattern has been observed in the complex of MAO-B and Hesperidin which showed strong hydrophobic interactions with Ile198, Ala263, Tyr398 and Tyr435 along with charged interactions with active amino acid Arg42 (Figure 4A). The interactions are potted in the protein–ligand contacts plot (**Figure 4B**). The RMSD graph of the Hesperidin-MAO-B complex was displayed versus time (20ns) after MD simulation investigations (Figure 4C).

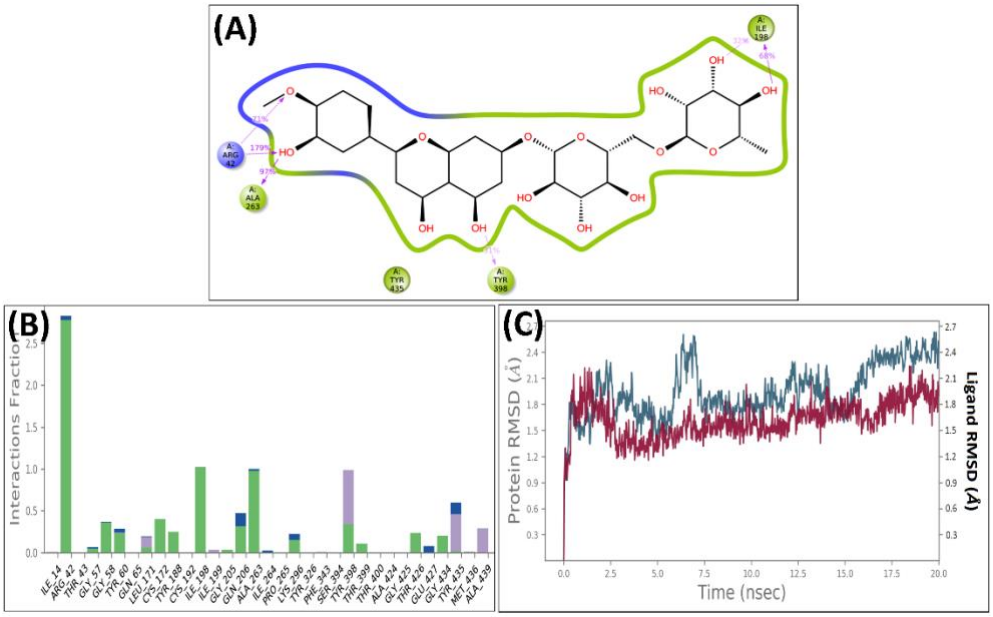


Figure 4. Post-MD interactions of Hesperidin with MAO-B; **(A)** Protein interactions fractions with the ligand (Hesperidin) plot throughout the simulation; **(B)** Protein-ligand contacts plot of compound Hesperidin with MAO-B; (C) RMSD trajectory plot for compound Hesperidin.

The root mean square deviation (RMSD) plots of Hsp with AChE and MAO-B demonstrated the stability of the complexes, with little variations (ranging from 1.2 to 2.5) seen within a 20 nanosecond time interval. The results obtained from the simulation demonstrate the inhibitory capacity of Hsp against AChE and MAO-B. The Hsp -MAO-B complex exhibited enhanced stability, as shown by its minor or negligible pulsation within the specified time frame. Conversely, the Hsp -AChE complex exhibited oscillation within the first 20 units of the binding cavity, after which it reached a stable conformation. During the simulated time necessary to elicit the therapeutic response, the complexes formed between Hsp and both target proteins exhibited remarkable stability inside the active site.

3.2.3. Drug Likeliness and ADME Studies

The pharmacokinetic studies ensure the anticipated pharmacological profile of targeted molecules. In order to identify new drugs, it is necessary to inquire absorption, distribution, metabolism, and excretion (ADME) profile at progressively earlier stages of the discovery process. In that situation, *in silico* models are appropriate substitutes for experimentation (Doogue et al., 2013). Our results (Table 3) demonstrated that Hsp has low GI absorption and has no blood brain barrier permeation. Furthermore, it also violates the Lipinski's rules. Therefore, there are challenges with Hsp molecule to develop it as a drug.

Table 3. The predicted Pharmacokinetic (ADME) profile of Hesperidin and Hesperetin molecules

Drug	MW	HBA	HBD	TPSA	Consensus Log P	Silicos-IT LogSw	GI absorption	BBB permeant	Lipinski #violations
Hesperidin	610.56	15	8	234.29	-0.72	-0.58	Low	No	3

3.3.Discussion

Brain disorders refer to a range of illnesses characterized by the progressive decline or death of neurons, particularly in the central nervous system, particularly the brain. These disorders often have a chronic trajectory and usually result in a gradual decline in cognitive, motor, emotional, and sensory functions. The potential development of various brain disorders can be attributed to the disruption of the cholinergic system, excessive activity of metabolizing enzymes such as monoamine oxidase (MAO) and acetylcholinesterase (AChE), and the presence of reactive oxygen species (Lawrence et al., 1995; Feinberg et al., 2016; Duncan et al., 2012; Liu et al., 2015; Pohanka, 2014; Jenner, 2003). Thus drugs targeting oxidative stress, AChE and MAO-B could be beneficial in treating brain disorders.

In this study, hesperidin, a plant flavonoid, was studied for its neuroprotective effect through in-vitro studies which were further validated using in-silico methods to explore its possible underlying mechanism of neuroprotection. The in-vitro experiments demonstrated that hesperidin exhibits favorable antioxidant properties, as evidenced by its strong DPPH and H_2O_2 inhibitory effects. Additionally, hesperidin has shown inhibitory activity against AChE and MAO-B enzymes, suggesting its potential therapeutic application in treating brain disorders. Further, in-silico molecular docking and molecular dynamics studies demonstrated that hesperidin interacted with crucial residues in the active areas of acetylcholinesterase and MAO-B enzymes. Molecular dynamics simulation research examines the functioning of biomolecular processes, including ligand binding, conformational changes generated by ligands or voltage, protein folding, and membrane transport. The results obtained from computational and laboratory experiments indicate that hesperidin has potential as a viable therapeutic strategy or supplementary treatment for managing brain disorders. The impact of oxidative stress on regulating enzyme activity, namely AChE and MAO-B, is well recognized. The AChE plays a crucial role in suppressing acetylcholine transmission at cholinergic synapses (Schallreuter et al., 2003). The rise in AChE activity may be attributed to many factors, including the loss of cholinergic neurons, the deposition of amyloid plaques, the formation of tau protein tangles, neuroinflammation, and the altered expression of acetylcholinesterase variations (Schallreuter et al., 2003; Uddin et al., 2020). ROS may oxidize the amino acid residues of AChE, leading to direct damage. The oxidative modification process can potentially disrupt the enzyme's active site, affecting its ability to bind to acetylcholine and facilitate effective breakdown. Excessive oxidative stress has capillary peroxidation, leading to the production of lipid peroxides that may cause damage to proteins, including AChE. The reduced function of enzymes may be indirectly attributed to the presence of damaged lipids and their breakdown products. According to Hasselmo (2006), acetylcholine is crucial in memory consolidation because it converts temporary memories into durable long-term memories. The regulation of acetylcholine levels inside the synaptic cleft by AChE plays a vital role in maintaining the proper functioning of neuronal circuits involved in memory formation. AChE does this by hydrolyzing excessive quantities of acetylcholine, thereby preserving the optimal equilibrium of this neurotransmitter (Lehmann and Fibiger 1979; Dańşman et al. 2022; Sharma et al. 2020). Therefore, changes in the functioning of AChE might result in impairments in memory, which is a

significant factor in the development of memory-related illnesses such as Alzheimer's. An inhibitory assay is conducted to evaluate the inhibitory activity hesperidin on AChE. The present investigation revealed that the IC_{50} value of hesperidin was comparatively lower than that of the donepezil. Consistent with previous research done by Singh et al. (2016), the present study suggests that hesperidin may possess efficacy in suppressing the activity of AChE, hence establishing its potential as a potent therapeutic agent for addressing diseases resulting from AChE dysregulation. Similarly, the activity of MAO-B is impacted by oxidative stress, a factor linked to the pathogenesis of several brain illnesses (Seif-El-Nasr et al., 2008). The enzymatic destruction of monoamine neurotransmitters, such as serotonin, dopamine, norepinephrine, and epinephrine, is attributed to monoamine oxidase, leading to the production of their respective metabolites (Tipton et al., 2004) which functions as a preventative strategy against the excessive activation of monoamine receptors. The maintenance of appropriate amounts of neurotransmitters inside synapses relies heavily on controlling monoamine breakdown by MAO-B. Thus, MAO-B inhibitors are often used in the treatment of several brain illnesses, including depression, epilepsy, and Parkinson's disorders (Jazvinġćak et al., 2023). In the present investigation, the IC_{50} value of hesperidin were determined to be 28.49 ±1.72 μg/ml, indicating its efficacy as a therapeutic agent for addressing illnesses associated with the modified activity of MAO-B.

Oxidative stress leads to a decrease in energy production and an increase in the synthesis of reactive oxygen species (ROS) (Federico et al. 2012; Niki et al. 2008; Tramutola et al. 2017; Barzilai et al. 2004). The process of neuronal death is expedited by oxidative stress since neurons possess an increased vulnerability to ROS due to their elevated metabolic rate, significant oxygen consumption, and restricted antioxidant defense systems (Niizuma et al., 2009). Flavonoids have the ability to act as scavengers of free radicals, hence assisting in the neutralization of these intrinsically unstable molecules via electron donation, leading to their eventual stability (Gupta et al., 2010). The antioxidant activities of hesperidin were assessed using the DPPH and H_2O_2 assays in this study. Numerous in vitro investigations have shown evidence that several plant flavonoids can mitigate the DPPH radical scavenging activity. Consistent with previous research, the present study found that hesperidin successfully scavenged DPPH radicals, with the extent of scavenging being dependent on the concentration of the radicals. In the H_2O_2 experiment, hesperidin demonstrated antioxidant characteristics. Therefore, it may be inferred that hesperidin has antioxidant capabilities, potentially providing neuroprotective benefits in the context of brain diseases.
In addition to inhibitory tests, the binding ability of hesperidin with AChE and MAO-B was assessed by molecular docking and molecular dynamic investigations. The investigations revealed that hesperidin displayed interactions with key amino acid residues located in the active region of the target proteins AChE and MAO-B. Consequently, hesperidin has shown the ability to block the receptor, castigating the symptoms associated with brain disorders. Further, Hsp molecule was further assessed for the determination of its drug-likeliness and ADME characteristics. The Lipinski's rule of five was used to evaluate the drug-like potential of the Hsp. Various parameters such as physicochemical properties, lipophilicity and solubility behaviors were predicted on the basis of topological polar surface area (TPSA), consensus log P, and ESOL LogS. Hsp showed optimal pharmacokinetic characteristics but did not comply with Lipinski's rules, indicating potential challenges in oral bioavailability, possibly due to sugar moieties hindering gastrointestinal absorption. Despite this, some drugs like erythromycin and rituximab also defy Lipinski's rules yet are clinically prescribed. Therefore, alternative administration routes may be explored to develop hesperidin as a viable drug candidate, considering its predicted ADME

properties and strong enzyme inhibition capabilities.

4. Conclusion and Future Perspectives

The present work confirmed by in-vitro and in-silico investigations that hesperidin has strong inhibitory effects on AChE and MAO-B, indicating its potential as a medication or adjunctive treatment for memory and mood disorders. In addition, it has strong antioxidant characteristics which reveal potential use in many neurological illnesses. Moreover, the computational investigations confirmed the in-vitro effects on the target proteins. Hesperidin had the excellent docking scores and substantial interactions with the target proteins AChE and MAO-B, as shown by the molecular docking experiments. Post-molecular dynamics simulations revealed the preservation of significant interactions with crucial amino acids in the active site. Therefore, it is necessary to conduct more in-vivo investigations on hesperidin to examine its pharmacological and mechanical properties.

5.References

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Ovais, M., Ullah, I., Ahmed, J., Shahid, M. (2019) Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. *Frontiers in Aging Neuroscience* 26;11,155.

Bai, R., Guo, J., Ye, X.Y., Xie, Y., Xie, T. (2022) Oxidative stress: The core pathogenesis and mechanism of Alzheimer's disease. *Ageing Research Reviews*, 1;77, 101619.

Barzilai, A., Yamamoto, K.I. (2004) DNA damage responses to oxidative stress. *DNA repair* 1;3,1109-15.

Bekdash, R.A. (2021) The cholinergic system, the adrenergic system and the neuropathology of Alzheimer's disease. *International Journal of Molecular Sciences* 28;22,1273.

Benzi, G., Moretti, A. (1998) Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease? *European Journal of Pharmacology* 3;346(1):1-3.

Bhatia. S., Singh, M., Sharma, P., Mujwar, S., Singh, V., Mishra, K.K., Singh, T.G., Singh, T., Ahmad, S.F. (2023) Scaffold morphing and *in silico* design of potential BACE-1 (β-secretase) inhibitors: A hope for a newer dawn in anti-alzheimer therapeutics. *Molecules* 28;16, 6032.

Bhatt, S., Nagappa, A.N., Patil, C.R. (2020) Role of oxidative stress in depression. *Drug discovery today* 1;25,1270-6.

Blois, M.S. (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 26;181,1199-200.

Can, N.O., Osmaniye, D., Levent, S., Saglık, B.N., Inci, B., Ilgın, S., Ozkay, Y., Kaplancıklı, Z.A. (2017) Synthesis of new hydrazone derivatives for MAO enzymes inhibitory activity. *Molecules* 22,1381.

Classics, E.G.L., Courtney, K.D., Andres, V., Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7;2,88-95.

Danışman, B., Akçay, G., Gökçek, S.Ç., Kantar, D., Aslan, M., Derin, N. (2022) The role of acetylcholine on the effects of different doses of sulfite in learning and memory. *Neurochemical Research* 47;11,3331-43.

Doogue, M.P., Polasek, T.M., 2013. The ABCD of clinical pharmacokinetics. *Therapeutic advances in drug safety* 4;1,5-7.

Duncan, J., Johnson, S., Ou, X.M. (2012) Monoamine oxidases in major depressive disorder and alcoholism. *Drug Discoveries and Therapeutics* 30;6,112-22.

Federico, A., Cardaioli, E., Da, P.P., Formichi, P., Gallus, G.N., Radi, E. (2012) Mitochondria, oxidative stress and neurodegeneration. *Journal of Neurological Sciences* 15;322,254-62.

Furlan, V., Bren, U. (2021) Insight into inhibitory mechanism of pde4d by dietary polyphenols using molecular dynamics simulations and free energy calculations. *Biomolecules* 11;3,479-485.

Gupta, V.K., Rachna, K., Munish, G., Monika, G. (2010) Recent updates on free radicals scavenging flavonoids: An overview. *Asian Journal of Plant Sciences* 9;3,108-17.

Hajialyani, M., Hosein, F.M., Echeverría, J., Nabavi, S.M., Uriarte, E., Sobarzo, S.E. (2019) Hesperidin as a neuroprotective agent: A review of animal and clinical evidence. *Molecules* 12;24,648.

Han, Y., Zhang, J., Hu, C.Q., Zhang, X., Ma, B. and Zhang, P., (2019) *In silico* ADME and toxicity prediction of ceftazidime and its impurities. *Frontiers in pharmacology 10*, 434.

Hasselmo, M.E. (2006) The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology* 1;16,710-5.

Hayashi, M., Miyata, R., Tanuma, N. (2012) Oxidative stress in developmental brain disorders. Neurodegenerative Diseases 1,278-90.

Hoidal, J.R. (2001) Reactive oxygen species and cell signaling. *American Journal of Respiratory Cell and Molecular Biology* 1;25,661-3.

Jenner, P. (2003) Oxidative stress in Parkinson's disease. *Annals of Neuroloy* 53;S3,S26-38.

Kaur, R., Singh, V., Shri, R. (2017) Anti-amnesic effects of Ganoderma species: A possible cholinergic and antioxidant mechanism. *Biomedicine and Pharmacotherapy* 1;92,1055-61.

Kim, J., Wie, M.B., Ahn, M., Tanaka, A., Matsuda, H., Shin, T. (2019) Benefits of hesperidin in central nervous system disorders: A review. *Anatomy & Cell Biology* 1;52,369-77.

Kim, J., Wie, M.B., Ahn, M., Tanaka, A., Matsuda, H., Shin, T. (2019) Benefits of hesperidin in central nervous system disorders: A review. *Anatomy & cell biology*. 52;4,369-77.

Klimek, V., Roberson, G., Stockmeier, C.A., Ordway, G.A. (2003) Serotonin transporter and MAO-B levels in monoamine nuclei of the human brainstem are normal in major depression. *Journal of Psychiatric Research* 37;5,387-97.

Lawrence, A.D., Sahakian, B.J. (1995) Alzheimer disease, attention, and the cholinergic system. *Alzheimer Disease & Associated Disorders* 1;9,37-49.

Lehmann, J., Fibiger, H.C. (1979) Acetylcholinesterase and the cholinergic neuron. *Life Sciences* 25;23,1939-47.

Li, A., Zheng, N., Ding, X. (2022) Mitochondrial abnormalities: A hub in metabolic syndrome-related cardiac dysfunction caused by oxidative stress. *Heart Failure Reviews* 27;4,1387-94.

Li, Q., Zhang, H., Guan, S., Du, J., Zhang, Y., Wang, S. (2023) Molecular dynamics simulation of the inhibition mechanism of factor XIa by Milvexian-like macrocyclic inhibitors. *Computational and Theoretical Chemistry* 1225,114131.

Liu, J., Duan, W., Deng, Y., Zhang, Q., Li, R., Long, J., Ahmed, W., Gu, C., Qiu, Y., Cai, H., Hu, Y. (2023) New insights into molecular mechanisms underlying neurodegenerative disorders. *Journal of Integrative Neuroscience* 22;3,58.

Liu, T., Zhong, S., Liao, X., Chen, J., He, T., Lai, S., Jia, Y. (2015) A meta-analysis of oxidative stress markers in depression. *PloS one* 10;10,e0138904.

Mahajan, P., Kaushal, J. (2020). Epidemic trend of COVID-19 transmission in India during lockdown-1 phase. *Journal of Community Health* 45;6,1291-1300.

Marucci, G., Buccioni, M., Dal Ben, D., Lambertucci, C., Volpini, R., Amenta, F. (2021) Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology*1;190,108352.

Morrison, J.H. (1997) Life and death of neurons in the aging brain. *Science* 278;5337,412-9.

Nagu, P., Parashar, A., Behl, T., Mehta, V. (2021) CNS implications of COVID-19: A comprehensive review. *Reviews in the Neurosciences* 32;2,219-234.

Niizuma, K., Endo, H., Chan, P.H. (2009) Oxidative stress and mitochondrial dysfunction as determinants of ischemic

neuronal death and survival. *Journal of Neurochemistry*109,133-8.

Niki, E. (2008) Lipid peroxidation products as oxidative stress biomarkers. *Biofactors* 34;2,171-80.

Pohanka, M. (2014) Alzheimer's disease and oxidative stress: A review. *Current Medicinal Chemistry* 21;3,356-64.

Rao, P.S., Kalva, S., Yerramilli, A., Mamidi, S. (2011) Free radicals and tissue damage: Role of antioxidants. *Free radicals and Antioxidants* 1;4,2-7.

Rehman, M.U., Sehar, N., Dar, N.J., Khan, A., Arafah, A., Rashid, S., Rashid, S.M., Ganaie, M.A. (2022) Mitochondrial dysfunctions, oxidative stress and neuroinflammation as therapeutic targets for neurodegenerative diseases: An update on current advances and impediments. *Neuroscience & Biobehavioral Reviews* 14:104961.

Roohbakhsh, A., Parhiz, H., Soltani, F., Rezaee, R., Iranshahi, M. (2014) Neuropharmacological properties and pharmacokinetics of the citrus flavonoids hesperidin and hesperetin—A mini-review. *Life sciences*. 15;113,1-6.

Schallreuter, K.U., Elwary, S.M., Gibbons, N.C., Rokos, H., Wood, J.M. (2004) Activation/deactivation of acetylcholinesterase by H_2O_2: More evidence for oxidative stress in vitiligo. *Biochemical and Biophysical Research Communications* 315;2,502-8.

Seif-El-Nasr, M., Atia, A.S., Abdelsalam, R.M. (2008) Effect of MAO-B inhibition against ischemia-induced oxidative stress in the rat brain. *Arzneimittelforschung* 58;4,160-7.

Sharma, P., Singh, M., Singh, V., Singh, T.G., Singh, T., Ahmad, S.F. (2023) Recent development of novel aminoethyl-substituted chalcones as potential drug candidates for the treatment of alzheimer's disease. *Molecules* 28;18,6579.

Sharma, P., Singh, V., Singh, M. (2023) N-methylpiperazinyl and piperdinylalkyl-O-chalcone derivatives as potential polyfunctional agents against Alzheimer's disease: Design, synthesis and biological evaluation. *Chemical Biology & Drug Design* 102;5,1155-75.

Sharma, V.K., Mehta, V., Singh, T.G. (2020) Alzheimer's disorder: epigenetic connection and associated risk factors. *Current Neuropharmacology* 18;8,740-53.

Shih, J.C., Chen, K., Ridd, M.J. (1999) Monoamine oxidase: from genes to behavior. *Annual Review of Neuroscience* 22;1,197-217.

Singh, V., Kahol, A., Singh, I.P., Saraf, I., Shri, R. (2016) Evaluation of anti-amnesic effect of extracts of selected Ocimum species using *in-vitro* and *in-vivo* models. *Journal of Ethnopharmacology* 4;193,490-9.

Singh, M., Kaur, M., Singh, N. and Silakari, O., (2017). Exploration of multi-target potential of chromen-4-one based compounds in Alzheimer's disease: Design, synthesis and biological evaluations. *Bioorganic & Medicinal Chemistry 25*;24,6273-6285

Sun, A.Y., Chen, Y.M. (1998) Oxidative stress and neurodegenerative disorders. *Journal of Biomedical Science* 5;6,401-14.

Tipton, K.F., Boyce, S., O'Sullivan, J., Davey, G.P., Healy, J. (2004) Monoamine oxidases: Certainties and uncertainties. *Current Medicinal Chemistry* 11;15,1965-82.

Tramutola, A., Lanzillotta, C., Perluigi, M., Butterfield, D.A. (2017) Oxidative stress, protein modification and Alzheimer disease. *Brain Research Bulletin* 1;133,88-96.

Uddin, M.S., Rahman, M.A., Kabir, M.T., Behl, T., Mathew, B., Perveen, A., Barreto, G.E., Bin-Jumah, M.N., Abdel-Daim, M.M., Ashraf, G.M. (2020) Multifarious roles of mTOR signaling in cognitive aging and cerebrovascular dysfunction of Alzheimer's disease. *Iubmb Life* 72;9,1843-55.

Wu, G., Robertson, D.H., Brooks III, C.L., Vieth, M. (2003) Detailed analysis of grid-based molecular docking: A case study of CDOCKER—A CHARMm-based MD docking algorithm. *Journal of computational chemistry* 24;13, 1549-1562.

Acknowledgment

The authors acknowledge and extend their appreciation to Chitkara University, Punjab, India for providing resources for completion of this research article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

M.K.: Data Collection; AKG: Study concept and design; MJ: Data analysis and interpretation; VS: Writing original draft preparation; TGS: Conceptualization; KKM: Writing, review and editing.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

PHYSICOCHEMICAL ANALYSIS AND ANTIOXIDANT BENEFITS OF YOGURT ENRICHED WITH BETALAINS FROM RED BEET (*BETA VULGARIS* L.)

S. Djabali [1,2]✉, M. Barkat [1], E. Sabba [1], L. Boussouf [2], Z. Chelaghema [2] and N. Haif [2]

[1]BIOQUAL Laboratory, INATAA, Brothers Mentouri Constantine University 1, Rue Aïn El Bey, 25000, Constantine 25000, Algeria.

[2]University Mohamed Seddik Ben Yahia, faculty of natural and life sciences, department of applied microbiology and food science, Jijel 18000, Algeria.

✉saliha.djabali@univ-jijel.dz; ORCID ID : https://orcid.org/0009-0003-5953-7232

https://doi.org/10.34302/crpjfst/2024.16.4.16

Article history:
Received
October 23th, 2024
Accepted
November 30th, 2024

Keywords:
Yoghurt;
Betalain;
Phenolic compound;
Antioxidant activity;
Sensory analysis.

ABSTRACT

Food color is a key purchasing factor for consumers and a quality indicator. Recently, there has been a shift towards natural food colors, leading to increased use of red beet as a colorant. Beet betalains are valued in the food industry for their natural coloring, high water solubility, and non-toxic nature. This study aimed to compare two types of yogurt: one with natural beetroot juice colorant at concentrations of 6 g/L and 12 g/L (chosen based on preliminary tests), and a control yogurt with no colorant.

Yogurts were analyzed for physicochemical properties (pH, acidity, ash content, moisture content, syneresis), nutritional content (protein and sugar), phytochemical properties (phenolic and betalain content, antioxidant activity), and sensory attributes over 21 days of refrigerated storage at 4°C. Statistical analysis was performed using Tukey-Kramer HSD test (Minitab software) with a significance level of 0.05.

pH decreased while acidity increased in betalain-enriched yogurt during storage, yet pH remained between 4.5 and 4.8. Significant differences in ash and moisture content were noted. Phytochemical parameters and antioxidant potential improved with betalain enrichment at both 6 g/L and 12 g/L. Sensory evaluation after 21 days showed that yogurt with beetroot juice at 6 g/L and 12 g/L was preferred for its pink color and sweet taste.

The study highlights the benefits of adding beetroot betalain to yogurt. This plant provides valuable polyphenols and betalains, which are beneficial for health. Betalain-enriched yogurt can thus enhance health and offer protection against free radical damage

1.Introduction

Yogurt is a widely consumed fermented dairy product, known for its nutritional benefits and rich in essential nutrients like protein, calcium, potassium, phosphorus, and vitamins (Yadav et al., 2015; Fisberg and Machado, 2015). Its color is a crucial sensory attribute that influences consumer attraction and product acceptance. However, synthetic colors, used as additives, pose serious health risks due to their carcinogenic effects (Alshehry, 2019), leading to a shift towards natural colors or 'bio-colors,' which are derived from vegetables, fruits, roots, and microorganisms (Ravichandran et al., 2011; Haddar, 2016).

Beetroot, known for its antimicrobial, antiviral properties, and its ability to inhibit tumor cell proliferation, contains bioactive compounds such as betalains (Shivangi et al., 2019; Débia et al., 2023). Betalains are water-soluble nitrogen pigments, classified into betacyanins (red-violet) and betaxanthins

(yellow) (Nirmal et al., 2021). Due to their glycosylation and acylation, betalains show significant structural diversity and are suitable for use in acidic foods like dairy products. However, they are temperature-sensitive and degrade at high temperatures, with their stability decreasing with increased temperature and heating duration (Herbach et al., 2006).

Betalains have garnered scientific interest due to their high antioxidant activity, which is seven times greater than that of vitamin C, making them valuable not only as natural dyes but also for their potential to enhance food packaging and provide additional benefits (Castro-Enríquez et al., 2020). Considering the nutritional value and seasonal availability of beetroot, this study aims to formulate stirred yogurts incorporating crude betalain extracts from red beetroot as a natural colorant and antioxidant at two concentrations. The study will evaluate their physicochemical, nutritional, and sensory characteristics, as well as their antioxidant activity during storage, comparing them to a control yogurt with no added colorant.

2. Materials and methods

2.1.Plant material

The plant material used was beetroot (Beta vulgaris L.), characterized by its round shape, reddish-purple color, and long, thin taproot. The beetroots were hand-sorted and washed to remove impurities and damaged specimens. This variety was selected due to its excellent antioxidant properties and high betalain content, which are less studied compared to other natural pigments.

2.2.Extraction of concentrated beetroot juice (betalains)

The washed beetroot was cut into small pieces and placed in an electric mixer for chopping. The chopped beetroot (5 g) was then shaken for 10 minutes in a beaker with 15 ml of distilled water. The resulting solution was filtered, centrifuged at 10,000 rpm for 20 minutes (HB110, Behsan Company, Iran), and subsequently concentrated using a Rotavapor (Laura et al., 2016).

2.3.Milk and lactic ferments

The raw materials for making the yogurt were purchased from a grocery store and underwent acceptance tests, including the boiling stability test and the methylene blue reduction test. The freeze-dried lactic acid bacteria culture used (Termophilic YoFlex Mild 1.0) was supplied by the COLAITAL group (Bir Khadem, Algeria). It contains a mixture of two bacterial species: Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (200 U/1000 L).

2.4.Preparation of yoghurt

The enriched stirred yogurts were prepared separately following the recipe provided by the Colaittal Group, Algeria. A mixture of skimmed and whole cow's milk, adjusted for solids by adding milk powder (0% fat) and sugar, was heated to 95°C for 5 minutes and then cooled to 45°C. The mixture was inoculated with the freeze-dried lactic acid bacteria culture and beetroot betalain at different concentrations (6 g/L and 12 g/L) and incubated at 42°C. The resulting gel was vortexed, packed into jars, and rapidly cooled to a temperature between 4°C and 6°C. The negative control was prepared using the same steps, but without the addition of beetroot betalain.

2.5.Physico-chemical analyses

2.5.1.pH

The pH was measured using a pH meter (OHAUS, Germany). This measurement reflects the concentration of H+ ions resulting from the production of organic acids by lactic acid bacteria (Amirdivani and Baba, 2011).

2.5.2.Titratable acidity

Titratable acidity measures the amount of lactic acid in the yogurt. To determine it, an acid-base titration was performed using a sodium hydroxide (NaOH) solution. Ten milliliters of distilled water were added to 5 grams of each sample. Two to three drops of

phenolphthalein were added, and the mixture was titrated with NaOH (N/9) until the color changed to pink (Amirdivani, 2015). Phenolphthalein is colorless below pH 8 and turns pink at pH 8.

A permanent pink color indicates the end of the titration. Results were expressed using the following formula:

$$\textbf{Dornic°=V×10} \quad (1)$$

With :

V: the volume of sodium hydroxide solution (ml).

2.5.3.Syneresis

Yogurt syneresis (whey separation) was determined using the centrifugation method described by Özturk and Öner (1999). Briefly, 20 grams of yogurt were centrifuged at 6000 rpm for 20 minutes. The supernatant was then collected and weighed. Syneresis was calculated using the following equation (Martha et al., 2021):

$$\textbf{Syneresis (\%) = weight of supernatant (g)/ weight of yoghurt sample (g) X 100} \quad (2)$$

2.5.4.Brix degree

The Brix of the samples was determined by placing a drop of the sample on the prism plate of the instrument, facing the light. The Brix value was then read through the eyepiece of the instrument (Azzouzi et al., 2022).

2.5.5.Moisture content

Moisture content was determined as follows: 1 gram of each sample was weighed and then placed in an oven (Memmert, Germany) at 105°C until the weight stabilized (Mahaut et al., 2000).

$$\textbf{H\% = M2÷M1×100} \quad (3)$$

With :

H : Humidity(%) ;

M1 : Mass of sample before drying(g) ;

M2: Mass of sample after drying (g).

2.6.6.Ash content

Ash content is determined by calcining a test sample until the organic matter is completely burned off in a muffle furnace (Thermolyne, France) at 550°C ± 5°C (Fekata et al., 2022). The mineral content was then calculated using the following formula:

$$\textbf{C\% = [residue weight ÷ sample weight]×100} \quad (4)$$

2.5.7.Nutritional analysis

2.5.7.1.Sugar content

Total sugars were determined using the phenol-sulfuric acid method. One gram of the sample was placed in a test tube with 1 ml of 5% phenol. Five milliliters of sulfuric acid were added quickly without allowing it to run down the sides, and the mixture was shaken immediately. A yellow coloration developed, which remained stable for several hours. The tubes were then placed in a water bath at 25-30°C for 20 minutes and subsequently cooled under running water to 20°C. Absorbance was measured at 488 nm using a spectrophotometer (UV-Visible S-2150, UNICO, USA) (Feller et al., 1991). Sugar levels were determined by referencing a standard glucose curve.

2.5.7.2.Protein content

The Bradford protein assay is a colorimetric method that uses Coomassie Blue as its primary reagent. In its free cationic form, this reagent absorbs light at a wavelength of 465 nm. Upon binding to proteins and their aromatic groups, the absorption maximum shifts to 595 nm. Briefly, 5 ml of Bradford reagent was added to 100 µl of yogurt and allowed to react in the dark for 5 minutes. The absorbance was measured at 595 nm. Protein levels were determined by referencing a bovine serum albumin (BSA) standard series (Azerdo et al., 2003).

2.5.7.3.Preparation of the yoghurt supernatant

The yogurt sample (10 g) was mixed with 2.5 ml of distilled water, and the pH was

adjusted to 4.0 with 1 M HCl (Honeywell, Germany). The yogurt was then incubated at 45°C for 10 minutes and centrifuged at 6000 rpm for 20 minutes. The supernatant was collected and its pH adjusted to 7.0 with 1 N NaOH. The neutralized supernatant was centrifuged again at 6000 rpm for 20 minutes at 4°C, and the resulting supernatant was used for analysis (Shori, 2020).

2.5.7.4.Betalain content

Ten grams (10 g) of each yogurt sample were added to 50 ml of distilled water. The mixture was shaken for 30 minutes, then centrifuged at 3000 rpm for 10 minutes, and filtered through filter paper. The absorbance was measured at 532 nm for betacyanins and 482 nm for betaxanthins (Khatabi et al., 2013). Betacyanin content was expressed in mg/100 g and calculated using the following equation:

$$\boldsymbol{B}=\mathbf{A}\times\mathbf{FD}\times\mathbf{PM}\times\mathbf{1000}/\boldsymbol{\varepsilon}\times\mathbf{L} \quad (5)$$

With :

A: Absorbance at 532 nm (betacyanins) and 482 nm (indicaxanthins);

FD: dilution factor;

PM: molecular weight (550 and 380 g/mol for betacyanin and indicaxanthin, respectively);

ε: molar extinction coefficients (60000l/mol.cm for betacyanins and 48000l/mol.cm for indicaxanthins);

L: optical path (1 cm).

2.5.7.5.Total phenolic content

Total polyphenols were determined colorimetrically using the Folin-Ciocalteu reagent (Merck, Germany). The intensity of the blue color produced is proportional to the amount of polyphenols present in the samples. The reaction mixture was prepared by mixing 1 ml of yogurt extract with 1 ml of 95% ethanol (Merck, Germany) and 5 ml of distilled water. Then, 0.5 ml of 50% Folin-Ciocalteu reagent was added to each sample, which was then mixed thoroughly by vortexing. After incubating at room temperature and in the dark for 60 minutes, the absorbance was measured at 725 nm (Shori et al., 2018). Total polyphenol content was estimated from a calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalents (mg EAG) per milliliter of yogurt supernatant.

2.5.7.6.Antioxidant activity

Total antioxidant capacity

0.3 ml of each yogurt extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After incubation at 95°C for 90 minutes and subsequent cooling, the absorbance of the solution was measured at 695 nm. A blank control, containing 3 ml of reagent solution and 0.3 ml of distilled water, was incubated under the same conditions as the sample. The total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per milliliter of yogurt supernatant (mg EAA/ml supernatant) (Prieto et al., 1999).

2.5.7.7.Hydrogen peroxide reduction

The hydrogen peroxide reduction assay was performed according to the method of Taşkın and Bağdatlıoğlu (2020) with slight modifications. One milliliter of each yogurt extract was mixed with 2.4 ml of phosphate buffer (pH 7.4) and 0.6 ml of 4 mM H_2O_2. After 40 minutes, the absorbance was measured at 230 nm. Water was used in place of the H_2O_2 solution for the blank. Hydrogen peroxide uptake was determined using the following equation:

$$\mathbf{H_2O_2\% = [(AC-AE)/AC] \times 100} \quad (6)$$

With :

AC: control absorbance;

AE: sample absorbance.

2.5.7.8.DPPH free radical test

To evaluate the scavenging effect of each yogurt extract on the DPPH radical (Sigma-Aldrich, Germany), 500 µl of the sample was mixed with 60 mM methanolic DPPH solution. After incubating for one hour at 25°C, the absorbance was recorded at 517 nm. The free

radical scavenging activity was estimated using the following equation (Shori, 2020).

$$\% \text{ inhibition} = [(AC-AE)/AC] \times 100 \quad (7)$$

With :

AC: control absorbance

AE: extract absorbance.

2.5.7.9.Sensory analysis

Sensory analysis was conducted by a qualified panel of 20 individuals of varying ages, who completed a questionnaire. The evaluation used a nine-point hedonic scale to assess color, odor, sweetness, acidic flavor, and consistency attributes. According to the nine-point scale: 9 = very much liked, 8 = liked very much, 7 = liked moderately, 6 = liked slightly, 5 = neither liked nor disliked, 4 = disliked slightly, 3 = disliked moderately, 2 = disliked very much, and 1 = extremely disliked (Watts et al., 1991).

2.6.Statistical analysis

Results were expressed as mean ± standard deviation (from three replicates), and data were compared based on these means. Differences between means were assessed using the Tukey-Kramer HSD test (Minitab software) at a significance level of 0.05.

3.Results and discussions

3.1.Physico-chemical analysis of prepared stirred yoghurts

3.1.1.pH values

The pH values (Figure 01) did not decrease significantly ($p > 0.05$) for the negative control yogurt (from 4.66 ± 0.06 to 4.54 ± 0.015) and the betalain-enriched yogurt at 12 g/L (from 4.83 ± 0.119 to 4.64 ± 0.04). In contrast, a non-significant increase ($p > 0.05$) was observed from day 1 to day 7 for the betalain-enriched yogurt at 6 g/L (from 4.64 ± 0.04 to 4.72 ± 0.06). From day 7 to day 14, there was a non-significant increase ($p > 0.05$) in pH values for the betalain-enriched yogurt at 12 g/L (from 4.64 ± 0.045 to 4.75 ± 0.006), the betalain-enriched yogurt at 6 g/L (from 4.72 ± 0.065 to 4.76 ± 0.005), and the negative control yogurt (from 4.54 ± 0.015 to 4.82 ± 0.006). From day 14 to day 21, a highly significant decrease ($p < 0.001$) was observed for all yogurts. The results indicated that yogurt enriched with betalain at both concentrations had a higher pH than the negative control during days 1-7, whereas the opposite was observed during days 14-21.

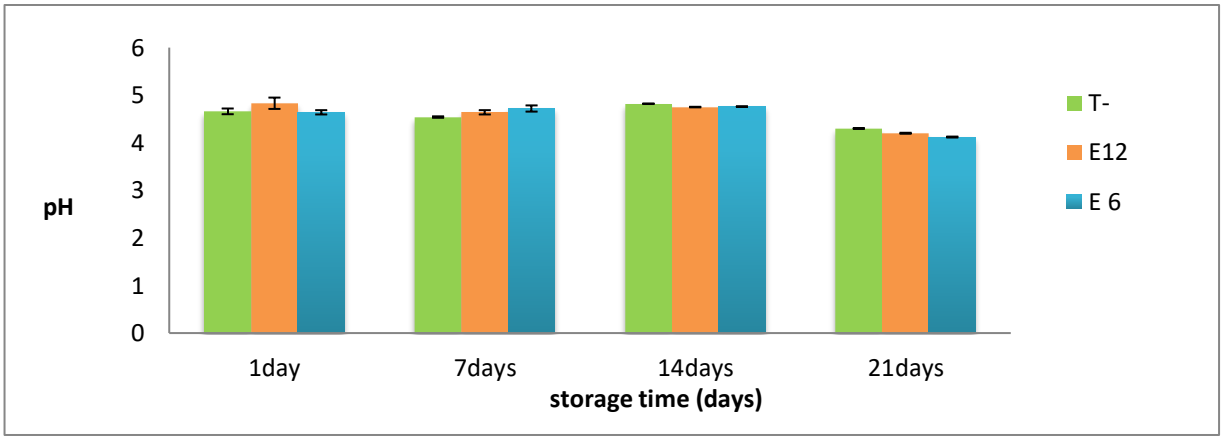


Figure 1. Changes in pH of formulated yoghurts during 21 days' storage at 4°C. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.1.2.Acidity

Analysis of Table 1 shows a non-significant decrease ($p > 0.05$) in the acidity of the negative control yogurt (from 0.99 ± 0.0057 Dornic to 0.76 ± 0.252 Dornic) and the betalain-enriched yogurt at 12 g/L (from 1.66 ± 0.577 Dornic to

0.83 ± 0.289 Dornic) during the period from day 1 to day 7. The period from day 7 to day 14 shows a non-significant increase ($p > 0.05$) in acidity for the negative control yogurt, the yogurt enriched with betalain at 12 g/L, and the yogurt enriched with betalain at 6 g/L. The period from day 14 to day 21 showed a non-significant decrease ($p > 0.05$) in acidity for the negative control yogurt and the yogurt enriched with betalain at 6 g/L. However, a non-significant increase ($p > 0.05$) was observed for the yogurt fortified with betalain at 12 g/L.

Table 1. Changes in acidity of formulated yoghurts during 21 days of storage at 4°C.

Types of yoghurt	1day (D°)	7days (D°)	14days (D°)	21days (D°)
Negative control	0.99± 0.005	0.76± 0.252	0.96± 0.057	0.93± 0.057
Betalain-enriched yoghurt at 12g/l	1.66± 0.577	0.83± 0.289	1.33± 0.577	1.83± 0.289
Betalain-enriched yoghurt at 6g/l	0.99± 0.005	1± 0.500	1.33± 0.577	0.96± 0.057

3.1.3.Brix level

The results of the Brix determination for the five prepared yogurts are summarized in Table 2.

The data indicate a non-significant decrease ($p > 0.05$) in Brix values for the negative control yogurt and a significant decrease ($p < 0.05$) for the yogurt fortified with betalain at 6 g/L during the period from day 1 to day 7. The yogurt fortified with betalain at 12 g/L remained stable during this period.

From day 7 to day 14, a non-significant increase ($p > 0.05$) was observed for the yogurt fortified with betalain at 12 g/L, while a significant increase ($p < 0.05$) was noted for the yogurt fortified with betalain at 6 g/L.

During the period from day 14 to day 21, all types of yogurt showed a decrease: significant ($p < 0.05$) for the negative control yogurt, and highly significant ($p \leq 0.01$) for both the yogurt enriched with betalain at 12 g/L and the yogurt enriched with betalain at 6 g/L.

Table 2. Brix evolution of formulated yoghurts during 21 days of storage at 4°C.

Types of yoghurt	1day (B°)	7 days (B°)	14 days (B°)	21 days (B°)
Negative control	14.66± 0.577	14.33± 0.577	14.33± 0.577	12.33± 0.577
Betalain-enriched yoghurt at 12g/l	14.33± 0.577	14.33± 0.577	14.66± 0.577	12.33± 0.577
Betalain-enriched yoghurt at 6g/l	14.99± 0.005	13.33± 0.577	14.66 ±0.577	12.33 ±0.577

3.1.4.Moisture content

The results of determining the moisture content of the five prepared yogurts are shown in Figure 02. Figure 02 indicates a highly significant decrease ($p \leq 0.01$) in moisture content for yogurts fortified with betalain at 12 g/L and 6 g/L, and a very highly significant decrease ($p \leq 0.001$) for the negative control yogurt during the period from day 1 to day 7.

From day 7 to day 14, there was a significant decrease ($p \leq 0.05$) in moisture content for the yogurt enriched with betalain at 12 g/L and a highly significant decrease ($p \leq 0.01$) for the yogurt enriched with betalain at 6 g/L. Conversely, a non-significant increase was observed in the negative control yogurt.

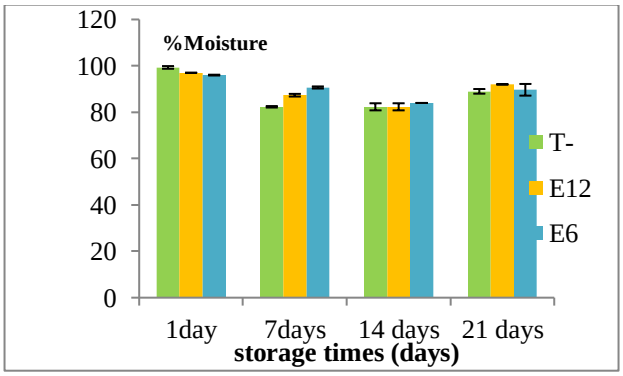


Figure 2. Moisture evolution of formulated yoghurts stored at 4°C for 21 days. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

From day 14 to day 21, a highly significant increase ($p \leq 0.01$) in moisture content was observed for the negative control yogurt and the yogurt fortified with betalain at 12 g/L, while a non-significant increase ($p > 0.05$) was noted for the yogurt with betalain at 6 g/L.

3.1.5.Ash content

The results for ash content are shown in the table below:

The data indicate a significant decrease ($p < 0.05$) in ash content for the negative control yogurt, while a non-significant decrease ($p > 0.05$) was observed for the other yogurts during the period from day 1 to day 7.

During the period from day 7 to day 14, there was a non-significant increase ($p > 0.05$) in ash content for the yogurts enriched with betalain at 12 g/L and 6 g/L, while the negative control yogurt showed a non-significant decrease ($p > 0.05$).

From day 14 to day 21, a highly significant decrease ($p \leq 0.01$) in ash content was observed for the negative control yogurt. The ash content of yogurt fortified with betalain at 12 g/L showed a highly significant increase ($p \leq 0.01$), while the ash content of yogurt fortified with betalain at 6 g/L increased significantly ($p < 0.05$).

Table 3. Change in ash content of formulated yoghurts stored at 4°C for 21 days.

Types of yoghurt	1day (%)	7 days (%)	14 days (%)	21 days (%)
Negative control	14.33 ±1.53	10.67 ±1.15	8.33 ±1.15	2 ±1.00
Betalain-enriched yoghurt at 12g/l	5 ±1.73	2.33 ±1.53	4 ±1.00	9 ±1.00
Betalain-enriched yoghurt at 6g/l	3±1.73	1.99±0.005	4.67± 1.15	10.67± 1.53

3.1.6.Syneresis

The results of the syneresis measurement are shown in Figure 3.

During the period from day 14 to day 21, a highly significant increase ($p \leq 0.01$) in syneresis was observed for the negative control yogurt (from 62.71 ± 0.247% to 68.06 ± 0.050%). A non-significant increase ($p > 0.05$) was noted for the yogurt enriched with betalain at 12 g/L (from 62.91 ± 0.843% to 62.93 ± 0.161%). Conversely, a highly significant decrease ($p \leq 0.01$) was observed for the yogurt enriched with betalain at 6 g/L (from 63.86 ± 0.473% to 60.45 ± 0.050%).

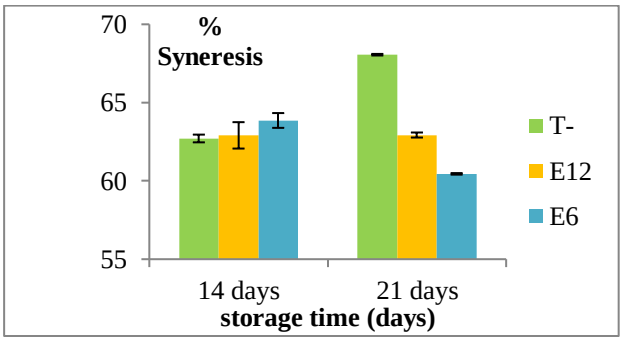


Figure 3. Syneresis of formulated yoghurt. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.2.Nutritional analysis of prepared stirred yoghurts

3.2.1.Sugar content

The sugar content measurements are shown in Figure 4.

From day 1 to day 7, a highly significant decrease ($p < 0.001$) in sugar content was observed for all types of yogurt.

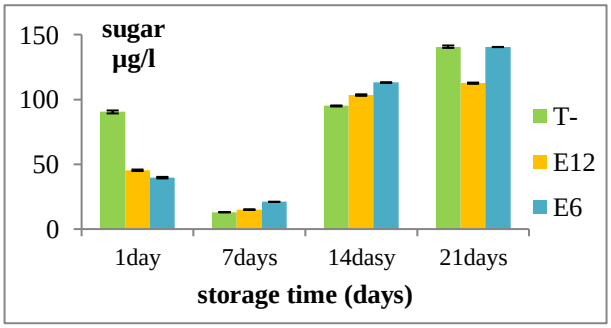


Figure 4. Changes in sugar content of formulated yoghurts during 21 days storage at 4°C.negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

During the period from day 7 to day 14, there was a highly significant increase ($p < 0.001$) in sugar content for all types of yogurt.

From day 14 to day 21, a highly significant increase ($p < 0.001$) in sugar content was again observed for all yogurts.

3.1.7.Protein content

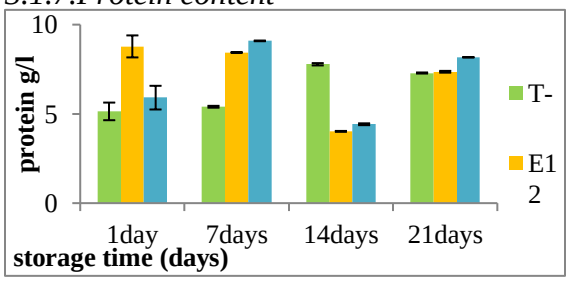


Figure 5. Changes in protein content of formulated yoghurts during 21 days storage at 4°C. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6)

The results of the protein analysis of the yogurts are shown in Figure 5.

The analysis reveals a non-significant decrease ($p > 0.05$) in protein content for the negative control yogurt and the yogurt enriched with betalain at 12 g/L during the period from day 1 to day 7. However, a significant increase ($p < 0.05$) was observed for the yogurt enriched with betalain at 6 g/L.

During the period from day 7 to day 14, there was a highly significant decrease ($p < 0.001$) in protein content for the yogurts enriched with betalain at 6 g/L and 12 g/L. The negative control yogurt showed a very highly significant increase ($p < 0.001$).

From day 14 to day 21, a highly significant decrease ($p < 0.01$) in protein content was observed for the negative control yogurt. In contrast, a very highly significant increase ($p < 0.001$) was noted for the yogurts enriched with betalain at 12 g/L and 6 g/L.

3.3.Phytochemical analyses

3.3.1.Betalain content

The results of the betalain determination in the yogurts are shown in Figure 6.

During the period from day 1 to day 7, a very highly significant decrease ($p < 0.001$) in betalain content was observed for the negative control yogurt and the yogurt fortified with betalain at 6 g/L. In contrast, a very highly significant increase ($p < 0.001$) was noted for the yogurt fortified with betalain at 12 g/L.

From day 7 to day 14, a very highly significant decrease ($p < 0.001$) in betalain content was observed for all types of yogurt. However, during the period from day 14 to day 21, a very highly significant increase ($p < 0.001$) in betalain content was observed for all yogurts.

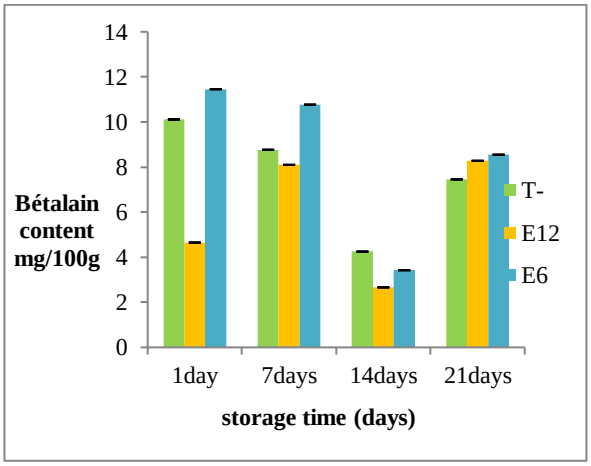


Figure 6. Evolution of betalain content in prepared yoghurts during 21 days of storage at 4°C. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.3.2.Total phenolic content

The test identified total polyphenols in the aqueous extracts of the prepared yogurts.

The results presented in Figure 7 show a non-significant decrease ($p > 0.05$) in polyphenol content for the supernatant of the yogurt enriched with betalain at 6 g/L. Conversely, a non-significant increase ($p > 0.05$) was observed in the supernatants of the negative control yogurt and the yogurt fortified with

betalain at 12 g/L during the period from day 1 to day 7.

From day 7 to day 14, a significant decrease ($p < 0.05$) in phenolic compound content was observed for the supernatants of the negative control yogurt, and the yogurts fortified with betalain at 12 g/L and 6 g/L.

During the period from day 14 to day 21, a very highly significant increase ($p < 0.001$) in phenolic compound content was observed for the supernatants of the yogurts enriched with betalain at 6 g/L and 12 g/L. In contrast, a significant decrease ($p < 0.05$) was noted for the supernatant of the negative control yogurt.

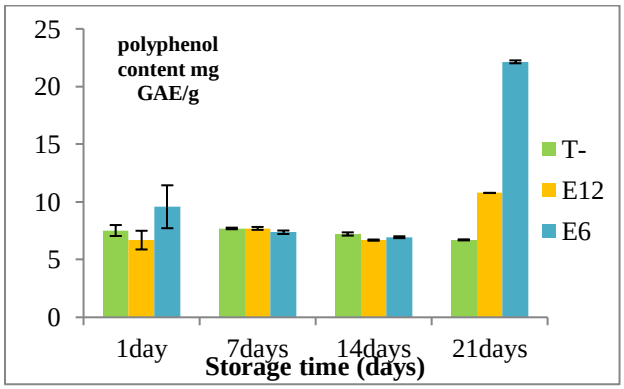


Figure 7. Changes in total phenolic compound content in the supernatants of yoghurts prepared during 21 days storage at 4°C.negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.3.Antioxidant activity

3.3.1.Total antioxidant capacity

The results obtained are presented in Figure 8.

During the period from day 1 to day 7, there was a significant increase ($p < 0.05$) in total antioxidant capacity for the supernatant of the negative control yogurt. Additionally, a highly significant increase ($p < 0.01$) was observed for the supernatants of the yogurts enriched with betalain at 6 g/L and 12 g/L.

From day 7 to day 14, a significant decrease ($p < 0.05$) in total antioxidant capacity was noted for the supernatant of the negative control yogurt. A highly significant decrease ($p \leq 0.01$) was observed for the supernatant of the yogurt enriched with betalain at 12 g/L. In contrast, a significant increase ($p < 0.05$) was found for the supernatant of the yogurt enriched with betalain at 6 g/L during this period. This could be attributed to syneresis or betalain instability.

During the period from day 14 to day 21, there was a significant decrease ($p < 0.05$) in total antioxidant capacity for the supernatant of the negative control yogurt and the supernatant of the yogurt enriched with betalain at 6 g/L. However, a very highly significant increase ($p < 0.001$) was observed for the supernatant of the yogurt enriched with betalain at 12 g/L.

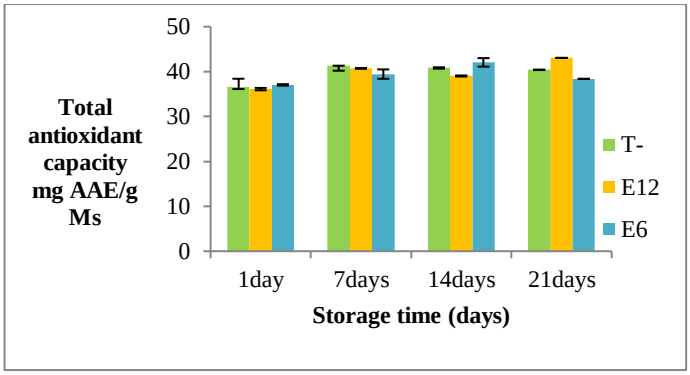


Figure 8. Changes in total antioxidant capacity in the supernatants of yoghurts prepared during 21 days' storage at 4°C.negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6)

3.3.2.Anti-free radical activity against the free radical DPPH·

The results are presented in Figure 9.

During the period from day 1 to day 7, there was a highly significant decrease ($p < 0.001$) in anti-free radical activity for the supernatants of the negative control yogurt and the yogurt enriched with betalain at 12 g/L. A highly significant decrease ($p \leq 0.01$) was also observed for the supernatant of the yogurt enriched with betalain at 6 g/L.

From day 7 to day 14, a highly significant decrease ($p \leq 0.01$) in anti-free radical activity was noted for the supernatant of the negative control yogurt, with a highly significant

decrease ($p < 0.001$) for the supernatants of the other yogurt samples.

During the period from day 14 to day 21, a highly significant increase ($p < 0.001$) in anti-free radical activity was observed for all yogurt supernatants.

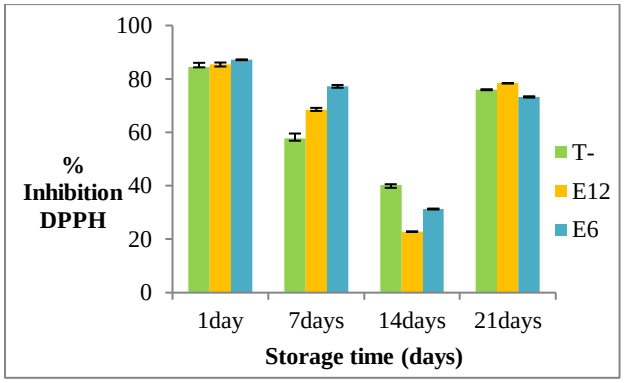


Figure 9. Changes in anti-free radical activity of supernatants prepared from yoghurts stored at 4°C for 21 days. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.3.3.Antioxidant activity by reduction of hydrogen peroxide

The results are presented in the table 4.The data show a highly significant decrease ($p < 0.001$) in the percentage reduction of hydrogen peroxide for all supernatants during the period from day 1 to day 7.

From day 7 to day 14, there was a significant increase ($p < 0.05$) in the percentage reduction of hydrogen peroxide for the negative control yogurt supernatant and the yogurt enriched with betalain at 12 g/L. A non-significant increase ($p > 0.05$) was observed for the yogurt enriched with betalain at 6 g/L.

From day 14 to day 21, a highly significant decrease ($p \leq 0.01$) in the percentage reduction of hydrogen peroxide was observed for the negative control yogurt supernatant, while a significant increase ($p < 0.05$) was noted for the supernatants of the other yogurts.

Table 4. Antioxidant activity measured by reduction of hydrogen peroxide in the supernatants of formulated yoghurts.

Percentage reduction in hydrogen peroxide (%)				
Types of yoghurt	1Day	7 Days	14 Dyas	21 Days
Negative control	78.59± 0.005	29.51±0.658	35.04±1.81	20.17±0.30
Betalain-enriched yoghurt at 12g/l	93.36± 0.05	33.13±0.520	35.74±0.250	46.58±1.21
Betalain-enriched yoghurt at 6g/l	92.06± 0.060	36.34±0.340	36.54±0.450	48.69±0.173

3.4.Sensory analysis

3.4.1.Colour

The color profile analysis of the formulated yogurts is shown in Figure 10. The results indicate the following:

-Days 1-7:Tasters reported a pink color for the yogurts fortified with betalain. Among these, the yogurt fortified with 6 g/L betalain was preferred over the others in sensory evaluations.

- Day 21:Five tasters noted that the color of the yogurt fortified with 6 g/L betalain had changed to beige.

3.4.2.Odour

The odor profile analysis of the formulated yogurts is presented in Figure 11. The sensory evaluation revealed distinct differences over the period:

-Days 1-14: The odor profiles varied significantly among the yogurts.

-Day 21: All tasters reported a strong odor in the formulated yogurts, except for the control yogurt, which was described as having a medium odor by four tasters.

3.4.3.Sweetness

The sweetness profile analysis of the formulated yogurts is presented in Figure 12. - Day 1 : Twelve tasters reported the control yogurt as having low sweetness, while eight found it to be medium.

- Day 7 : One taster perceived no sweetness, five noted low sweetness, and fourteen described it as medium.

- Day 14 : Twenty tasters rated the sweetness of the control yogurt as medium.

- Day 21: The sweetness was also rated as medium by twenty tasters.

For yogurts fortified with betalain at concentrations of 6 g/l and 12 g/l, the majority of tasters consistently rated the sweetness as medium throughout the period from Day 1 to Day 21.

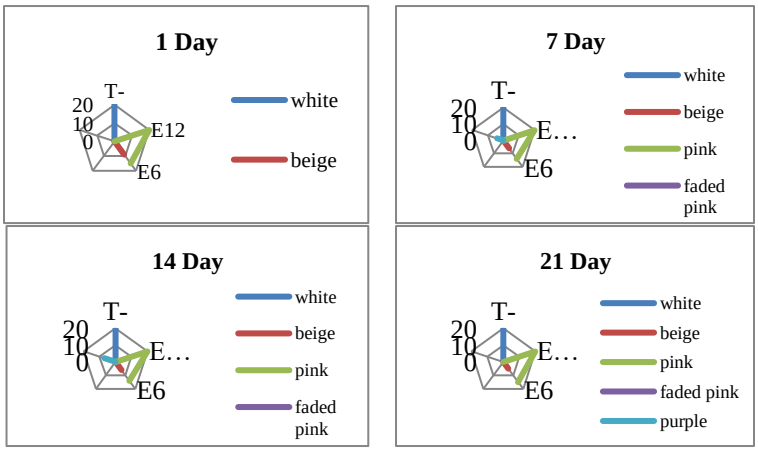


Figure 10. Colour profile of formulated yoghurts during storage. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6)

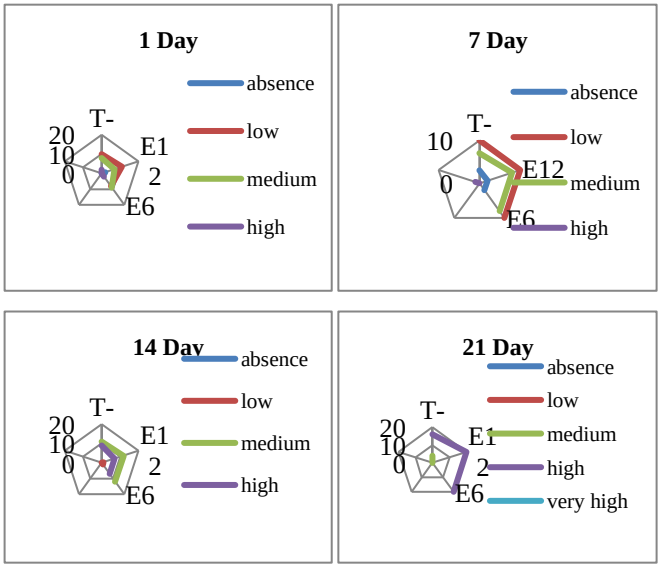


Figure 11. Odour profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

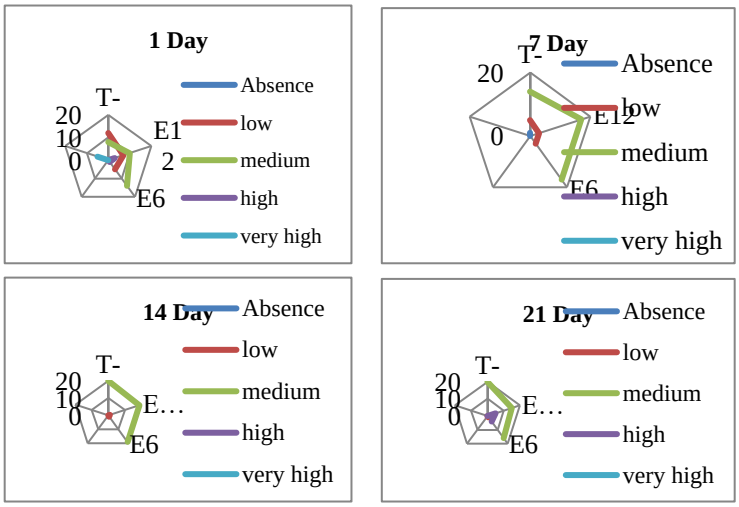


Figure12. Sweetness profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

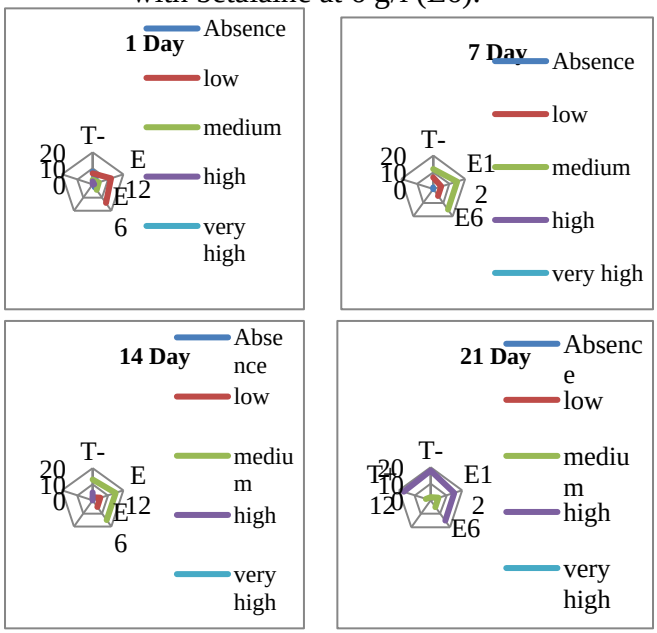


Figure 13. Acid flavour profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.4.4.Acidic flavour

The analysis of the acid flavor profile for the formulated yogurts is presented in Figure 13.

- Day 1: Most tasters perceived a low acid flavor in both the control yogurt and the yogurts fortified with betalain at 6 g/l and 12 g/l.
- Day 7: The majority of tasters noted a medium acid flavor in the yogurts fortified with betalain at both concentrations.
- Day 21: Most tasters reported a high acid flavor in the yogurts fortified with betalain at 6 g/l and 12 g/l.

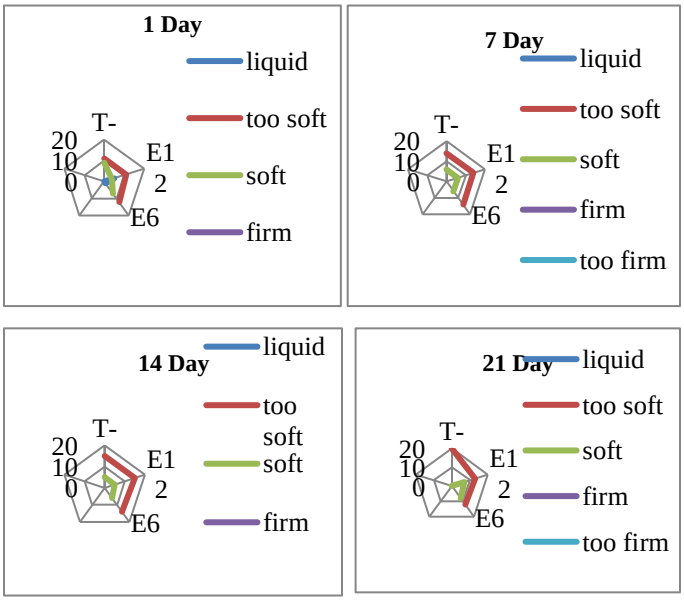


Figure 14. Consistency profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.4.5.Consistency

Analysis of the consistency profile of the formulated yoghurts is presented in Figure 14.

For the control yoghurt and according to the results in the figure above, we can see that for :

D 1: Eleven tasters found the consistency too soft, compared to nine tasters who found it moderately soft.

D 7: Most tasters found the consistency too soft.

D 14: Fifteen tasters found the consistency too soft and five said it was soft.

D 21: Twenty tasters found the consistency to be too soft.

For the yoghurt fortified with betalaine at 6g/l and 12g/l, most of the tasters noted a too soft consistency on days D1, D7, D14 and D21.

3.5.Discussion

The color analysis of the samples reveals that the color remains consistent with pH changes because betalains are relatively stable within a pH range of 3-7. Betacyanins, the natural pigments in question, are effective for coloring low-acid foods red to purple. Under anaerobic conditions, betanin is stable at a pH of 5.5–5.8, but this stability shifts to a lower pH range (4.0–5.0) in the presence of oxygen (Izabela Sadowska & Grzegorz, 2021).

The observed decrease in yogurt pH is attributed to the growth of lactic bacteria and continued production of lactic acid. Cold storage slows but does not entirely halt bacterial metabolic activity (Kaur et al., 2017). These findings are consistent with Jimoh and Kolapo (2007), who reported pH values between 3.39 and 5.68. However, the pH values for our enriched yogurt samples consistently ranged from 4.1 to 4.7, which helps maintain the product's functionality and flavor. Consequently, betalain enrichment helps stabilize pH during storage.

Our titratable acidity results are similar to those reported by Shalaby and Hassenin (2020), who found values between 1.09 D° and 1.11 D°. The decrease in titratable acidity may be due to the decomposition of fermentable substrates and sugars by microorganisms, particularly lactobacilli, which ferment carbohydrates to produce energy and lactic acid (Amirdivani, 2015). The increase in titratable acidity results from the accumulation of lactic acid produced by lactic acid bacteria (Kim et al., 2019).

Brix degrees in our study ranged from 0.55 to 1.30 °B, which is lower than the 7.64 to 10.36 °B reported by Madora et al. (2016) in their study on yogurt enriched with carrot powder. This discrepancy could be attributed to variations in sugar additions, climatic conditions, geographic location, soil type, and the vegetable varieties used. The decrease in Brix level is related to protein hydrolysis by lactic acid bacteria and the depletion of organic components (Won et al., 2018). It is worth noting that literature on Brix levels in yogurt enriched with betalains is limited, making comparisons challenging.

Betalain stability decreases exponentially with increasing moisture, likely due to the susceptibility of pigments to aldimine bond cleavage. The values found in our study fall within the range reported by Dhineshkumar and Ramasamy (2016) for beetroot juice-based yogurt, which ranged from 62.09 ± 0.4% to 91.08 ± 1.6%. Bourlioux et al. (2011) reported water content in yogurts and fermented milks between 80% and 90%, which is similar to the values observed for the negative control yogurt in this study.

Ash content reflects the mineral content in the food. Its decrease may be due to the activity of lactic ferments depleting mineral elements in yogurt (Oguneyemi et al., 2021). Limited studies on ash content in yogurt enriched with betalains make comparisons difficult. Our results differ from those of Martha et al. (2021), who reported syneresis ranging from 18.01 ± 0.7% to 32 ± 0.97%.

Syneresis in yogurt is influenced by the microstructure of the protein network ; insufficient water binding results in whey expulsion during storage (Oguneyemi et al., 2021). Total solids in yogurt help prevent or reduce syneresis, and high fat and protein content are associated with lower whey separation (Martha et al., 2021).

The enrichment of yogurt with plant proteins might improve protein content (Oguneyemi et al., 2021). Decreases in protein content could be due to the degradation of milk proteins, especially whey proteins (Oliveria et al., 2009).

The reduction in betalain content may be due to its hydrolysis to betalamic acid (Laura et al., 2016). Fermentation of red beets lowers betalain content, and lactic fermentation can cause isomerization and dehydrogenation of betanin. Additionally, glycosylation in betacyanins usually reduces antioxidant activity (Izabela Sadowska & Grzegorz, 2021).

The decrease in total polyphenol content could be attributed to the action of lactic acid bacteria during cold storage, which degrades polymerized phenolic compounds (Muniandy et al., 2017). High levels of phenolic compounds in the control yogurt supernatant reflect milk protein degradation. Tyrosine, with its phenolic side chains, can be used to increase polyphenol content in yogurt (Amirdivani, 2015). This increase can be explained by the gradual release of phenolic compounds associated with milk proteins (Muniandy et al., 2017).

Betalains' antioxidant activity increases when a hydroxyl group is present at the C-5 position of the aglycone. Betanin has been shown to scavenge various free radicals dose-dependently (Izabela Sadowska & Grzegorz, 2021). Our results are consistent with Atmani et al. (2009), who reported hydrogen peroxide reduction percentages between 22.5% and 75.11%. Taşkın and Bağdatlıoğlu (2020) found variable percentages between 1.07% and 4%, while EL-Haci (2016) reported a range of 4.25% to 39.50%.

Some lactic acid bacteria and bifidobacteria produce NADH oxidase, which forms H2O2 by oxidizing NADH (Taşkın & Bağdatlıoğlu, 2020). Fermented dairy products, including yogurt, may not effectively reduce H2O2 due to enzymatic activity (Taşkın & Bağdatlıoğlu, 2020). Decreased antioxidant activity during refrigerated storage is likely due to the degradation of phenolic compounds with antioxidant properties and/or increased interactions between milk proteins and polyphenols. Consuming yogurt within 7 days of manufacture is recommended to maximize

live bacterial content and antioxidant activity beneficial for cardiovascular health (Amirdivani, 2015).

The observed decrease in antioxidant activity could be due to increased degradation of phenolic compounds or enhanced interactions between milk proteins and polyphenols (Shori, 2020). An increase might be attributed to the formation of bioactive peptides with enhanced antioxidant activity following milk protein degradation (Virtanen, 2007).

Thus, a concentration of 12 g/l of betalain (from concentrated beetroot juice) is optimal for maintaining the color of betalain-enriched yogurt during refrigerated storage.

Odor is a subjective parameter influenced by individual perception and sensitivity. The fermentation of sugars, which starts near the end of the product's shelf life, could contribute to changes in odor.

Taste evaluations showed that the sweetness was medium, indicating that the amount of added sugar was appropriate. Beet sugar content did not negatively affect the taste, suggesting that the yogurt produced was well-received, as most consumers prefer dairy products that are not excessively sweet.

The results indicate that concentrated beetroot juice (betalain) acts as a preservative, helping to maintain pH levels, prevent the development of an acidic taste, and inhibit sugar fermentation compared to the control.

4. Conclusions

The aim of our study was to evaluate the impact of enriching yogurt with beetroot betalain on various physicochemical parameters, nutrient and polyphenol composition, sensory profile, and antioxidant activity.

Regarding physicochemical criteria, the addition of betalain extract resulted in a decrease in pH during refrigerated storage, while the acidity of the betalain-enriched yogurt increased compared to the control yogurt. However, over the same storage period, there were no significant differences in syneresis, Brix, moisture content, and ash content between the enriched yogurts and the control yogurt.

Our results also indicated that the protein content was not significantly affected by the addition of betalain, as the protein levels in all yogurts were comparable. Conversely, there was an increase in both sugar and phenolic compound levels in the fortified yogurt compared to the control yogurt during the period from day 1 to day 21.

Additionally, the fortification of yogurt with betalain positively impacted the percentage of DPPH free radical inhibition and hydrogen peroxide reduction.

Sensory analysis revealed that yogurts fortified with 6 g/l and 12 g/l of betalain were well-received by tasters, who appreciated their pink color and mild taste.

Overall, our findings support the beneficial effects of adding beetroot betalain to yogurt. Betalain is a valuable source of polyphenols and betalain, which contribute significantly to human health. Consuming betalain-enriched yogurt can thus enhance health and offer protection against the harmful effects of free radicals.

5.References

Alshehry, A. (2019). Utilization of beetroot as a natural antioxidant, pigment and antimicrobial in cupcake during the storage period, *International journal of engineering research and technology*, 8(10). DOI:10.17577/IJERTV8IS100053

Amirdivani, S., Baba, A.S. (2011). Changes in yogurt fermentation characteristics, and antioxidant potential and in vitro inhibition of angiotensin-1 converting enzyme upon the inclusion of peppermint ; dill and basil : *LWT-food science and technology* ,44(6) ,1458-1464. DOI:10.1016/j.lwt.2011.01.019

Amirdivani, S. (2015). Inclusion of *Allium sativum* in yogurt and its effects on inhibition of diabetes and hypertension-associated by enzymes *in vitro*, *Applied*

Food Biotechnology, 2(3), 29-37. https://doi.org/10.22037/afb.v2i3.7714

Atmani, D., Chaher, N., Berboucha, M., Ayouni, K., Lounis, H., Boudaoud, H. (2009). Antioxidant capacity and phenol, content of selected Algerian medicinal plants, *Food chemistry* , 303-309. https://doi.org/10.1016/j.foodchem.2008.05 .077

Azeredo, L.D.C., Azeredo, M.A.A., De Souza, S.R., Dutra, V.M.L. (2003). Proteins contents and physicochemical proprieties in honey samples of *Apis mellifera* of different floral origins , *Food Chemistry*, 80, 249-254. DOI:10.1016/S0308-8146(02)00261-3

Azzouzi, H.,Achchoub, M, Salmaoui, S., Elfazazi, K. (2022). Etude des critères de qualité d'une confiture à base de la clémentine marocaine fabriquée avec la pectine extraite des écorces de clémentine, *African and Mediterranean Agricultural Journal- Al Awamia*, (134), 275-291.

DOI:10.34874/IMIST.PRSM/afrimedi134.3173

Bourlioux, P., Braesco, V., Mater, D.D.G. (2011). Yaourts et autres laits fermentés, *Cahier de nutrition et de dietétique*, 46, 305-314. doi:10.1016/j.cnd.2011.07.001

Castro-Enríquez, D., Montaño-Leyva, B., Del Toro-Sánchez, C. L., Juaréz-Onofre, J. E.,. Carvajal-Millan, E., Burruel-Ibarra, S., Tapia-Hernández, J. A., Barreras-Urbina, C. G., Rodríguez-Félix, F. (2020). Stabilization of betalains by encapsulationa review. *Journal of Food Science and Technoly*, 57(5): 1587–1600. doi: 10.1007/s13197-019-04120-x

Débia, G.D.P.J., Castanheira, C.M., Brito, O.D.C., Barros, B.C.B., Dias-Arieira, C.R. (2023). Chemical composition and antioxidant properties of beetroot parasitized by Meloidogyne javanica and treated with elicitors, *Comunicata scientiae Horticultural journal*, 14. DOI:10.14295/cs.v14.3930

Dhineshkumar, V., Ramasamy, D. (2016). Studies on Development of Yoghurt Flavoured with Beetroot Juice *Beta vulgaris* L.) ; *International Journal of Advanced Research in Biological Sciences*, 3(2), 108-117. http://s-o-i.org/1.15/ijarbs-2016-3-2-17

Feller, C., Jeanson, P ., Giummelly, P., Bonaly. (1991). comparaison de différentes méthodes d'hydrolyse acide en vue du dosage des glucides totaux dans les sols ,*Science du sol*, 29(1), 13-22.

Fekat, A., Chimde, L., Yimam, K. (2022), Quality analysis of raw milk in west Guji Zone, oromia Regional state, Ethiopia Inv, *Entum biologicum*, 2(1), 41-49. DOI:10.5281/zenodo.6550829

Fisberg, M., Machando, R. (2015). History of yogurt and current pattern of consumption, *Nutrition reviews*, 73(S1), 4-7. DOI: 10.1093/nutrit/nuv020

Haddar, W. (2016). Valorisation des déchets de végétaux dans la teinture des fibres, textiles,*Thèse de doctorat de l'école nationale d'ingénieurs de monastir,* Tunisie, 7- 9 -10- 11.

Izabela Sadowska Bartosz., Grzegorz Bartosz. (2021). Biological Properties and Applications of Betalains, *Molecules*, 26 (9), 2520. https://doi.org/10.3390/molecules26092520

Jimoh, K.O., Kolapo, A.L. (2007). Effect of different stabilizers on acceptability and shelf- stability of soy- yogurt, *African Journal of biotechnology*, 6(8), 1000- 1003.

Kaur, R., Kaur, G., Mishra, S. K., Panwar, H., Mishra, K. K., Brar, G. S. (2017). Yogurt: A nature's wonder for mankind, *International Journal of Fermented Foods*, 6(1), 57-69. DOI:10.5958/2321-712X.2017.00006.0

Khatabi, O., Hanine, H., Elothmani, D., Hasib, A. (2013). Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (*Opuntia ficus indica*), *Arabian Journal of Chemistry*, 3. DOI:10.1016/j.arabjc.2011.04.001

Kim, Y., Kim, J.W., Cheon, S., Nam, M.S., Kim, K.K. (2019). Alpha-Casein and Beta-Lactoglobulin from Cow Milk Exhibit Antioxidant Activity: A Plausible Link to

Antiaging Effects, *Journal of Food Science*, 84(11):3083-3090. DOI: 10.1111/1750-3841.14812

Laura, B., Gheorghe, T., Verginica, T. (2016). La teneure en betalaines et la capacité antioxydante de betterave rouge, *université ovidius constanta, Faculté pharmacie*,64 (2).

Madora, E.P., Takalani, T. K., Mashau, M.E. (2016). Physicochemical, microbiological and sensory properties of low fat yoghurt fortified with carrot powder, *International Journal of Agricultural & Biological Engineering*, 9(1), 118－124. DOI:10.3965/j.ijabe.20160901.1874

Martha, A.F-M., Martha, G.R-G., Ana, L., Rogehio, S.-V., Juliana, J-M., Eduardo, S-E., América, C-M. (2021). Stirred yogurt added with beetroot extracts as an antioxidant source : rhelogical sensory and physicochemical characteristics. *Journal of Food Processing and Preservation* 45(1). DOI:10.1111/jfpp.15628

Mahaut, M., Romain, J., Schuck, P., Brulé, G. (2000). Les produits industriels laitiers, *Ed: Tec et Doc, Lavoisier* ; 1-74 , 2-7430-0429-

Muniandy, P., Shori, A. B., Baba, A.S. (2017). Comparison of the effect of green, white and black tea on *Streptococcus thermophilus* and *Lactobacillus* spp. in yogurt during refrigerated storage, *Journal of the Association of Arab Universities for Basic and Applied Sciences*, 22(1), 26-30. https://doi.org/10.1016/j.jaubas.2015.11.002

Nirmal, N.P., Mereddy, R., Maqsood, S. (2021). Recent developments in emerging technologies for beetroot pigment extraction and its food applications, *Food chemistry*, 15:356:129611. doi:10.1016/j.foodchem.2021.129611.

Özturk, B.A., Óner, M.D. (1999). Production and Evaluation of Yogurt with Concentrated Grape Juice, *Journal of food science*, 64, (3), 530-532.

Prieto, P., Pinesa, M., Aguilar, M. (1999). Spectrometric quantitation of antioxydant capacity through the formation of a phosphomolybdenum complex:specific application to the determination of vitamin E, *analytical biochemistry*, 269, 337-341. https://doi.org/10.1006/abio.1999.4019

Ravichandran, K., Saw, N.M.M.T., Mohdaly, A-A., Gabr, A-A., Kastell, A., Riedel, H., Cai, Z., Knorr, D., Smetanska, I. (2011). Impact of processing of red beet on betalain content and antioxidant activity, *Food Research International*, 50 (2), 670–675. DOI:10.1016/j.foodres.2011.07.002

Shalaby, H.S., Hassenin, A.S.H. (2020). Effects of fortification stirred yoghurt with red beet powder (RBP) on hypercholesterolemia rats, *European journal of agriculture and food sciences*, 2(5). DOI:10.24018/ejfood.2020.2.5.61

Shivangi, S., Shanta, P., Parimita, P., Trupti, P., Sarfraz, A. (2019). Studies on cup-cakes incorporated with beetroot and wheatgrass powder, *International Journal of Food Science and nutrition*, 4(4), 206-208. DOI:10.13140/RG.2.2.29619.17443

Shori, A.B., Rashid, F., Baba, A.S. (2018). Effect of the addition of phytomix-3+ mangosteen on antioxidant activity, viability of lactic acid bacteria, type 2 diabetes key enzymes, and sensory evaluation of yogurt, *Food science and technologie*, ; 94 (7), 33-39. DOI:10.1016/j.lwt.2018.04.032

Shori, A.B. (2020), Inclusion of phenolic compounds from diffrent medicinal plants to increase α amylase inhibition activity and antioxidants in yogurt, *Journal pf taibah university for science*, 14(1), 1000-1008. https://doi.org/10.1080/16583655.2020.1798072

Taşkın, B., Bağdatlıoğlu, B. (2020). Influence of Conventional Fermentation on Antioxidant Activity and Phenolic Contents of Two Common Dairy Products: Yogurt and Kefir ; Turkish *Journal of Agriculture - Food Science and Technology*, 8(6), 1277-1282. https://doi.org/10.24925/turjaf.v8i6.1277-1282.3290

Virtanen, T., Pihlanto, A., Akkanen, S., Korhonen, H. (2007). Development of antioxidant activity in milk whey dming fermentation with lactic acid bacteria, *Journal of applied microbiology*, 102(1), 106-115. DOI: 10.1111/j.1365-2672.2006.03072.x

Watts, B.M., Ylimaki, G.L., Jeffery, L.E., Elias, L.G. (1991). Méthodes de base pour l'évaluation sensorielle des aliments, Faculté d'écologie humaine, Université du Manitoba, *Centre de recherches pour le développement international*, Ottaw, canada.

Won, J. I., Lee, J. H., Park, H. I., Cho, Y.U., Choi, I.D., Lee, S.K., Choi, H.S. (2018). Quality characteristics of commercial semisolid type yogurt in Korea, *Journal of the Korean Society of Food Science and Nutrition*, 47(11), 1185-1190. DOI:10.3746/jkfn.2018.47.11.1185

Yadav, A., Jaiswal, P., Raghuwanshi, S., Prasads, G.B.K.S., Bisen, P.S. (2015). Importance of probiotics yogurt for human health improvement, *Journal of environmental science toxicology and food technology*, 9(7), 25-30. DOI:10.9790/2402-09722530

Acknowledgment

The author would like to thank the engineers Asma SABBA and Mokhtar KHERRAT from the laboratory of biology at the University of Jijel for their gracious assistance. They are also grateful to the INATAA Institute, especially Bioqual laboratory for their support.