CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 16(3)
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.(3) 2024

UNIVERSITATEA TEHNICĂ DIN CLUJ-NAPOCA

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

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ISSN-L 2066 -6845

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

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EFFECTS OF THE POST-FLOWERING TIMELINES ON THE NUTRITION, PHYTOCHEMICAL COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM.)

Chi Khang Van[1,✉], Thanh Ngan Truong[2], Thi Tuyet Lan Le[2], Trinh Thi Nhu Hang Nguyen[1], Binh An Pham[1], Cong Kha Nguyen[3]

[1]*Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Vietnam*

[2]*Department of Natural Products, Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City 700000, Vietnam*

[3] *Institute of Food and Biotechnology, Can Tho University, Can Tho City, Vietnam*

✉ *vckhang@ntt.edu.vn*

https://doi.org/10.34302/crpjfst/2024.16.3.1

Article history:
Received:
January 28th, 2024
Accepted:
August 1st, 2024

Keywords:
Jackfruit;
Artocarpus heterophyllus;
Lam;
Nutrition;
Phytochemical;
Antioxidant activities.

ABSTRACT

The nutrition, phytochemical compounds, and antioxidant activity of jackfruit (Artocarpus heterophyllus Lam.) are affected by the post-flowering timelines. Therefore, selection of appropriate harvest timing is an important factor to reduce the post-harvest losses. The objective of this study was to determine the nutritional, chemical, and antioxidant activity changes of jackfruit parts (e.g. pulp, fiber, and seed) collected from the top, middle, and bottom of the fruit at 3 different times after flowering (100 days, 110 days, and 120 days). Jackfruit parts were extracted from ethanol solvent before analysis. Results have shown that the seeds contained a high amount of starch (10.6±0.07% - 14.86±0.03%), while the sugar, total acidity (TA) and total soluble solids (TSS) are absent. The antioxidant activity was determined by three free radical scavenging methods ABTS·+, DPPH·, and phosphomolybdenum (TAC) method. At 120 days at the top of jackfruit, the highest total sugar and TSS content were 79.48 ± 2.8 (mg/gDM) and 25.25 ± 0.35% in jackfruit pulp, respectively, the highest TA content was 0.36. ± 0.02% in jackfruit fiber, the highest protein, ash and fat content in jackfruit seeds were obtained at 4.43 ± 0.01%, 1.12 ± 0.03%, 3.22 ± 0.01%, respectively. The highest carbohydrate content and pH in the jackfruit pulp at the end of the fruit at 110 days were 26.18%, 6.63 ± 0.03, respectively. The highest fiber content in jackfruit seeds at the end of the fruit at 100 days was 19.7±0.12.

1.Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) belongs to the genus Artocarpus, family Moraceae (the Mulberry family, Moraceae), and is commonly grown in Vietnam. It is native to the Western Ghats of India, Malaysia, Southeast Asia and the islands of the Pacific Ocean (Prakash et al., 2009). Jackfruit tree is an important component in the livelihoods of farmers in many eco-geographic regions around the world. The tree is the main source of food and essential products for the poor. The fruit, leaves, and barks of jackfruit tree have been extensively used in traditional medicine due to their anticarcinogenic, antimicrobial, antifungal, anti-inflammatory, wound healing, and hypoglycemic effects. Jackfruit contains a wide range of nutrients such as flavonoids, phenolic acids, organic acids, carotenoids, stilbene, triterpenes, and sterols, especially prenylflavonoids (Baliga et al., 2011; de Faria et al., 2009). In addition, vitamins and minerals, especially riboflavin (B_2), potassium, and phosphorus are present in the jackfruit pulp and fiber (Moke et al., 2017). Due to several health-promoting benefits, it is necessary to analyze the phytochemical components of jackfruit. The chemical compounds and antioxidant activities have been previously reported. The chemical compounds and antioxidant activities have been previously reported. Chavez-Santiago et al., 2022 analyze the polyphenolic content, as well as the antioxidant and antifungal properties of jackfruit extract on phytopathogenic fungi. In 2020, Adan et al. studied the phytochemical composition and essential mineral profile of the unutilized parts of jackfruit, which gave rise to the significant antioxidant and antimicrobial potentials. In 2021, Juan et al. evaluated the content of nutrients, minerals, and antioxidants (e.g. synthetic phenolics, flavonoids, vitamin C, and carotenes) found in jackfruit pulp. With an attempt to produce jackfruit of high quality to meet the high demand of consumers in the world, it is essential to select the optimal harvest ripeness. This study provides an essential insights in the effects of the harvesting stage on the content of nutrients, phytochemical

compounds and antioxidant activity of the jackfruit by-products, thereby diversifying the valuable products range of jackfruit, reducing the risk of environmental pollution, and promoting the development of the food industry towards the sustainable development of the agricultural production industry. Therefore, we chose jackfruit to analyze the chemical composition, and antioxidant activity of jackfruitpulp, fiber, and seed located at the top, middle and bottom of the fruit after 100 days, 110 days, and 120 days of flowering. The knowledge of the phytochemical composition of these fruit parts would provide a cost-effective alternative source of phytochemicals and essential minerals that exhibits high biological activity for application to other fields in the future.

2.Materials and methods

2.1.Materials

Jackfruit (Artocarpus heterophyllus Lam.) was obtained at commercial farms located in Can Tho City, Viet Nam (10.0452° N, 105.7469° E). The harvested fruit were immediately transported to the laboratory.

Jackfruit takes about 20 days for the flowers to successfully undergo the anthesis process, followed by about 95 – 100 days to mature for harvesting. Three batches of fruits were harvested after100, 110, and 120 days of flowering. The dimensions and mass of the whole fruit were measured, then washed thoroughly with water and the top, middle and bottom portions were equally sectioned. The peel, and core of the fruit were removed. Each part was evaluated for several physical characteristics such as shape, mass, dimensions, and distribution ratio of the fruit parts. Finally, the samples from each fruit part were randomly selected for chemical analysis. Chemical analyses were carried out in duplicate for each batch.

2.2.Analysis methods

2.2.1. Qualitative methods

Phytochemical groups, including alkaloids, flavonoids, carbohydrates, amino acids, organic acids, phenolic and tannins, proteins, saponins were determined follwing a method by Phung, (2007).

2.2.2. Determination of nutrition

Moisture content (expressed as a percentage by weight on a wet basis) was determined using the standard official methods of analysis (AOAC 1990). This involved drying to a constant weight at 105 ^{o}C at calculated moisture as the loss in weight of the dried samples. A total of 1 g of oven-dried sample was subjected to determination of protein content in sample following the method described by Hema et al. (2016). Total nitrogen content in samples was determined by the modified Kjeldahl method, involving H_2SO_4 salicylic acid digestion, distillation and titration (Bremner & Keeney, 1965). The fat content was measured using a partial drying of a weighed sample prior to Soxhlet extraction (Nielsen & Carpenter, 2017). Dry ash contents of all jackfruit samples were determined following standard procedures (AOAC, 1977). Crude fiber is determined following the standard procedures (AOAC, 1982). The starch content of jackfruit seed was measured by the polarimetry method, as described by Subroto (2020).

The total sugar content, pH, tritable acidcity (TA), and total soluble solids (TSS) was measured in the jackfruit fiber and pulp. The total sugar was determined as previously described by Hema (2015). Total soluble solids (TSS) content was measured following TCVN 4417 – 87 by using ATAGO digital refractometer (Atago Co.Ltd , Tokyo, Japan). The titratable acidity (TA) and pH measurement method followed the procedure of Rangana (1979) with some modifications.

2.2.3. Quantitative analysis

Determination of total carotenoid content (TCC)

TCC determination followed the method described by Wellburn (1994) [15]. The absorbance spectra of chlorophyll a, chlorophyll b and total carotenoids were measured at 664 nm, 647 nm and 470 nm, respectively.

Determination of total polyphenol content (TPC)

TPC was measured by Folin-Ciocalteu assay, as previously described in Jagtap (2010), followed by absorbance measurement at 765 nm using Shimadzu UV160A UV-Vis spectrophotometer (Kyoto, Japan).

Determination of total flavonoid content (TFC)

TFC was determined following the procedure described by Park et al. (2008) [17] with some modifications, followed by absorbance measurement at 510 nn.

2.2.4. Antioxidant activity methods

Determination of total acidity capacity (TAC)

TAC was determined by using the phosphomolydenum method as previously described by Saha et al. (1970) with slight modifications. The reagent solution was prepared by mixing 0.6 M H_2SO_4 (95-97%) with 4 mM ammonium molybdate (98%) and 28 mM sodium dihydrogen phosphate using a glass rod. 3 mL of the prepared reagent was taken into separate test tubes and added with 0.3 mL of extract samples at various concentrations (100 - 500 μg/mL). Methanol was used as negative control (blank). All test tubes subjected to incubation in an oven at 95 °C for 90 min, then allowed to cool down at room temperature. The absorbance was measured at 695 nm using UV-Vis spectrophotometer (Shimadzu Corp., Kyoto, Japan). Investigation of free radical scavenging activity by DPPH· method.

The inhibitory activity of the samples against DPPH free radicals followed the previously described procedure by Sharma et al.

(2009). A total of 1.5 mL of 0.1 mM DPPH solution was mixed with 0.5 mL of sample solution of the diluted concentrations, followed by 30-min incubation without light and absorbance measurement at 517 nm. The antioxidant activity of the sample is indicated by 50% antioxidant efficiency (IC_{50}) (Miliauskas et al., 2004).

Investigation of free radical scavenging activity by ABTS·⁺ method

The antioxidant activity was determined by the ABTS free radical scavenging assay described by Nikolaos et al. (2004). ABTS·+ free radical solution was prepared by adding 10 mL of 7.4 mM ABTS·⁺ solution to 10 mL of 2.6 mM $K_2S_2O_8$ solution, then incubating for 24 h in the dark and adjusting the solution absorbance at 734 nm to 1.1 ± 0.02. 5 g of sample was diluted on 100 mL and 0.5 mL of which was drawn and placed into the test tube. Ethanol (99.5%) was used as the control. Then, 1.5 mL of ABTS·⁺ solution ($OD_{517\ nm}$ = 1.1±0.02) was added into the test tube and leave in the dark for 30 min. Absorbance measurement was measured at 734 nm using Shimadzu UV-Vis spectrophotometer (Japan). Vitamin C (ascorbic acid) was used as the standard for comparison.

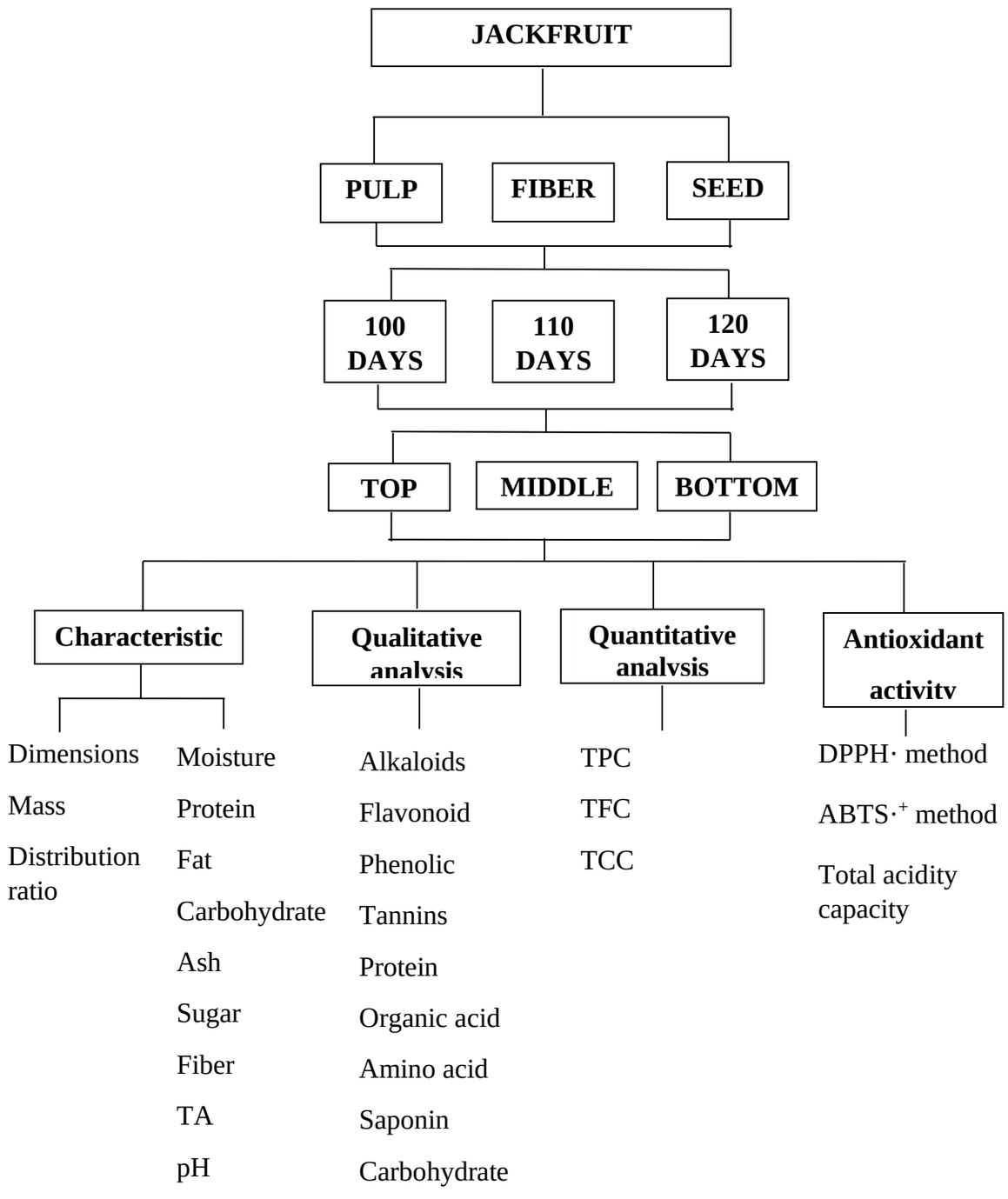

Figure 1. Schematic diagram of the experiment

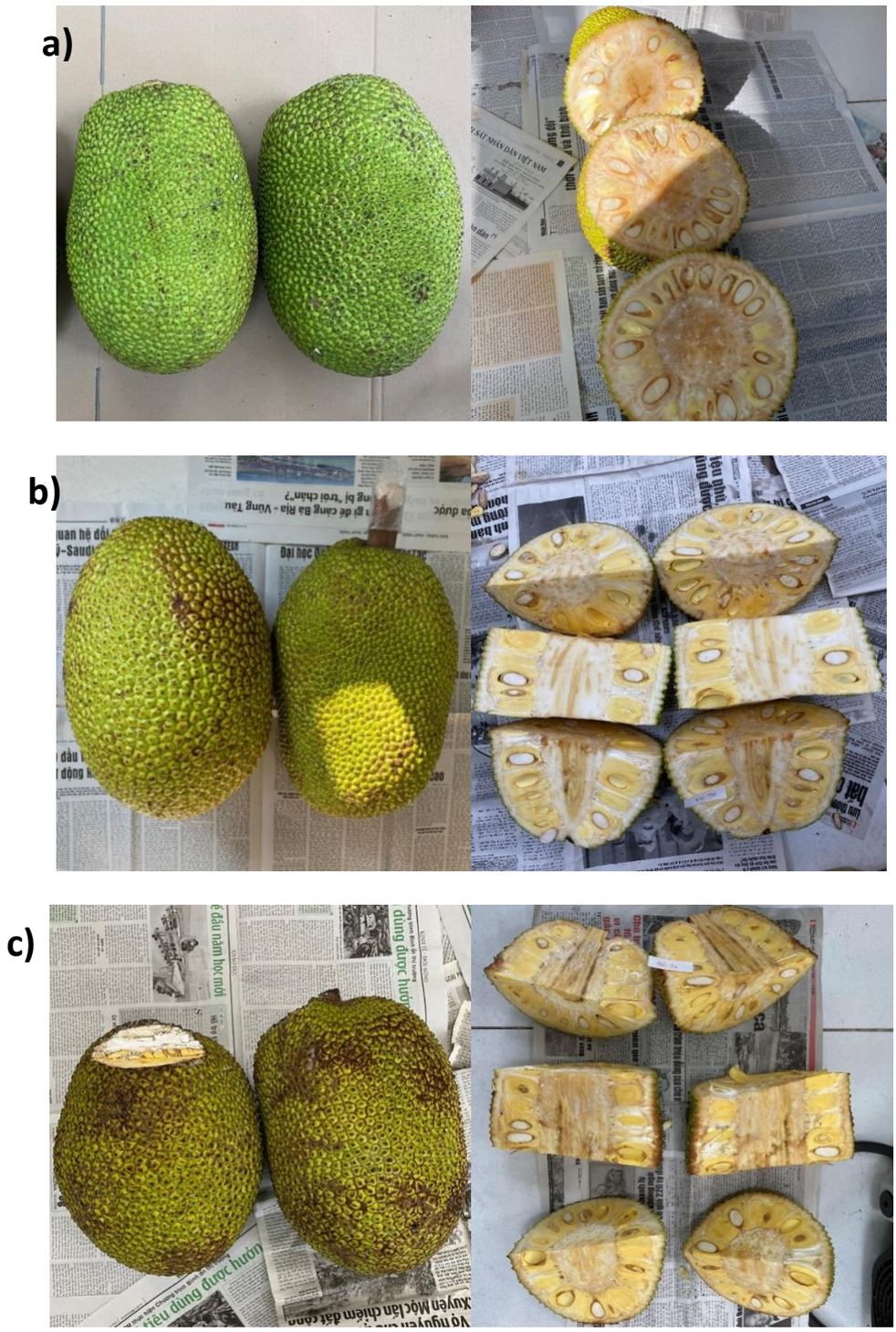

Figure 2. Jackfruit in the different days after flowering
(a) 100 days, (b) 110 days, (c) 120 days

3. Results and discussion

3.1. Determination of chemical compounds in jackfruit

The phytochemical profile of jackfruit pulp, fiber, and seed at different post-flowering timelines (i.e. 100 days, 110 days and 120 days) were shown in Table 1. Results have shown that alkaloids, flavonoids, phenolics, tannins, proteins, organic acids, carbohydrates and saponins are present in all jackfruit parts. This preliminary chemical quantification is very useful in finding the chemical components in plant materials and the quantitative part can be performed in the next indicators. Alkaloids are a large group of naturally occurring organic compounds that contain a nitrogen atom or atom in their structure and are widely used in pharmaceuticals and medicine because of their antibaterial properties (Omulokoli et al., 1997). Flavonoids and tannins are phenolic compounds, a major class of compounds that act as major antioxidants or free radical scavengers (Bhandary et al., 2012). These compounds were detected in the extract of jackfruit pulp with ethanol as a solvent, so it can be demonstrated

that jackfruit has strong antioxidant capacity. Tannins are recognized to inhibit the growth of many molds, yeasts, bacteria and viruses (Hegde et al., 2012). Secondary metabolites and other chemical constituents have been reported in the pulp. For example, saponins have antihypertensive and heart failure properties. The presence of saponins in jackfruit pulp may play a role in cardioprotective potential (Olaleye, 2007). Bhandary et al., (2012) also had a study comparing extracts from two different solvents, ethanol and methanol, to qualitatively identify chemical compounds. As a result, ethanol solvents have better extraction capacity and recover many natural antioxidants that can be applied in medicine, promoting supplements for the food industry. The ethanol extract was also selected for this study. Besides, this preliminary chemical quantification is very useful in analyzing the chemical components in plant materials, thereby performing subsequent quantification of other indicators.

Table 1. Phytochemical compounds of jackfruit portions in the different days after flowering

Portions	**Number of day** / **Compounds**	**100**	**110**	**120**
Pulp	Alkaloids	+	++	+++
	Flavonoids	+ ++	+++	+ +
	Phenolic & Tannin	+ +	++	+
	The protein	+	++	+++
	Amino Acids	+	++	+++
	Organic Acids	+	++	+++
	Carbohydrates	++	+	+
	Saponins	++	+++	+++
Fiber	Alkaloids	+	++	+++
	Flavonoids	+	++	+++
	Phenolic & Tannin	++	+ +	+
	The protein	+	++	++
	Amino Acids	+	++	+++
	Organic Acids	+	++	+++
	Carbohydrates	+ +	+	+
	Saponins	+	+	+
Seed	Alkaloids	+	+	+
	Flavonoids	+	+	+
	Phenolic & Tannin	++	+ +	+
	The protein	+	++	++
	Amino Acids	+	++	+++
	Organic Acids	+	++	++
	Carbohydrates	+ +	+	+
	Saponins	+	++	++

Table 1 describes the presence of compounds in jackfruit parts, including the pulp, fiber, seed at different post-flowering timelines 100 days, 110 days and 120 days. The phytochemical compounds include alkaloids, flavonoids, phenolics, tannins, proteins, organic acids, carbohydrates and saponins are all exist in jackfruit parts. This preliminary chemical quantification is very useful in finding the chemical components in plant materials and the

quantitative part can be performed in the next indicators. Alkaloids are a large group of naturally occurring organic compounds that contain a nitrogen atom or atom in their structure and are widely used in pharmaceuticals and medicine because of their antibaterial properties (Omulokoli et al., 1997). Flavonoids and tannins are phenolic compounds, a major class of compounds that act as major antioxidants or free radical scavengers (Bhandary et al., 2012). These compounds were detected in the extract of jackfruit pulp with ethanol as a solvent, so it can be demonstrated that jackfruit has strong antioxidant capacity. Tannins are recognized to inhibit the growth of many molds, yeasts, bacteria and viruses (Madhuri et al., 2012). Secondary metabolites and other chemical constituents have been reported in the pulp. For example, saponins have antihypertensive and heart failure properties. The presence of saponins in jackfruit pulp may play a role in cardioprotective potential (Tolulope, 2007). Bhandary et al. (2012) also had a study comparing extracts from two different solvents, ethanol and methanol, to qualitatively identify chemical compounds. As a result, ethanol solvents have better extraction capacity and recover many natural antioxidants that can be applied in medicine, promoting supplements for the food industry. The ethanol extract was also selected for this study. Besides, this preliminary chemical quantification is very useful in finding the chemical components in plant materials, from which the quantification of the next indicators can be performed.

3.2.Evaluation of the characteristics of jackfruit

3.2.1.Physical characteristics

Table 2. Physical characteristics of jackfruit at different the post-flowering timelines

The post-flowering timelines / **Indicators**		**100**	**110**	**120**
Shape		Oblong	Oblong	Oblong
Dimensions (cm)	**Length**	35	36	30
	Width	24.5	21.5	23.5
Weight (kg)		8.3	8	8.5
Outside color		Light green	Dark green	Dark green, black spots, and streaks appear
Inside color		Light yellow	Light yellow	Yellow
Aroma		No scent	Light scent	Clear scent
Taste		No taste	Light sweetness	Clear sweetness

Table 2 describes the appearance and morphological characteristics of jackfruits harvested at different post-flowering timelines (100 days, 110 days, and 120 days). The jackfruits generally have the same weight, size and color to evaluate the next indicators. These fruits are generally oblong or pear-shaped with thick green peel. The outer color and aroma increased in the post-flowering timelines. The fruit colour changes from yellowish green to yellow due to the conversion of chlorophylls, anthocyanins, and carotenoids like pigments during ripening (Ranasinghe et al., 2019). Ong et al. (2006) have shown an increase in the color of the jackfruit at days 1, 3, 5, and 6 after harvest. The strong aroma is due to the presence of aromatic compounds that are esters in the fruit (Ong et al., 2006). Sword et al. (1978) have confirmed that isopentyl isovalerate was particularly dominant in jackfruit. The taste of fruits is effected by the TSS:TA ratio (Krüger et al., 2012). Kruger et al. (2012) reported that fruit flavor is mainly determined by total acid (TA) and total soluble matter (TSS) content. Saxena et al. (2011) also reported that jackfruit had TSS greater than 25°brix and TA could be titrated

0.3% suitable for consumer taste. As for dimension and mass, the fruits selected above have a weight of 6 - 8 kg and a length of 30 - 36 cm, generally not too much difference in days. The average weight of jackfruit ranges from 2 to 20 kg, some fruits reaching up to 50 kg have been published previously (Ranasinghe et al., 2019). The differences in shape, size and weight are due to factors such as variety, soil, fertilizers, weather, crops, cultivation and storage.

Table 3. Mass of jackfruit parts as percentages of total weight at different the post-flowering timelines

Jackfruit part weights (%)			
	100 days	**110 days**	**120 days**
Pulp	26.54	30.50	27.69
Fiber	16.05	12.50	17.69
Seed	14.81	13.25	14.62
Peel	17.28	25.00	16.92
The other	22.22	18.75	23.08

Table 3 describes the weight of jackfruit parts as percentages of total weight, including peel, fiber, pulp, seed, and the other at different the post-flowering timelines. According to the results, jackfruit pulp had the highest distribution rate in 100 days, 110 days and 120 days at 26.54%, 30.5% and 27.69%, respectively. Besides, the weight of fiber, seed, peel, and other parts fluctuated from 12.5% to 17.69%, from 13.25% to 14.81%, from 16.92% to 25% and from 18.75% to 23.08% respectively. The means followed by the different superscript letters in the same row within the column of each individual portion are significantly different ($p < 0.05$ by Duncan's multiple range test).

3.2.2.Nutritional characteristics

3.2.2.1.Moisture

Moisture is an important parameter affecting the quality and appearance of fruit, and the post-flowering timelines and portions significantly affect moisture content ($p<0.05$). In general, the moisture content tended to increase gradually on different days of ripening, yet decrease slightly at the top, middle, and bottom parts. Similar results were alo reported by Ong et al. (2006). This can be explained by the fact that the ripening process of the jackfruit proceeds from the top to the bottom, so the first ripening part contained higher moisture than the remaining. The highest moisture content was found in the fiber (from 72.87 ± 0.3% to 88.52 ± 0.22%), followed by the pulp (from 72.23 ± 0.06% to 79.54±0.08%), and seed (from 50.96 ± 0.71% to 69.87 ± 0.25%).

This result of the pulp was similar to the study by Goswami et al. (2011) (from 79.62% to 84.44%). However, the obtained results were different from Ranasinghe's study, which was from 70.94 to 89.21% (Ranasinghe & Marapana, 2019). The differences can be attributed to the differences in internal factors (e.g. genus, and varieties) and external factors (e.g. environment, and cultivation) that affected the tested subjects. ANOVA results showed that the fruit parts, portions and the post-flowering timelines significantly affect moisture content at 95% confidence level ($p<0.05$).

3.2.2.2.Fat

The fat contents of pulp, fiber, and seed tended to increase as the fruits ripened, yet decreasing from the top to the bottom part of the fruit. This change could be explained based on the ripening process of jackfruit The seed had the highest fat content (from 0.44 ± 0.01 to 3.22 ± 0.01%), followed by the pulp (from 0.37 ± 0.01 - 1.52 ± 0.06%) and fiber (from 0.3224 ± 0.09 to 2.2498 ± 0.03%). The fat content of the pulp tended to increase as the ripening proceeded, which was similar to the results of Shamla et al. (2019). In addition, ANOVA results also showed that the fruit parts, portions and the post-flowering timelines significantly affected fat content ($p<0.05$).

3.2.2.3.Protein

Similar to the fat content, the protein content was also significantly affected by the post-flowering timelines and fruit parts ($p<0.05$). The fiber had the lowest value, as compared to the pulp and seed. The protein values of pulp, fiber and seed were reported to increase from 0.48± 0.12 to 1.22 ± 0.02%, 0.81 ± 0.01 to 1.11 ± 0.02% and 3.95 ± 0.014 to 4.44 ± 0.01%, respectively. This value of the pulp is similar to the study of Goswami et al. (2011). Sabahelkhier et al. (2010) reported that the albumin, globulin and protein content increased proportionally with the mature stage in pineapple.

3.2.2.4.Ash

The ash content of the pulp, fiber and seed were 0.61 ± 0.03 - 0.97 ± 0.03%, 0.67 ± 0.03 - 0.88 ± 0.03%, and 0.78 ± 0.02 - 1.12 ± 0.03%, respectively (Table 4).

Table 4. Nutritional characteristics change at different days after flowering in different portions of jackfruit parts

		Top			Middle			Bottom		
		100 days	**110 days**	**120 days**	**100 days**	**110 days**	**120 days**	**100 days**	**110 days**	**120 days**
Pulp	**Moisture (%)**	73.65^{cdh}±0.04	75.14^{ceh}±0.02	79.54^{cfh}±0.00	73.19^{bdh}±0.01	73.65^{beh}±0.04	78.47^{bfh}±0.06	72.41^{adh}±0.08	72.23^{aeh}±0.08	74.11^{afh}±0.02
	Sugar (mg/gDM)	15.87^{cdg}±2.04	39.17^{ceg}±1.18	79.48^{cfg}±2.8	16.77^{bdg}±0.77	35.42^{beg}±1.77	76.82^{bfg}±0.05	16.50^{adg}±0.01	33.06^{aeg}±0.01	75.93^{afg}±0.06
	Protein (%)	0.65^{cdg}±0.1	1.1^{ceg}±0.03	1.22^{cfg}±0.02	0.6^{bdg}±0.06	0.98^{beg}±0.05	1.19^{bfg} ±0.05	0.48^{adg}±0.12	0.82^{aeg}±0.04	1.05^{afg}±0.03
	Ash (%)	0.67^{cdh}±0.03	0.87^{ceh}±0.02	0.97^{cfh}±0.03	0.66^{bdh}±0.02	0.82^{beh}±0.03	0.93^{bfh}±0.03	0.61^{adh}±0.03	0.78^{aeh}±0.02	0.92^{afh}±0.02
	Fat (%)	0.28^{cdh}±0.04	0.76^{ceh}±0.11	2.34^{cfh}±0.06	0.26^{bdh}±0.06	0.67^{beh}±0.07	2.24^{bfh}±0.17	0.32^{adh}±0.09	0.50^{aeh}±0.09	2.25^{afh}±0.03
	Fiber (%)	10.18^{adh}±0.22	8.89^{afh}±0.15	7.93^{aeh} ±0 .03	11.42^{bdh}±0.13	9.36^{bfh}±0.07	8.15^{beh}±0.04	12.14^{cdh}±0.06	9.96^{cfh}±0.05	8.23^{ceh}±0.1
	Carbohydrate (%)	24.76	22.14	15.93	25.29	23.88	17.17	26.18	25.67	21.67
	TA (%)	0.11^{cdg}±0.02	0.19^{ceg}±0.01	0.23^{cfg}±0.02	0.09^{bdg}±0.01	0.16^{beg}±0.01	0.19^{bfg}±0.01	0.08^{adg}±0.01	0.15^{aeg}±0.01	0.18^{afg}±0.01
	pH	6.08^{afh}±0.04	5.73^{aeh}±0.01	5.50^{adh}±0.02	6.52^{bfh}±0.02	5.95^{beh}±0.04	5.55^{bdh}±0.03	6.63^{cfh}±0.03	5.93^{ceh}±0.02	5.60^{cdh}±0.02
	TSS (%)	6.55^{cdh}±0.07	14.00^{ceh}±0.00	25.25^{cfh}±0.35	5.38^{bdh}±0.07	12.85^{beh}±0.49	23.75^{bfh}±1.06	4.70^{adh}±0.14	11.90^{aeh}±0.14	21.70^{afh}±0.42
Fiber	**Moisture (%)**	74.49^{cdi}±0.31	79.16^{cei}±0.24	88.52^{cfi}±0.22	73.64^{bdi}±0.27	78.48^{bei}±0.33	87.17^{bfi}±0.34	72.87^{adi}±0.3	76.22^{aei}±0.21	85.41^{afi}±0.13
	Sugar (mg/gDM)	14.28^{cdh}±1.27	16.77^{ceh}±0.77	27.92^{cfh}±0.59	13.02^{bdh}±0.00	16.5^{beh}±0.39	24.07^{bfh}±0.8	12.23^{adh}±0.38	15.04^{aeh}±0.57	21^{afh}±1.17
	Protein (%)	0.87^{xdh}±0.01	0.99^{ceh}±0.02	1.11^{cfh}±0.02	0.84^{bdh}±0.01	0.95^{beh}±0.02	1.06^{bfh}±0.02	0.81^{adh}±0.01	0.91^{aeh}±0.01	1.02^{afh}±0.02
	Ash (%)	0.68^{cdg}±0.02	0.77^{ceg}±0.03	0.88^{cfg}±0.03	0.68^{bdg}±0.02	0.77^{beg}±0.028	0.88^{bfg}±0.03	0.67^{adg}±0.03	0.76^{aeg}±0.03	0.87^{aeg}±0.02
	Fat (%)	0.48^{cdg}±0.10	0.86^{ceg}±0.00	1.52^{cfg}±0.06	0.48^{bdg}±0.01	0.86^{beg}±0.00	1.52^{bfg}±0.06	0.37^{adg}±0.01	0.69^{aeg}±0.02	1.39^{afg}±0.04
	Fiber (%)	9.79^{adg}±0.05	7.61^{afg}±0.14	6.40^{aeg}±0.15	10.84^{bdg}±0.00	7.55^{bfg}±0.14	7.07^{beg}±0.03	10.8^{cdg}±0.21	7.57^{cfg}±0.06	7.07^{ceg}±0.03
	Carbohydrate (%)	23.48	18.23	7.98	24.48	19.12	9.52	24.48	19.12	9.52
	TA (%)	0.15^{cdh}±0.01	0.26^{ceh}±0.04	0.36^{cfh}±0.02	0.13^{bdh}±0.01	0.20^{beh}±0.01	0.34^{bfh}±0.02	0.10^{adh}±0.01	0.17^{aeh}±0.02	0.29^{afh}±0.01
	pH	5.59^{afg}±0.04	5.30^{aeg}±0.02	4.98^{adg}±0.02	5.66^{bfg}±0.03	5.34^{beg}±0.02	5.18^{bhg}±0.02	5.77^{cfg}±0.02	5.39^{ceg}±0.04	5.23^{cdg}±0.04
	TSS (%)	5.52^{cdg}±0.25	8.2^{ceg}±0.28	17.50^{cfg}±0.14	4.75^{bdg}±0.49	8.20^{beg}±0.28	16.30^{bfg}±0.42	3.95^{adg}±0.07	6.80^{aeg}±0.28	11.60^{afg}±0.28
Seed	**Moisture (%)**	54.49cdg±0.20	62.39ceg±0.12	69.87cfg±0.25	54.66^{bdg}±0.55	61.07^{beg}±0.24	69.45^{bfg}±0.20	63.05^{adg}±0.21	59.84^{aeg}±0.87	50.96^{afg}±0.71
	Protein (%)	3.98^{cdi}±0.01	4.19^{cei}±0.01	4.43^{cfi}±0.01	3.97^{bdi}±0.01	4.19^{bei}±0.01	4.43^{bfi}±0.00	3.95^{adi}±0.01	4.18^{aei}±0.00	4.43^{afi}±0.01
	Ash (%)	0.83^{cdi}±0.03	0.99^{cei}±0.02	1.12^{cfi}±0.03	0.8^{bdi}±0.02	0.97^{bei}±0.02	1.1^{bfi}±0.03	0.78^{adi}±0.02	0.95^{aei}±0.02	1.02^{afi}±0.02
	Fat (%)	0.57^{cdi}±0.00	2.47^{cei}±0.00	3.22^{cfi}±0.01	0.45^{bdi}±0.01	1.84^{bei}±0.03	2.62^{bfi}±0.07	0.44^{adi}±0.01	1.61^{aei}±0.04	2.61^{afi}±0.04
	Fiber (%)	17.97^{adi}±0.21	15.21^{afi}±0.21	2.861^{aei}±0.02	18.11^{bdi}±0.09	15.23^{bfi}±0.04	2.93^{bei}±0.01	19.7^{cdi}±0.12	15.45^{cfi}±0.07	3.02^{cei}±0.09
	Carbohydrate (%)	20.99	17.2	11.68	21.6	19.36	13.38	22.43	21.04	17.83
	Starch (%)	10.6^{ad}±0.07	15.01^{af}±0.02	14.02^{ae}±0.00	11.1^{bd}±0.07	15.4^{bf}±0.02	14.75^{be}±0.00	11.38^{cd}±0.10	15.71^{cf}±0.06	14.86^{ce}±0.03

The means followed by the different superscript letters in the same row within the column of each individual portion are significantly different ($p < 0.05$

The ash content of jackfruit samples followed a similar trend with the protein and fat contents, which increased as the fruit ripened and decreased from the top to the bottom part of jackfruit.This result was similar to the report of by Shamla et al. (2019), in which prolonging ripening time would increase the ash content from 0.59 ± 0.02% to 1.86 ± 0.06%. ANOVA results showed that the fruit parts, portions and the ripening time of the fruit significantly affected the ash content ($p<0.05$).

3.2.2.5.Fiber

In contrast to the contents of fat, protein and ash, the fiber content tended to decrease from 100 to 120 days after flowering, while increasing from the top to the bottom part of jackfruit. The fiber content of the pulp, fiber, and seed were from 7.93 ± 0 .03 to 12.14 ± 0.06%, from 6.30 ± 0.12 to 10.90 ± 0.07%, from 2.861 ± 0.02 to 19.70 ± 0.12%respectively. However, this result is different from the study of Shamla et al. (2019), which reported that the fiber content of the pulp was recorded from 3.97 ± 0.03% to 0.96 ± 0.10%. This difference could be due to several factors such as breed, environment, and preservation. ANOVA results showed that parts, portions and the post-flowering timelines significantly affect fiber content at 95% confidence level ($p<0.05$).

3.2.2.6.Carbohydrate

Similar to the conclusion of fiber content, total carbohydrate content also tended to decrease gradually at the different post-flowering timeline and increase in the different portions, that due to the maturation of fruit. The highest value was recorded in the pulp that were to be 15.93 – 26.18%, the fiber and the seed had values from 7.98 to 24.48% and 11.68 to 22.43% respectively. This result is similar to the report of Shamla et al. (2019) that recorded the carbohydrate value from 18.37±0.26% to 61.34±0.09%.

3.2.2.7.Starch

Total starch content was only in the seed. Starch was measured lowest at the beginning of 100 days (10.6±0.07%) and highest at the end of 120 days (14.86±0.03%). There is a tendency to gradually increase at 100 and 110 days then decrease at 120 days. The reason is jackfruit seeds are not fully developed at 100 days, leading to lowest starch content. At 110 – 120 days, jackfruit seeds convert starch into sugar, so the slight decrease in starch content. The results obtained were different from previous studies, including jackfruit seed starch (26.13%), corn starch (22.20%) and potato starch (25.2%) (Alvani et al., 2011; Tulyathan et al., 2002). ANOVA results showed that parts, portions and the post-flowering timelines significantly affect starch content ($p<0.05$).

3.2.2.8.Sugar

Table 4 shows the sugar content of the pulp and the fiber that was ranged from 15.87 to 79.54 mg/g DM and 12.23 to 27.92 mg/g DM respectively. The pulp had the value was higher than the fiber, but there was none in the seed. The sweetness of fruit is largely dependent on the amount of sugar that is inversely proportional to the acidity. Therefore, the sugar content is increase that usually imparts sweetness to the fruit, which means decreasing acidity by decreasing in organic acid and phenol content. The total sugar content of jackfruit parts also tended to increase gradually at the different timelines and decreasing at the top, middle, and bottom. This trend was also reported by Ong et al. (2006) and Ranasinghe & Marapana (2019). ANOVA results showed that parts, portions and the post-flowering timelines significantly affect total sugar content at 95% confidence level ($p<0.05$).

3.2.2.9. pH and total acidity (TA)

Table 4 shows the pH and total acid (TA) values of jackfruit parts (pulp, fiber, seed) at the portions (top, middle, bottom) and the post-flowering timelines (100, 110 and 120 days). They had an opposite trends: the pH values of pulp, fiber decrease from 6.63±0.03 to 5.50±0.02, 5.77±0.02 to 4.98±0.02 respectively; the TA values of pulp, fiber increase from 0.10±0.01 to 0.36±0.02, 0.08±0.01 to 0.23±0.02% respectively. This trend was similar to the results of Ong et al. (2006) that had pH values were highest in unripe jackfruit and significantly decreased in all parts of the fruit (top, middle, bottom) while TA increased significantly, fluctuating in the range of 0.3-0.9%. The increase in acidity during ripening can be attributed to formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances and uronic acid (Ranasinghe & Marapana, 2019). The results of ANOVA analysis showed that parts, portions and the post-flowering timelines significantly affected on the acidity and pH at the 95% confidence level ($p<0.05$).

3.2.2.10.Total soluble solids

The highest TSS values of the pulp, fiber were reported at the top of 120 days and the lowest values were at the bottom of 100 days jackfruit after flowering (4.70±0.14-25.25±0.35% and 3.95±0.07-17.50±0.14%) That means the TSS was tended to increase in the post-flowering timlines and decrease at the portions. The increase in TSS during ripening may be due to the conversion of starch into sugar (Deepthi, 2017; Shamsudin et al., 2009). The recorded value of pulp is similar to the study of Ong et al. (2006), from 1.33±0.52% to 20±1.26%. Several studies have shown that TSS content of guava (Deepthi, 2017; Mercado-Silva et al., 1998) and sweet peppers (Tadesse et al., 2002) also had a similar trend during the ripening process. ANOVA results showed that the fruit parts, portions and the post-flowering timelines significantly affect TSS at 95% confidence level ($p<0.05$).

3.3.Quantification of chemical components in jackfruit parts

3.3.1.TCC

TCC plays an important role in determining the characteristic yellow color of all fruits, particularly in jackfruit. As shown in Table 5, TCC increased at different post-flowering timelines and decreased in the different fruit portions. The highest value was reported in the pulp and the lowest was in the seed. When the fruits ripens, the temperature, moisture, and pressure all decrease. For the reason, this reduces environmental influences and the relationships between fatty acids and carotenoids leading to carotenoid production. Carotenoids are used to increase the protective power of fruits, so they are important for recovery and protection from the effects of solar radiation (De Azevedo & Rodriguez-Amaya, 2005). The TCC values of the pulp, fiber, seed were to be 0.30±0.00 - 0.63 ±0.01 µg/mL, 0.19±0.00 - 0.44±0.00 µg/mL, and 0.11±0.01 - 0.30±0.03 µg/mL, respectively. This result is similar to some previous reports: TCC of the pulp from 0.06 to 0.63 µg/g published by Shamla et al. in four different ripening stage (Shamla et al., 2019), TCC value of tropical jackfruit pulp is 0.3±0.00 mg/100g reported by Barreto et al. (2009). ANOVA results showed that parts, portions and the post-flowering timelines significantly affect total carotenoid content at 95% confidence level ($p<0.05$).

3.3.2.TPC

Meanwhile, TPC tended to decrease from 100 to120 days, yet increase from the top to the bottom portions of the fruit. TPC of the seed had the highest value (from 0.13 ± 0.02 to 0.28 ± 0.04 mgGAE/g DM) due to the presence of an antioxidant compound, followed by pulp (from 0.12 ± 0.02 to 0.25 ± 0.04 mgGAE/g DM) and fiber (from 0.07 ± 0.02 to 0.23 ± 0.02 mgGAE/g DM).(Table 5). When the fruits ripen, TPC decreased since the polyphenols were degraded into small molecules by the enzymatic reactions (Krüger et al., 2012). The obtained results were relatively similar to Jagtap et al. (2010), which reported that the ripe Brazilian jackfruit samples extracted from ethanol solvent exhibited a TPC of 34.1±1.0 mg GAE/100 g DM. ANOVA results showed that the post-flowering timelines and different fruit parts significantly affected total polyphenol content of jackfruit extract ($p<0.05$).

3.3.3.TFC

Similar to TPC, TFC was reduced due to enzymatic reactions as the fruits ripe. TFC also decreased from 100 to 120 days. The values of the pulp, fiber, seed were to be 0.10±0.00 - 0.31±0.01 mgQE/g DM, 0.15±0.04 - 0.44±0.02 mgQE/g DM, 0.03±0.01 - 0.06±0.02 mgQE/g DM (Table 5). In there, the pulp had the highest value. The results were similar to the studies of Barreto et al. (2009), Jagtap et al. (2010b). However, the results obtained were lower than those of Shamla et al. (2019) (1,744 – 0.302 mg QE/g DM). ANOVA results showed that parts, portions and the post-flowering timelines significantly affect total flavonoid content at 95% confidence level ($p<0.05$).

3.4.Evaluation of antioxidant activity in jackfruit parts

3.4.1.DPPH· method

Results have shown that the DPPH scavenging activity of jackfruit pulp, fiber and seed extracts tends to decrease as the post-flowering pprolonged from 100 to 120 days, as indicated by the increasing IC_{50} values from 108.59 ± 9.75 to 202.34 ± 17.40 µg/mL, from 4.28 ± 0.53 to 367.33 ± 4.72 µg/mL, from 3.29 ± 0.37 to 19.55 ± 1.30 µg/mL, respectively. This can be explained that the jackfruit extractcontains antioxidants which are capable of converting hydrogen molecules into free radicals to get antioxidant capacity. This result was according to Li et al. (2021), the published IC50 value is 2.871 mg/mL that was different to this result. ANOVA results showed that parts, portions and the post-flowering timelines significantly affect IC50 value of DPPH· at 95% confidence level ($p<0.05$).

3.4.2.$ABTS^{\cdot+}$ method

Similar to DPPH· method, the results of $ABTS^{\cdot+}$ free radical scavenging capacity were shown in Table 6 The lower the OD value measured at 734 nm, the higher the free radical scavenging capacity of the antioxidant. IC50 of $ABTS^{\cdot+}$ radical of pulp, fiber, seed extractions tended to increase in the post-flowering timelines which recorded at 56.60±6.33 - 81.54±3.04 µg/mL, 3.70±0.15 - 287.07±18.78 µg/mL, 2.96±0.07 - 19.55±1.30 µg/mL. The increased IC50 value means the antioxidant capacity of jackfruit in the post-flowering timelines 100 days, 110 days, 120 days will be decrease. This can be explained by the increased respiration capacity as jackfruit ripens, leading to reduced antioxidant activity due to enzymatic reactions or external factors (such as oxidation or high temperature) affecting enzyme production (Quirós-Sauceda et al., 2019). The IC50 value was similar to that recorded in the Brazilian ripe jackfruit (9.39±0.18 mg/100 g) but they was lower than the value of IC50 was published by Z.Li (0.259 mg/mL) (Barreto et al., 2009; Li et al., 2021).

3.4.3.Phosphomolybdenum method

The total antioxidant capacity of the jackfruit parts and its portions were measured spectrophotometrically through the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex with maximum absorption at 695 nm. The antioxidant capacity of jackfruit parts and its portions was found to decrease in 100 days < 110 days < 120 days and increase in order top > middle > bottom.

Table 5. TCC, TPC, and TFC change at different days after flowering in different portions of jackfruit parts

		Top			Middle			Bottom		
		100 days	**110 days**	**120 days**	**100 days**	**110 days**	**120 days**	**100 days**	**110 days**	**120 days**
Pulp	**TCC (mg/mL)**	0.31^{cdi}±0.00	0.44^{cei}±0.01	0.61^{cfi}±0.01	0.29^{bdi}±0.02	0.41^{bei}±0.00	0.63^{bfi}±0.01	0.30^{adi}±0.00	0.33^{aei}±0.01	0.53^{afi}±0.01
	TPC (mgGAE/g DM)	0.2^{afh}±0.02	0.14^{aeh}±0.02	0.12^{adh}±0.02	0.24^{abfh}±0.02	0.16^{abeh}±0.05	0.14^{abdh}±0.04	0.25^{bfh}±0.04	0.19^{beh}±0.06	0.14^{bdh}±0.02
	TFC (mgQE/g DM)	0.23^{afh}±0.02	0.18^{aeh}±0.05	0.10^{adh}±0.00	0.25^{bfh}±0.00	0.24^{beh}±0.05	0.15^{bdh}±0.01	0.31^{cfh}±0.01	0.29^{ceh}±0.05	0.19^{cdh}±0.03
Fiber	**TCC (mg/mL)**	0.24^{cdh}±0.00	0.24^{ceh}±0.00	0.44^{cfh}±0.00	0.20^{bdh}±0.00	0.21^{beh}±0.00	0.33^{bfh}±0.01	0.19^{adh}±0.00	0.21^{aeh}±0.00	0.32^{afh}±0.02
	TPC (mgGAE/g DM)	0.18^{afg}±0.02	0.14^{aeg}±0.00	0.07^{adg}±0.02	0.21^{abfg}±0.03	0.14^{abeg}±0.01	0.08^{abdg}±0.00	0.23^{bfg}±0.02	0.19^{beg}±0.05	0.11^{bdg}±0.01
	TFC (mgQE/g DM)	0.27^{afh}±0.01	0.18^{aeh}±0.05	0.15^{adh}±0.04	0.32^{bfh}±0.09	0.21^{beh}±0.09	0.16^{bdh}±0.00	0.44^{cfh}±0.02	0.24^{ceh}±0.06	0.16^{cdh}±0.02
Seed	**TCC (mg/mL)**	0.11^{cdg}±0.02	0.15^{ceg}±0.01	0.30^{cfg}±0.03	0.11^{bdg}±0.02	0.15^{beg}±0.01	0.30^{bfg}±0.03	0.11^{adg}±0.01	0.14^{aeg}±0.01	0.30^{afg}±0.01
	TPC (mgGAE/g DM)	0.25^{afi}±0.04	0.22^{aei}±0.00	0.13^{adi}±0.02	0.27^{abfi}±0.05	0.23^{abei}±0.00	0.14^{abdi}±0.04	0.28^{bfi}±0.04	0.24^{bei}±0.02	0.14^{bdi}±0.03
	TFC (mgQE/g DM)	0.04^{afg}±0.01	0.03^{aeg}±0.01	0.03^{adg}±0.01	0.05^{bfg}±0.01	0.05^{beg}±0.01	0.04^{bdg}±0.01	0.06^{cfg}±0.02	0.05^{ceg}±0.00	0.04^{cdg}±0.00

The means followed by the different superscript letters in the same row within the column of each individual portion are significantly different ($p < 0.05$)

Table 6. IC50 of DPPH·, IC50 of $ABTS^{\cdot+}$, and TAC change at different days after flowering in different portions of jackfruit parts

		Top			Middle			Bottom		
		100 days	**110 days**	**120 days**	**100 days**	**110 days**	**120 days**	**100 days**	**110 days**	**120 days**
Pulp	**IC50 $ABTS^{\cdot+}$ (µg/mL)**	58.65^{afh}±8.48	77.32^{aeh}±1.18	81.54^{adh}±3.04	58.61^{bfh}±11.27	75.36^{beh}±0.33	78.55^{bdh}±0.56	56.60^{cfh}±6.33	71.40^{ceh}±7.08	77.64^{cdh}±4.23
	IC50 DPPH· (µg/mL)	128.29^{cdh}±2.37	135.10^{ceh}±4.61	202.34^{cfh}±17.40	116.52^{bdh}±3.16	124.23^{beh}±4.66	170.21^{bdh}±8.22	108.59^{adh}±9.75	114.08^{aeh}±5.30	165.16^{afh}±1.39
	TAC (mgGAE/g DM)	87.21^{afh}±4.94	32.80^{aeh}±1.59	29.15^{adh}±0.69	93.13^{bfh}±7.38	36.71^{beh}±2.04	34.65^{bdh}±7.37	113.52^{cfh}±10.33	44.02^{ceh}±2.17	35.90^{cdh}±7.33
Fiber	**IC50 $ABTS^{\cdot+}$ (µg/mL)**	122.91^{afi}±33.88	130.06^{aei}±18.15	287.07^{adi}±18.78	97.55^{bfi}±16.78	108.82^{bei}±3.66	264.97^{bdo}±12.41	3.70^{cfi}±0.15	3.89^{cei}±0.07	4.23^{cdi}±0.01
	IC50 DPPH· (µg/mL)	311.03^{cdi}±15.37	315.86^{cei}±4.72	367.33^{cfi}± 4.72	294.79^{bdi}±4.42	304.60^{bei}±3.11	366.62^{bfi}±3.08	4.28^{adi}±0.53	11.51^{aei}±0.29	19.55^{afi}±1.30
	TAC (mgGAE/g DM)	110.80^{afi}±14.33	62.56^{aei}±3.78	42.87^{adi}±15.83	115.40^{bfi}±8.47	80.09^{bei}±20.18	48.43^{bdi}±18.62	123.67^{cfi}±12.70	88.11^{cei}±16.82	56.03^{cdi}±14.82
Seed	**IC50 $ABTS^{\cdot+}$ (µg/mL)**	3.70^{afg}±0.15	3.89^{aeg}±0.07	4.23^{adg}±0.01	3.23^{bfg}±0.25	3.44^{beg}±0.27	4.13^{bdg}±0.10	2.96^{cfg}±0.07	3.24^{ceg}±0.36	3.43^{cdg}±0.35
	IC50 DPPH· (µg/mL)	4.28^{cdg}±0.53	11.51^{ceg}±0.29	19.55^{cfg}±1.30	3.99^{bdg}±0.21	9.45^{beg}±1.13	17.29^{bfg}±2.18	3.29^{adg}±0.37	8.39^{aeg}±0.35	11.24^{afg}±0.40
	TAC (mgGAE/g DM)	23.47^{afg}±6.40	16.44^{aeg}±4.01	13.04^{adg}±0.13	31.33^{bfg}±2.41	20.42^{beg}±0.79	16.05^{bdg}±3.14	35.28^{afg}±1.73	32.16^{aeg}±2.06	17.84^{adg}±3.80

ANOVA results showed that parts, portions and the post-flowering timelines significantly affect IC50 value of $ABTS^{\cdot+}$ at 95% confidence level ($p<0.05$).

However, the bottom portions of 100-day and the top portions of 120-day showed the highest and lowest antioxidant capacities respectively (Table 6). In there, the seed had the lowest values were to be 13.04±0.13 - 35.28±1.73 mgAAE/g DM and the pulp, fiber were recorded at 29.15±0.69 - 113.52±10.33 mgAAE/g DM, 42.87±15.83 - 123.67±12.70 mgAAE/g DM.This change may be due to a decrease in antioxidant compounds such as phenolic acids and flavonoids. As jackfruit ripens, these compounds are degraded leading to reduced antioxidant capacity. Kumari et al. (2013) also showed the decrease of TAC in lemons at different mature stage and they reported the unripe lemons had a higher TAC than ripening (0.178 and 0.127 mg/mL).

The antioxidant capacity of jackfruit pulp was determined based on the ABTS·$^{+}$, DPPH· free radical scavenging methods and phosphomolybdenum method (TAC). Both ABTS·$^{+}$ and DPPH· free radical scavenging methods can use the IC50 value to calculate the free radical scavenging level, but the TAC method cannot achieve the IC50 value. The TAC method was found to be inappropriate in determining the antioxidant capacity of jackfruit pulp in this study. The IC50 value of the DPPH· free radical scavenging method (108.59±9.75 - 202.34±17.40 µg/mL) was higher than the IC50 value of the capture method. ABTS·$^{+}$ free radicals (56.60±6.33 - 81.54±3.04 µg/mL). Therefore, ABTS·$^{+}$ method was better than DPPH· and phosphomolybdenum method. This difference can be explained by the type of antioxidant used as the standard. Antioxidants include endogenous (enzymes and nonenzymes) and exogenous (vitamin E, vitamin C, β-carotene, flavonoids, Se minerals, vitamin D and vitamin K3) (Biochem et al., 2011). In which vitamin E (Trolox) and vitamin C (ascorbic acid) were selected as standard substances in methods to determine the antioxidant capacity of jackfruit. In this study Trolox was used as a standard in the DPPH· and ABTS·$^{+}$ free radical scavenging methods, and ascorbic acid was used in the phosphomolybdenum (TAC) method. Borut and Peter (2008) compared these two antioxidant compounds and reported that Trolox has many advantages over the other, while the auto-oxidation of ascorbic acid produces a quantity of H_2O_2 that prevents the determination of determine the antioxidant capacity of the compound. This may explain the poor free radical scavenging ability of the TAC method. The ABTS·+ free radical scavenging method is better than DPPH· because ABTS·+ free radicals are detected at 734 nm far from the visible region (with wavelengths from 380 to 760 nm), while DPPH· radicals are detected at 517 nm wavelength may be attenuated due to potential interference. Another advantage of the ABTS·+ method is that antioxidants in the aqueous and oily phases can both capture ABTS·+ free radicals, while only lipophilic antioxidants can capture DPPH· radicals in the environment field (Arnao et al., 2001).

4.Conclusion

The effects of post-flowering timelines (i.e. 100 days, 110 days and 120 days) on the nutritional, chemical, and antioxidant values of jackfruit parts (pulp, fiber, and seed) were analyzed to gain useful insights in determining the optimal harvesting time to obtain high-quality jackfruit. Results have shown that most of the nutritional values of the tested jackfruit parts increased as the post-flowering timelines prolonged and decrease gradually from the top to the bottom. The value of fiber and carbohydrate content tended to be the opposite. In particular, increasing TSS, sugar content and TCC provides a better palatability for the ripened fruit, thus improving consumer tastes. Meanwhile, chemical constituents, TPC, TFC, and antioxidant activity tended to decrease from 100 to 120 days and increased from the top to the bottom part of jackfruit due to enzymatic reaction. The results also showed that 120 days is the most appropriate harvesting time to obtain jackfruit with high nutritional content. Further studies are required to optimize the antioxidant activity to exploit the biological potentials and extend the applications of jackfruit.

5. References

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Acknowledgements

This research was supported by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam and the Department of Science and Technology of Can Tho City, Vietnam

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

IMPACT OF GRANULAR ACTIVATED CARBON AND MAGNETIC FIELD IN SLOW SAND FILTER ON WATER PURIFICATION FOR RURAL DWELLERS

Oloriegbe Y. A.[1], Saad A. O.[1], Baiyeri M. R.[2], Moshood M. A.[3], Sanusi H. O.[1] and Yusuf K.O.[1]✉

[1]Department of Agricultural and Biosystems Engineering, University of Ilorin, Nigeria
[2]Department of Agricultural and Bio-Environmental Engineering, Kwara State Polytechnic, Ilorin, Nigeria
[3]Department of Civil Engineering, University of Ilorin, Ilorin, Nigeria
✉*yusuf.ok@unilorin.edu.ng*

https://doi.org/10.34302/crpjfst/2024.16.3.2

Article history:
Received:
January 16th, 2024
Accepted:
August 12th, 2024

Keywords:
Activated carbon;
Magnetic water;
Pathogen;
Potable water;
Slow sand filter.

ABSTRACT

Most farmers that produce food crops in Nigeria live in rural areas where potable water is not available. The farmers in some areas have problem of water borne diseases which could affect their health and could lead to death. This study was conducted to determine the impact of incorporating Granular Activated Carbon (GAC) and Magnetic Field (MF) in Slow Sand Filter (SSF) on the purification of water for rural dwellers. The SSF was constructed using PVC pipe (152.4 mm diameter and 1100mm long) with layers of fine sand (size 0.25mm and 350mm depth), followed by GAC (10mm size and 100 mm depth), fine sand (0.25mm with 500mm depth) and gravel (grain size 10-14 mm and 100mm depth). The SSF was kept moist for 21 days for biofilm layer (*schmutzdecke)* to fully develop which is essential for trapping bacteria. Two SSFs fabricated consist of SSF+GAC+MF as Filter 1, SSF+GAC as Filter 2 and Control (Raw water without passing through the filter. Water samples were collected from the filter and analyzed. The discharge capacity of the filter was 25 litres/h. Total bacteria counts (TBC) for Filter 1 and Filter 2 and control were 2.4, 4.6 and 8.1 cfu/mg, respectively. Total coliform count for Filter 1 and Filter 2 and control were 1.7, 3.0 and 6.4 cfu/100mL, respectively. The filters reduced water hardness, turbidity, lead, copper, electrical conductivity and reduced TCC by 53.13-73.44% but increased pH from 5.8 to 7.1-7.3. The SSF is recommended for water purification in the rural area.

1.Introduction

Most rural dwellers especially the farmers that produce food crops in Nigeria live in rural areas and most of them don't have access to potable water. Yusuf and Murtala (2020) reported that rural dwellers depend mostly on surface water such as ponds, open wells, springs and streams for drinking water. Water from these sources are usually contaminated with disease-causing organism that could cause certain diseases to man. Hammer and Hammer (2012) reported that contaminated water from ponds and streams could be contaminated with toxic chemicals and pathogens which could cause water-borne diseases such as cholera and typhoid to man. Ashbolt (2004) also reported that lack of potable water and proper sanitation in rural areas is the main cause of gastrointestinal diseases in some developing countries in Africa. Ankidawa and Tope (2007) reported that water purification in the rural areas is difficult and expensive because of bad roads for transportation and lack of electricity for water treatment plant. Therefore, purification of water for farmers in the rural areas using simple

and low-cost water treatment equipment is needed.

Slow sand filter is a simple equipment for purifying water which does not require electricity and is effective for removal of debris and disease-causing organism from the water (LeChevallier and Au, 2004; Yusuf *et al*., 2019). WHO (1996) indicated that SSF is effective in the removal of 98 to 99% of microorganism from water. El-Harbawi *et al*. (2010) stated that SSF could remove particles and some toxic chemicals from the water. The removal of contaminants from water by SSF and its purification efficiency depends on the filter media, the depth of the media especially the fine sand and the discharge of water during filtration (Abudi, 2011). Bagundol *et al*. (2013) reported that the depths of filter bed for fine sand with 60 and 90 cm could reduce the turbidity by 90% and remove E. Coli from the water.

EPA (2016) indicated that activated carbon is effective for the removal of some compounds that could cause taste, odour, colour and toxic to man by adsorption which the filter sand could not remove. Basha *et al*. (2023) reported that activated carbon from walnut shell and green leaves are good adsorbent agent and they are efficient in the removal of Lead and Arsenic. GAC is an adsorbent agent that has ability to remove physical, chemical and some organic compounds from the contaminated water while sand medium traps the pathogens from the water during the filtration. Activated carbon is commonly produced from agricultural materials such as wood, coconut shell and rice husk. Chandarin *et al*. (2023) reported that solid adsorbents are environmentally friendly materials for the removal polluants from the wastewater. Tobarameekul *et al*. (2022) reported that common adsorbent agents that could be used for water purification are zeolite, activated carbon and metal-organic frameworks.

The first step in production of activated carbon is carbonization of the agricultural material to obtain charcoal under anaerobic environment (without oxygen) at a temperature of 700 °C (Dileck and Oznuh, 2008). Das *et al*. (2015) reported that activated carbon is produced by heating charcoal in a furnace with temperatures ranging from 800 to 900 °C. Das *et al*. (2015) also stated that activated carbon could be prepared by using chemicals like KOH, NAOH, ZCl_2 and H_2SO_4.

Magnetic field could be used to enhance water purification, modify the molecular structure of water and energize the water. Magnetized water is produced by allowing water flow through magnetic field in a pipe and the water must flow vertically to the magnetic field according to Fleming's Right-hand rule (Chern, 2012). Kozic *et al*. (2006) reported that magnetic field could be used to purify water, reduce the water hardness, modify the water clusters and soften the water for human consumption and good health. Babu (2010) pointed out that magnetic field enhances water purification efficiency, modifies the water structure and reduces the surface tension which is more useful to both plant and animal. (LeChevallier *et. al*, 2004) stated that incorporating magnetic treatment unit in slow sand filter could reduce bacteria, algae, E. coli and scale formation in water. The objective of this study was to develop and determine the impact of incorporating GAC and MF treatment unit in SSF on water purification of some selected physical, chemical and bacteriological properties of water.

2. Materials and Methods

2.1. Preparation of fine sand for the slow sand filter

A 40 kg of fine sand was collected within Ilorin, the sand was sun dried for 2 days, then sieved through 0.25 mm and soaked in water for 2 days for softening and easy separation of any foreign particles from the sand. The sand was thoroughly washed with cleaned water to remove particles, soluble element sand then sun dried for another 4 days. Washing of the sand is essential to have a clean filter sand material that is free from organic matter, clay particles, insects and some soluble chemicals. Gravels were collected and sieved through 15 mm and 6 mm sieves; the gravels retained were thoroughly washed and used as the supporting layer. A

perforated cover with 4 mm in diameter holes was put below the gravel through which the filtered water passes to the lower chamber.

2.2. Granular activated carbon for the filter

Granular activated carbon (GAC) was prepared using coconut shell which was burnt to have the charcoal. The charcoal was ground, sieved through 10 mm standard sieve, put in a crucible and then heated in a furnace at temperature of 700 ^{0}C for 2 hours. The GAC was washed with distilled water to reduce the pH level and then dried in an electric oven at 105 ^{0}C for 2 hours to have a dry granular activated carbon as shown in Figure 1.

Figure 1. Granular activated carbon for the filter

2.3. Magnetic treatment unit incorporated in the slow sand filter

Magnetic treatment unit incorporated in the filter was a rectagular pipe (15 mm by 60 mm and 600 mm long) constructed using a transparent perspex glass (2 mm thick). A total of 12 pieces of 50 × 25 × 10 mm neodymium magnet were arranged on the two sides of the rectangular pipe as shown in Figure 2. The rectangular plastic pipe was folded to form 3 layers to have a longer magnetic treatment unit with 12 pieces of magnet. The neodymium magnet covers a total length of 450 mm and it has magnetic flux density of 1.0Tesla (10,000 Gauss).

Figure 2. Pictorial view of neodymium magnet

2.4. Description of the slow sand filter

The SSF was constructed using locally available materials in Ilorin, Kwara State, Nigeria. The filter chamber was constructed from 6 inches (152.4 mm internal diameter) PVC pipe and 1100 mm long. A diffuser of 20 mm openings is placed at the inlet pipe to the filter chamber for even distribution of water over the filter medium. The drain pipe and other fittings were fitted to the base that serves as drain through which the water would be collected.

The gravel (grain size of 14 mm) was put at the bottom of the filter to 60 mm height and gravel of 10 mm grain size was also put after the 14 mm gravel to a height of 40 mm. The two layers of the gravel allow easy flow of filtered water after the fine sand medium. Water would flow from the raw water storage tank (40 litres capacity) through 12.7 mm PVC pipe to the filter chamber. Both the inflow and outflow pipes have control taps for regulating the inflow discharge of water from the water storage tank into the filter chamber and from the filter to the outlet when water is needed for drinking. The SSF has a discharge (capacity) of 25 litres/h.

The total cost of the SSF as at March, 2021 using the available materials in Ilorin was sixty-seven thousand (₦67,000) which is equivalent to 175.85 U.S. Dollar (USD175.85).The orthographic drawing of the SSF is shown in Figures 3 and 4. The order of arrangement of the media in the SSF from the top of the filter was granular activated carbon, fine sand, gravels. The description and the arrangements of the filter media from the top of the filter were as follows:

i. Supernatant with 50 mm depth (space above the filter bed)
ii. Fine sand of grain size 0.25 mm with a depth of 50 mm
iii. Granular activated carbon of 10 mm size with depth of 100 mm
iv. Fine sand of effective grain size 0.25 mm with filter depth of 500 mm
v. Gravel of grain sizes of 10 -14 mm of depth 100 mm

vi. Filtered water chamber of depth 350 mm.

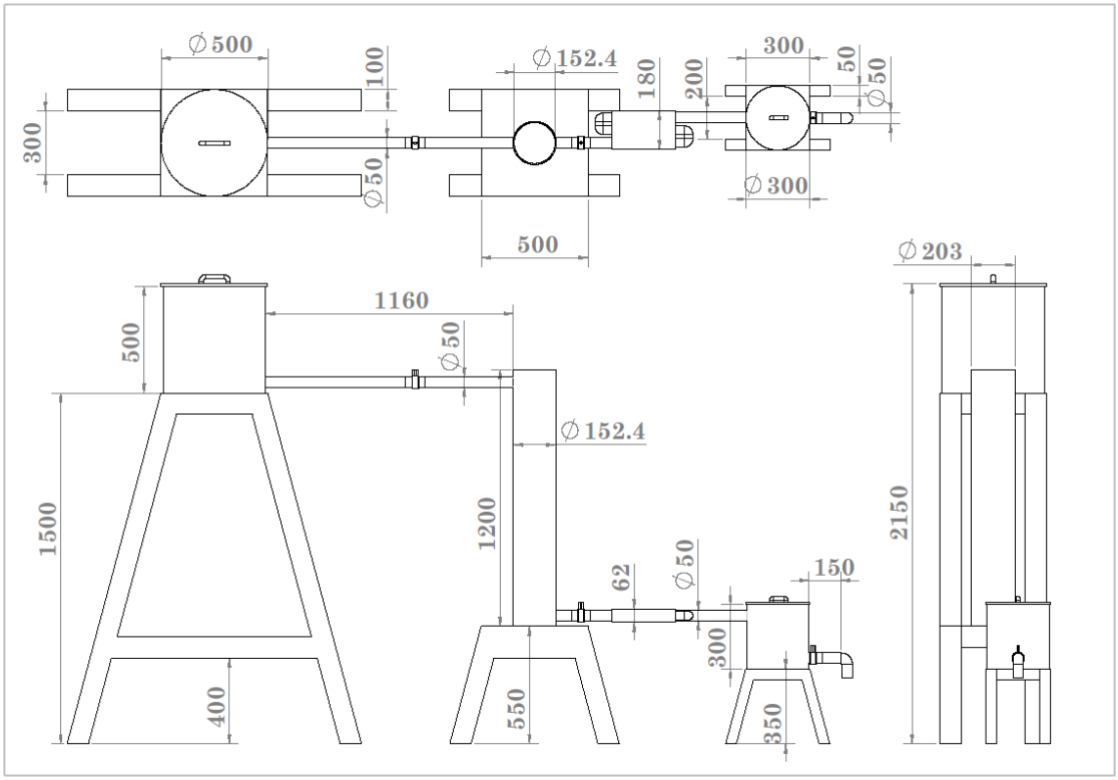

Figure 3. Orthographic drawing of the slow sand filter

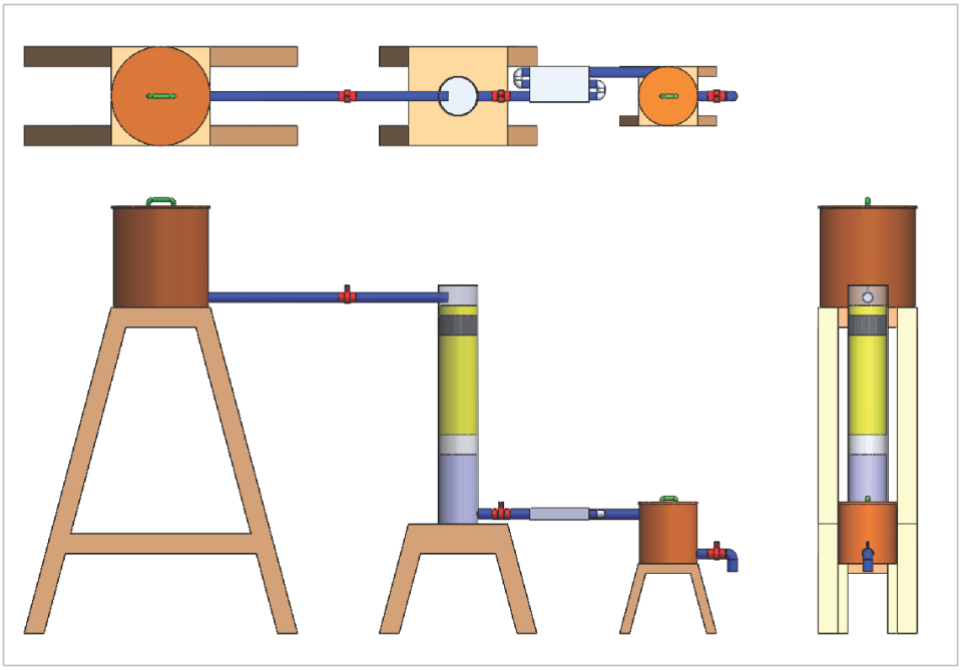

Figure 4. Orthographic view of the slow sand filter

2.5. Determination of flow discharge and water analysis of the filtered water

The discharge of the SSF was determined to know the quantity of water which the filter can produce per hour and water quality analysis was conducted to know if the filter is working satisfactorily. The discharge of the SSF evaluated by determining the volume of water it could filter per hour using Equation (1). The performance evaluation of the SSF on water purification was carried out using well water within the university campus at male hostel. Water samples were collected using sterilized 750 ml plastic bottle.

The physical, chemical and bacteriological properties analyzed in the water were Turbidity (appearance), pH, Electrical conductivity, Total hardness, Lead, Cadmium, Manganese, Copper, Calcium, Total bacteria counts, E. Coli and total coliform counts. The physicochemical and bacteriological properties of the water were determined using the standard methods (AOAC, 2000).

$$Q = \frac{V}{T} \qquad (1)$$

where Q is the discharge of the SSF (litre/h), V is the volume of water collected from the outlet (litre) and T is the time taken to obtain the volume of water (h).

2.6 Statistical analysis using paired t-test for the water purification of the slow sand filter

A paired t-test was used to check the effect of magnetic field and GAC incorporated in the SSF were significant or not on the purification of water. The mean, standard deviation, standard error and t-test values were computed using Equations (2), (3), (4) and (5), respectively as reported by Montgomery (1998). The data used for determining the Paired t-test is presented in Table 1 which was extracted from Table 2. The calculated values of t-test and that of table values of the t-test were compared at α = 5 % (α = 0.05).

$$\bar{x} = \frac{\sum x}{n} (2)$$

$$\delta = \sqrt{\frac{\sum x^2 - n\bar{x}^2}{n-1}} \qquad (3)$$

$$\delta_{Er} = \frac{\delta}{\sqrt{n}} (4)$$

$$t_{cal} = \frac{\bar{x}}{\delta_{Er}} (5)$$

where $\bar{x}$ is the mean of difference of the data x_1 and x_2, Σx is the summation of x, n is the number of observations, δ is the standard deviation, δ_{Er} is the standard error and t_{cal} is the calculated value.

Table 1. Determination of the Paired t-test

x_0	x_2	$x = x_0 - x_2$	x^2
5.8	7,3	-1.5	2.25
3.35	3.24	0.11	0.0125
420	390	30	900
342	305	37	1369
32	24	8	64
80	40	40	1600
320	260	60	3600
1.20	1.04	0.16	0.0256
0.01	0.01	0.00	0.00
1.03	1.01	0.02	0.0004
0.05	0.06	-0.01	0.0001
0.00	0.01	-0.01	0.0001
9.03	9.01	0.02	0.0004
175.14	174.71	0.43	0.1849
6.90	5.90	1.00	1.00
3.24	2.80	0.44	0.1936
8.10	2.40	5.70	32.49
6.4	1.6	4.80	23.04
N = 18		**∑d= 186.16**	**∑ d^2= 7,592.1972**

x_0 = Raw water, x_1 = water parameters that filtered through filter 1 (SSF+GAC+MF)

$$\bar{x} = \frac{186.16}{18} = 10.342 \quad (2)$$

$$\delta = \sqrt{\frac{7{,}592.1972 - 18(10.342)^2}{18-1}} = 18.258 \quad (3)$$

$$\delta_{Er} = \frac{18.258}{18} = 4.303 \quad (4)$$

$$t_{cal} = \frac{10.342}{4.303} = 2.403 \quad (5)$$

Similarly, Raw water versus water parameters filtered through filter 2 (SSF + GA), t_{cal} was 2.340 but table value of t-test is 2.110 at 17 degree of freedom and at $\alpha \leq 0.05$.

3. Results and discussion

The fabricated SSF shown in Figure 5 has a discharge of 25 litres/h of clean drinkable water for people especially in rural areas where potable water are not available. The results of the performance evaluation of the slow sand filters 1 and 2 on the removal of cations mainly the heavy metals, anions, bacteriological properties and water hardness are shown in Figures 6, 7, 8 and 9, respectively. The total bacteria counts (TBC) for filter 1 (SSF + GAC + MF), filter 2 (SSF + GAC) and the raw water without passing through the filter (control) were 2.4, 4.6 and 8.1 cfu/mg, respectively while the total coliform counts (TCC) for Filter 1, filter 2 and control were 1.7, 3.0 and 6.4 cfu/100mL as shown in Table 2. The filter 2 with GAC only reduced the TBC by 43.21% but the filter 1 with GAC and MF reduced TBC by 70.37%. The filter 2 with GAC only also reduced TCC by 53.13% while slow sand filter 1 GAC and MF reduced TCC by 73.44% as presented in Table 2.

The result of this study is in agreement with the study of LeChevallier and Au (2004) which reported that slow sand filter could remove pathogens in the water to minimum value with the removal of total coliforms varied from 84.35 to 99.5% and faecal coliforms varied from 48.1 to 70.0%. WHO (1996) reported that SSF could remove microbial pathogens that can cause diseases to man in the rural and urban areas by 98 to 99 %. The percentage removal of TBC in this study was 43.21 to 70.37% compared to 84.35 to 99.5% which depends on the quality of the raw water, filtration rate, media sizes and depths of the media as reported by LeChevallier and Au (2004).

Magnetic field incorporated in the slow sand filter enhanced the removal of TBC and TCC and this agreed with the study of Nasher and Hussein (2008) that magnetic field is a simple physical method for eliminating bacteria from a system. Brkovic *et al*. (2014) also concluded that magnetic field reduced number of microbes in the oral cavity.

The filters reduced the water hardness, turbidity, lead, copper and electrical conductivity as shown in Table 2. The slow sand filter 1 reduced the TDS, Turbidity, Hardness and Sodium by 10.82, 3.28, 25.00 and 13.33%, respectively while the slow sand filter 2 also reduced the TDS, Turbidity, Hardness and Sodium by 9.36 and 3.28%, 25.00 and 13.33%, respectively. The two filters reduced the values of Electrical conductivity, BOD, COD, reduced concentrations of Copper, Nitrite, Nitrate, Phosphorus and Sulphate after filtration. The results of the filtration in this study agreed with the findings of Bagundol *et al*. (2013) that SSF could reduce turbidity by 90% which depends on the depths of filter bed (fine sand). This study was also in agreement with the study of Chatterjee (2007), Hammer and Hammer (2012) that SSF improved the physical and chemical properties of water.

The effect of the filtration of water through filter 1 and filter 2 were significant on the purification of the water with values of t-test of 2.403 and 2.340 compared to the table value of t-test of 2.110 at $\alpha \leq 0.05$ as presented in Table 3. All the properties of raw water and water after filtration were within the permissible limits of SON (2007) and WHO (2008).

Figure 5. Pictorial view of the fabricated slow sand filters

Table 2. Properties of water before and after filtration

Water properties	Filter 1	Filter 2	Raw water	SON 2007	WHO 2008
pH	7,3	7.1	5.8	6.5-8.5	6.5-8.5
Turbidity (NTU)	3.24	3.24	3.35	5.00	5.00
EC (µS/cm)	390	400	420	1000	1000
TDS (mg/l)	305	310	342	500	500
Hardness (mg/l)	24	24	32	150	150
BOD (mg/l)	40	50	80	-	-
COD (mg/l)	260	280	320	-	-
Sodium (mg/l)	1.04	1.04	1.20	200	200
Calcium (mg/l)	0.00	0.00	0.00	200	200
Cadmium (mg/l)	0.01	0.01	0.01	0.003	0.003
Copper (mg/l)	1.01	1.02	1.03	1.000	2.000
Iron (mg/l)	0.02	0.03	0.05	0.300	0.030
Lead (mg/l)	0.00	0.00	0.00	0.010	0.010
Manganese (mg/l)	0.00	0.00	0.00	0.20	0.400
Zinc (mg/l)	0.01	0.01	0.01	3.00	-
Nitrate (mg/l)	9.01	8.57	9.03	50.00	50.00
Nitrite (mg/l)	174.71	173.29	175.14	0.200	3.000
Phosphate (mg/l)	5.90	5.80	6.90		
Sulphate (mg/l)	2.80	3.10	3.24	100	100
Total bacteria counts(cfu/mg)	2.40	4.60	8.10	10	10
E. Coli (cfu/100ml)	0.0	0.0	0.0	0	0
Total coliform counts (cfu/100ml)	1.6	3.0	6.4	10	10

Filter 1 = SSF + GAC + MF, Filter 2 = SSF + GAC
SSF = Slow sand filter, GAC = Granular activated carbon, MF = Magnetic field
SON = Standard Organization Nigerian, WHO = World Health Organisation

Table 3. Values of t-test for the purification of water through the slow sand filters

Treatment	Degree of freedom	Calculated value of t (t_{cal})	Table value of t at $\alpha \leq 0.05$	Effect of passing the water through the filters
Raw water versus Filter 1	17	2.403^{S}	2.110	Significant
Raw water versus Filter 2	17	2.340^{S}	2.110	Significant

Filter 1 = SSF + GAC + MF, Filter 2 = SSF + GAC, Raw water (unfiltered water)
S = the value is significant, SSF, GAC and MF were as defined in Table 2

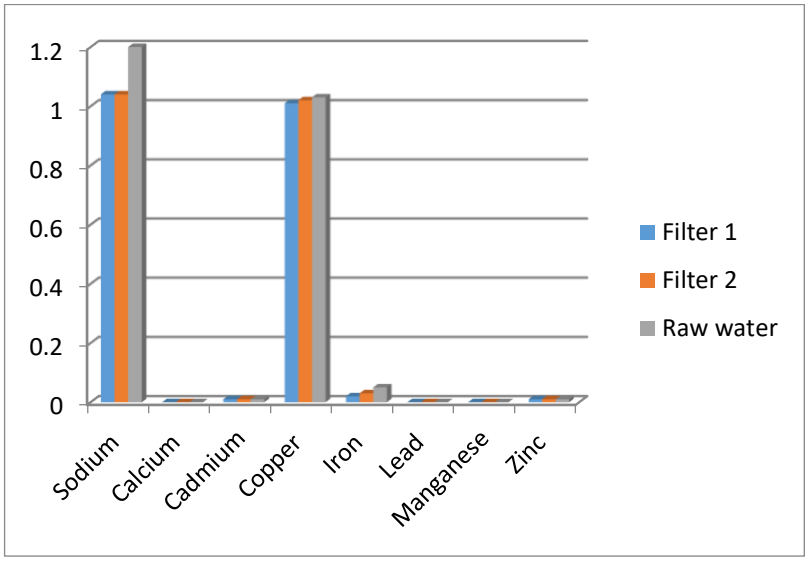

Figure 6 . Properties of cations after filtration through Filters 1, 2 and the Rawwater Filter 1, Filter 2 and Raw water were defined in Table 2.

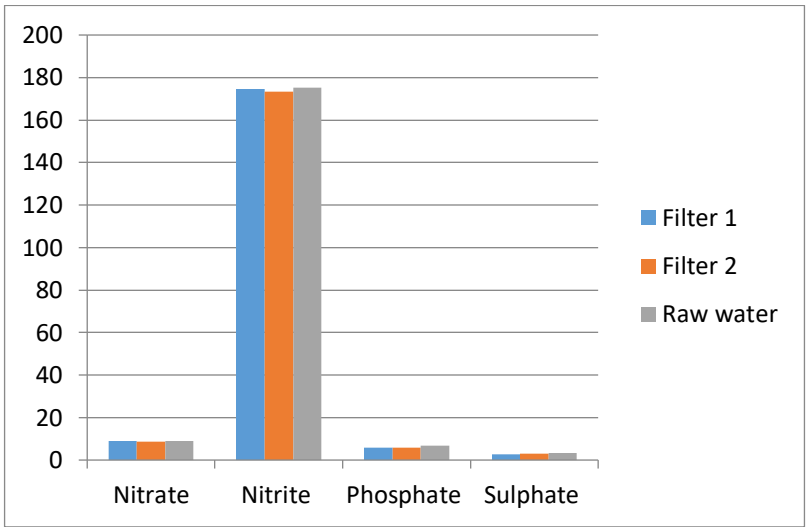

Figure 7 . Properties of anions after filtration through Filters 1, 2 and the Rawwater

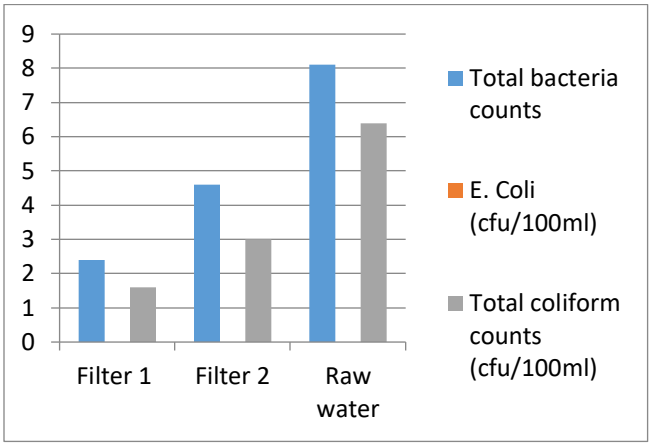

Figure 8. Bacteriological properties of water after filtration through Filters 1, 2 and the Rawwater

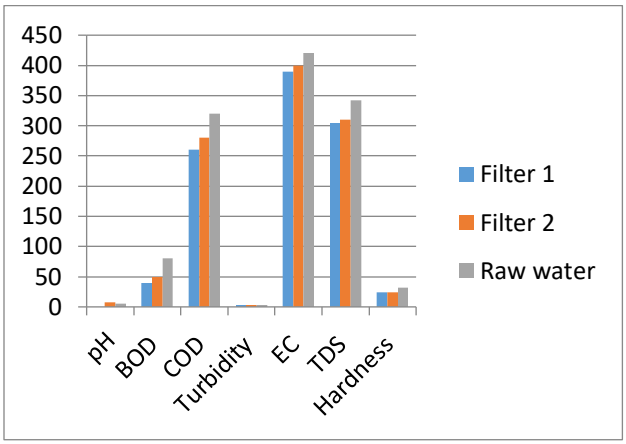

Figure 9. Water hardness and electrical conductivity of water after filtration through the filters compared with the raw water

4.Conclusions

Two slow sand filters were fabricated, one filter was incorporated with GAC only and the second filter was incorporated with GAC and magnetic treatment unit. The two slow sand filters can produce 25 litres/h of clean drinkable water. The slow sand filter with granular activated carbon only reduced the total bacteria counts by 43.21% while the filter with GAC and MF treatment unit reduced the total bacteria counts by 70.37%. The two filters reduced the values of Electrical conductivity, BOD, COD, reduced concentrations of Copper, Nitite, Nitrate, Phosphorus and Sulphate after filtration. The slow sand filter with GAC and magnetic treatment unit reduced the TDS, Turbidity, Hardness and Sodium by 10.82, 3.28, 25.00 and 13.33%, respectively while slow sand filter granular activated carbon only reduced the TDS and Turbidity by 9.36 and 3.28%. Combination of GAC and MF treatment unit in the slow sand filter enhanced the removal of total bacteria counts and total coliform counts.

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CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

PROTEIN, ENERGY, AND ANTIOXIDANT-DENSE FLAKED BREAKFAST CEREAL BY RESPONSE SURFACE OPTIMISATION OF COMPOSITE FLOUR COMPONENTS (YELLOW MAIZE, SOYBEAN, AND MANGO PEEL)

Oludolapo Akinyemi Osunrinade[1]✉, John Olanrewaju Babalola[2], Oluwole Adeola Adeoti[2], and Akeem Olayemi Raji[1]

[1] *Department of Food Science and Technology, Kwara State University, Malete, Ilorin, Kwara State, Nigeria*
[2] *Department of Food Science and Technology, The Oke-Ogun Polytechnic Saki, Oyo State, Nigeria*
✉*oludolapo.osunrinade@kwasu.edu.ng*

https://doi.org/10.34302/crpjfst/2024.16.3.3

Article history:
Received:
May 14th, 2024
Accepted:
September 15th, 2024

Keywords:
Flakes;
Antioxidant;
Nutrient;
Mango peel;
Optimization.

ABSTRACT

Breakfast flakes are a popular choice among consumers for a quick and nutritious meal. However, to enhance their nutritional value, supplementation with nutrient-dense ingredients is essential. In this study, soybean and mango peel were utilized to develop protein, energy, and antioxidant-rich flakes through an optimized formulation process. A three-component constrained optimal mixture experimental design was employed, utilizing yellow maize (0-100%), soybean (0-50%), and mango peel flour (0-50 %) blends. Standard procedures were employed to analyze the proximate composition, antioxidant properties, and colour of the flake samples. Sensory analysis using a 9-point hedonic scale was performed by thirty panellists to evaluate the flakes. Statistical significance was determined at $\alpha \leq 0.05$, and optimization was achieved by maximizing protein, energy, and antioxidant properties. The moisture content of the flake samples ranged from (7.03-9.78 %), protein (8.05-35.37 %), fat (3.13-13.74 %), ash (2.72-5.88 %), crude fibre (1.75-5.55 %), and carbohydrate (39.41-70.94 %). The energy value ranged from 341.95 to 422.71 kCal. Mango peel flour increment significantly influenced the antioxidant properties of the flakes, while changes in protein, fat, and energy were dependent on the quantity of soybean flour. The inclusion of up to 33.33% mango peel flour did not significantly alter the sensory ratings compared to the control sample (100% maize flour). The optimized composite formulation for nutrient-dense flakes consisted of 50 % yellow maize, 28.45 % soybean, and 21.45 % mango peel. Enriching flakes with soybean and mango peel flour significantly improved their protein, energy, and antioxidant properties, making them a healthier choice for consumers of all ages.

1.Introduction

Ready-to-eat cereals have become a significant commodity in the global market, catering to the fast-paced lifestyles of consumers worldwide. Researchers have delved into the trend of ready-to-eat cereals by diversifying cereal products, including breakfast cereal flakes (Kince *et al.*, 2017; Tay *et al.*, 2021). Traditional flakes are predominantly derived from cereals, rich in carbohydrate content. This emphasis on carbohydrates may underlie the assertion that cereal-based foods are often labelled as unhealthy or "junk" foods (Bolanho *et al.*, 2015). Research, such as that by Jones & Poutanen (2020), suggests that the protein content in breakfast cereal flakes plays a pivotal role in conferring health benefits. Evidently, high-protein and antioxidant-rich breakfast meals are superior to low-protein and

low-antioxidant breakfast meals in terms of maintaining normal blood sugar and resolving oxidative stress (Osunrinade et al., 2022; Ademosun et al., 2023).

The nutritional benefit obtainable from the consumption of a cereal flake is dependent on the characteristics of the flour samples (Okache et al., 2020). Researchers have explored the use of cereals, legumes, and tubers to determine their influence on the flakes produced (Adebanjo et al., 2020; Juniour et al., 2022; Olorunsogo & Adejumo, 2023; Sumczynski et al., 2015). Maize holds a prominent position as a primary cereal utilized in flake production, boasting a myriad of applications. It stands as the second most widely cultivated cereal globally, trailing only wheat in terms of production volume (Mir et al., 2019; Oladapo et al., 2017). In developing countries, maize plays a vital role, meeting more than half of the total caloric and protein requirements (Serna-Saldivar, 2015). Whole grain flour and maize-based products are sought after for their flavour profile and nutritional advantages (Žilić et al., 2010). Nevertheless, maize is deficient in lysine and tryptophan, and its protein content typically falls below 10% (Chaudhary et al., 2013). This deficiency underscores the necessity for enrichment and supplementation strategies when incorporating maize into nutrient-dense food products.

Enrichment and supplementation of cereal products have emerged as common practices aimed at addressing nutritional deficiencies and producing functional food items tailored to meet the populace's dietary requirements. Research indicates that combining cereals with legumes results in products with enhanced nutritional profiles and calorific values compared to those made solely from cereals or legumes (Tanyitiku & Petcheu, 2022). Soybeans, a commonly utilized legume for supplementation in cereal products, offer significant nutritional benefits. The formulation of food products with inherent nutritional properties has become increasingly vital as a strategy to mitigate various common diseases associated with oxidative stress in humans. By incorporating ingredients rich in antioxidants and other essential nutrients, food items can contribute to maintaining overall health and well-being.

Due to its nutritional value and cost-effectiveness, mango peel has gained attention as a possible nutritional source for dietary supplementation and enrichment. According to Jahurul et al. (2015), by-products from the mango business, such as the peel, make up between 35% and 60% of the entire fruit weight. Mango peels are frequently thrown away, although they contain a wealth of nutrients like phenolic compounds, carotenoids, vitamin C, and dietary fiber. According to Dorta et al. (2014), the extractable and non-extractable polyphenol (NEP) contents of mango peel are notably high at 7.22% and 5.54%, respectively. These polyphenols exhibit potent antioxidant activity, making mango peel a valuable dietary addition for combating oxidative stress and promoting overall health.

Given the growing urban population's preference for convenient breakfast options and the prevalent low protein and antioxidant content in ready-to-eat cereal-based meals, it is imperative to investigate optimization strategies for producing protein, energy, and antioxidant-rich breakfast cereal flakes. Therefore, this study utilized the D-optimal response surface methodology to determine the composite mixture of yellow maize, soybean, and mango peel flours that would produce protein, energy, and antioxidant-rich breakfast cereal flakes.

2.Materials and Methods

2.1. Materials

For this research, yellow maize grain (*Zea mays*) and soybeans (*Glycine max*) were sourced from the Sango market in Saki. Mangoes were hand-plucked from The Oke-Ogun Polytechnic, also located in Saki, Oyo State. Additional ingredients such as instant-filled milk (Dano™), granulated sugar, salt, and transparent plastic containers were procured from a local market.

2.2. Sample Preparation

2.2.1. Preparation of maize flour

The methodology outlined by Odimegwu et al. (2019) was followed with minor adjustments for this study. Initially, about 4 kg of yellow maize grain was carefully sorted, cleaned, and subsequently milled using an attrition milling machine. The resulting yellow maize flour was then carefully packaged into well-labelled, airtight polyethylene bags to maintain its freshness and integrity for subsequent usage and analysis.

2.2.2. Preparation of Soybean flour

The methodology outlined by Tanyitiku & Petcheu (2022) was employed with minor adjustments in this study. Briefly, about 4 kg of soybeans were sorted and wet cleaned. Subsequently, the soybeans were boiled in a pot of water for 20 min. Following the boiling process, the soybeans were manually dehulled, thoroughly washed, and then subjected to oven drying. Once dried, the soybeans were milled into flour using laboratory attrition mill. Finally, the resulting soybean flour was carefully packaged in a well-labelled, airtight, high-density polyethylene bag for further usage and subsequent analysis.

2.2.3. Preparation of Mango peel flour

The freshly harvested mango fruits underwent initial physical sorting to identify and remove any peel defects and spots. Following this, the mangoes were thoroughly washed with clean water and peeled using a knife. The resulting fruit peels were then cut into small pieces and subjected to drying at 50°C in a force-draught oven. Once adequately dried, the peel pieces were milled into fine flour using an attrition mill. Finally, the dried mango peel flour was carefully packaged in a well-labelled, airtight polyethylene bag for subsequent use and analysis.

2.2.4. Sample Formulation for the production of composite flour

Composite flour for preparing flakes made from maize, soybean, and mango peel flours was interacted using design expert 6.0.2 software, which lays out a strategy for mixture design of experiments that provides maximum efficiency and effectiveness. A three-component constrained 0-100 % (yellow maize), 0-50 % (soybean), and 0-50 % (mango peel flour) using D-optimal mixture response surface methodology was employed, and 14 randomized experimental runs were generated, which consisted of 10 factorial runs and four replicates.

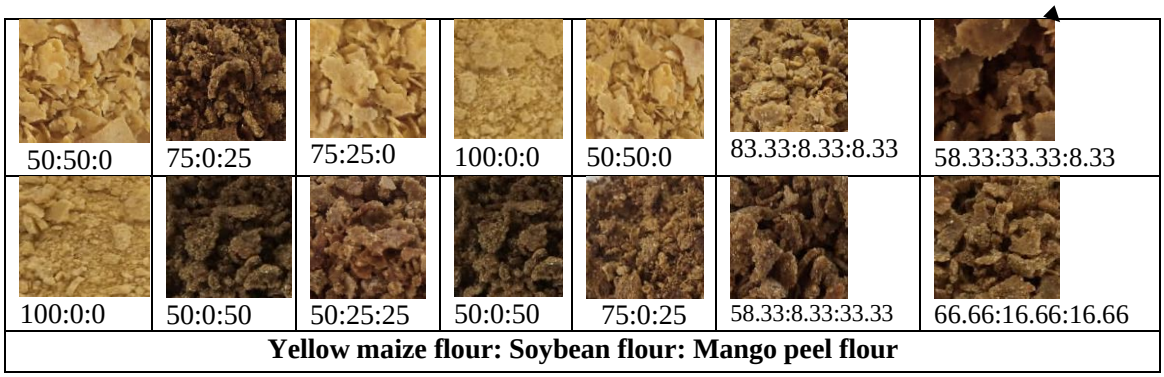

Figure 1. Flakes samples from composite of yellow maize, soybean and mango peel

2.2.5. Breakfast Cereal Flakes Production

A minor variation of the Tay et al. (2021) and Odimegwu et al. (2019) methods was used to prepare the breakfast cereal (flakes). The prepared composite flour (250 g) was combined with 750 ml of water, 10 g of sugar, 2 g of salt, and 6 g of milk. To get a semi-dried product, the batter was thinly put onto a cleaned, flat, and greased steel tray and baked at 125^{o}C for 20 minutes. After being partially dried, the items were broken up into flakes and put back in the oven to toast and dry further at 130 °C for 15 minutes. After being cooled, the flake samples (Figure 1) were placed in an airtight container for subsequent laboratory analysis and sensory assessment.

2.3. Determination of Proximate Composition of Flakes and Flours

The proximate compositions of both flour and flake samples were assessed following the guidelines outlined by the Association of Official Analytical Chemists (AOAC, 2012). Protein content was quantified using the Kjeldahl method as specified by AOAC (2012). Flake samples ash content was quantified by subjecting the samples to a muffle furnace at 600 °C for 3 hours, while moisture content was determined through a forced air draft oven set at 105°C to operate for 4 h. Crude fiber and fat contents were also determined following the procedures outlined in AOAC (2012). Carbohydrate content was calculated through the difference method, while the Atwater factor that gives the energy value per unit mass for protein (4), carbohydrate (4), and fat (9) was used to estimate the energy content of flake samples in Kilo-calories per gram.

2.4. Determination of Antioxidant Properties

2.4.1. Extraction method for antioxidant analysis

The extraction of antioxidant components from flour and flake samples followed the method outlined by Osunrinade et al. (2022). In summary, samples were initially ground using a hand-operated attrition mill. Subsequently, 1g of the sample was subjected to extraction with 20 mL of 80 % ethanol for 48 hours with intermittent agitation. Following extraction, the mixture underwent filtration using Whatman No 1 filter paper. The filtrate was kept at 4 °C in a refrigerator prior to subsequent analysis of antioxidant properties.

2.4.2. Determination of the total phenolic content (TPC)

Total phenolic content (TPC) in flake samples was measured using the procedure described by Osunrinade et al. (2022). In short, 0.8 mL of 7.5% Na_2CO_3 and 0.2 mL of the extract were mixed with 1 mL of five-fold diluted Folin-Ciocalteu's reagent. After 20 minutes of reaction time at room temperature in the dark, the absorbance at 765 nm was measured in comparison to a blank sample. The findings were measured and reported as milligrams of gallic acid equivalent (GAE) per gram of dry weight.

2.4.3. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of flake samples was calculated using the approach described by Saikia et al. (2012). First, 1.25 mL of distilled water was used to dilute 0.25 mL of the flake sample extract and standard catechin solution, each in triplicate. After adding 75 µL of a 5% $NaNO_3$ solution, the mixture was allowed to sit at room temperature for six minutes. 150 µL of a 10% $AlCl_3$ solution was added after the first incubation, and the mixture was then incubated for an additional five minutes. After that, 0.5 mL of a 1 M NaOH solution was added, and then distilled water was added to bring the volume up to 3 mL. The absorbance was determined with a spectrophotometer (JENWAY, Model 7305) set at 510 nm. The concentration of TFC in the flake samples was calculated using a calibration curve and the resulting equation, which was represented in milligrams per gram (mg/g).

2.4.4. Determination of Total Antioxidant Capacity (TAC)

Flake extracts' total antioxidant capacity was evaluated using the methodology proposed by Osunrinade et al. (2022). The phosphomolybdenum reagent was made by mixing 3.3 mL of sulfuric acid, 335 mg of sodium phosphate, and 78.4 milligrams of ammonium molybdate with 100 mL of distilled water. After that, the phosphomolybdenum reagent and 0.1 mL of the flake extract were combined, and the combination was put in a water bath that was heated to 95 °C. After 90 minutes of boiling the combination, the absorbance of the resultant solution was measured at 695 nm. Instead of using the sample, different amounts of gallic acid were used to create a standard curve. The total antioxidant capacity of the flake extract was determined using the equation derived from the standard curve, and the findings were

represented in milligrams of gallic acid equivalent per gram of sample.

2.4.5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay for flake samples

The DPPH radical scavenging assay, described by Osunrinade et al. (2022), was used to evaluate the flake extract's capacity to scavenge radicals. A 0.004% DPPH solution was first made. After that, 0.3 mL of the DPPH reagent and 0.1 mL of the flake extract were mixed, and the mixture was exposed to darkness for 30 minutes. At 516 nm, the absorbance of the resultant combination was measured. Without the sample extract, a control was made with the DPPH reagent. Equation 1 was used to calculate the extract's percentage inhibition.

$$Percentage\ inhibition = \frac{Ac - Ae}{Ac} x100\% \times 100 \quad (1)$$

Where:
Ac = Absorbance of control
Ae = Absorbance of extract

2.4.6. Determination of Ferric Reducing Antioxidant Power (FRAP)

In order to calculate the ferric-reducing antioxidant power (FRAP) for flake samples, the procedure outlined by Sukrasno et al. (2017) was used. First, 0.3 mL of the flake extract was diluted with 0.7 mL of distilled water, and then 2.85 mL of the FRAP reagent (acetate buffer, TPTZ, and $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1, respectively) was added. The reaction mixture was then incubated at 50°C for 20 minutes, and its absorbance at 700 nm was measured. A standard curve was constructed using ascorbic acid to estimate the antioxidant power of both flour and flake samples.

2.5. Sensory Evaluation of Flakes

The sensory evaluation of flake samples from the composite of yellow maize, soybean, and mango peel flour was carried out by a panel of 30 semi-trained panellists who are familiar with the sensory attributes of flakes. The panellist used a nine-point hedonic scale—where 1 denotes "extremely dislike" and 9 denotes "extremely like"—to evaluate the sensory qualities based on appearance, taste, texture, aroma, and overall acceptability.

2.6. Statistical Analysis

Version 20.0 of the Statistical Package for Social Science (SPSS) (SPSS Inc. Chicago, IL, USA) was used to analyze the data. The Duncan multiple range test was used to determine the mean differences. The Design Expert 6.0.2 software determined composite mixture and optimisation that produced highly nutrient-dense flakes.

3. Results and discussion

3.1. Proximate, antioxidant and colour properties of composite flour components

The results of the proximate composition, antioxidants, and colour properties of yellow maize, soybean, and mango peel flour are presented in Table 1. The proximate composition of the flour samples (yellow maize, soybean, and mango peel) used for the production of flakes ranged as moisture (3.62 to 9.38%), Protein (3.13 to 47.80%), fat (2.5 to 21%), ash (2.22-4.79), crude fibre (1.41-10.27%), and carbohydrate (21% to 70.73%) while the energy value is between 317.94 and 464.20 kCal.

As presented, there is a significant ($p<0.05$) wide variation in the proximate compositions of yellow maize, soybean, and peel flours. Therefore, this variation is a major consideration for their choice to produce nutrient-dense flakes.

As expected, soybean flour had the highest Protein (47.80%), fat (21%), and Energy value (464.20 kCal). The high values of Protein, fat, and energy for soybean flour obtained in this work are in the range of those reported by Tanyitiku & Petcheu (2022) for soaked soybean flour [Protein (38.69 – 48.61%), Fat (21.93- 27.93%) and Energy (485.97-519.81 kCal)].

Table 1. Nutritional, antioxidant, and colour properties of maize, soybean, and mango peel flour

SAMPLE	FGP	MFF	WPS
Moisture (%)	9.38 [b]	10.18 [a]	3.62 [c]
Protein (%)	3.13 [c]	12.90 [b]	47.80 [a]
Fat (%)	2.50 [c]	9.89 [b]	21.00 [a]
Ash (%)	3.99 [b]	2.22 [c]	4.79 [a]
Crude fiber (%)	10.27 [a]	1.41 [c]	1.79 [b]
Carbohydrate (%)	70.73 [a]	63.40 [b]	21.00 [c]
Energy (kCal)	317.94[c]	394.21[b]	464.20[a]
Ferric Reducing Antioxidant Power (mg/g)	4.44 [a]	2.40 [b]	1.72 [c]
Total Flavonoid Content (mgQuarcetin/g)	6.45 [a]	2.32 [b]	1.59 [c]
Total Phenolic Content (mgGAE/g)	29.14 [a]	12.56 [b]	7.67 [c]
Total Antioxidant Content (mgGAE/g)	2.66 [a]	0.93 [b]	0.55 [c]
DPPH (%)	80.65 [a]	19.86 [c]	39.41[b]
l	47.66[b]	67.88 [a]	68.47 [a]
a	2.86 [a]	0.78 [c]	1.58 [b]
b	12.50 [c]	25.18 [a]	15.24 [b]
ΔL	29.74 [c]	49.28 [b]	50.54 [a]
Δa	-0.71 [a]	-2.80 [c]	-1.99 [b]
Δb	10.82 [c]	23.83 [a]	13.55 [b]
ΔE	31.65 [c]	54.54 [a]	52.03 [b]

Values are in mean +SD of duplicate determination. Means differently superscripted along the horizontal columns are significantly different (P<0.05).
FGP - mango peel, MFF- yellow maize, WPS – soybean

Soybean is recognized for its high protein content in comparison to cereal crops, as reported by Qin et al. (2022). Its favourable agronomic traits and protein quality akin to that of animal sources have led to its extensive utilization in food formulation. The proximate composition reported for Mango peel is similar to that reported [Moisture (10.66%), Ash (2.38%), fat (3.43%), protein (1.04%), Crude fibre (10.36%) and Carbohydrate (71.63%)] by Nur Azura et al. (2019) who used mango peel flour to substitute wheat in the production of alkaline noodles. The study of Bertha et al., (2019) on the in-vitro gastrointestinal digestion of mango by-product snacks, reported a very close percentage of carbohydrate (68.6%) and fat (3%) in mango peel which is comparable to the result obtained in this study.

Mango peel flour had significantly ($p<0.05$) higher antioxidant properties than soybean and maize flour. The antioxidant properties studied had their range for flour samples as TPC (7.67 - 25.14 mgGAE/g), TFC (1.59-6.45 mg quercetin/g), TAC (0.55-2.66 mgGAE/g), FRAP (1.72-4.44 mg/g) and DPPH (19.86-80.65%). The result of TPC (29.14 mgGAE/g) obtained for mango peel flour in this work is not significantly different from the value obtained for the palmer variety (30.53 mgGAE/g) but higher than the keith variety (26.20 mgGAE/g) of mango peel studied by Troiani et al. (2022).

The result of lightness (L), redness (a), and yellowness (b) showed that soybean had the highest lightness (68.47), which was higher but not significantly different (P<0.05) from that obtained for yellow maize flour (67.88). However, yellow maize had a significantly higher yellowness than soybean and mango peel flours. In addition, mango peel flour's redness (a) was significantly higher compared to yellow maize and soybean flour. The lower values of lightness (L) and yellowness observed in mango peel flour, as reported in

this study, have previously been linked (Nur Azura et al., 2019) to the primary cause of the brown colouration observed in food products incorporating mango peel flour. This browning phenomenon in mango peel flour could be attributed to the oxidation of polyphenols present in the peel by enzymes such as polyphenol oxidase and peroxidase, resulting in a transition from yellow to brown colour (Priyadarshini et al., 2023; Sogi et al., 2013). The lightness of mango peel flour obtained in this work is within the range reported for the Palmer variety (43.47) mango peel flour but lower than that reported for the Keith variety of mango peel flour by Troiani et al. (2022)

3.2. Proximate Composition of Flake Samples

The result of the proximate composition of the flake sample is presented in Table 2. The moisture content range (7.03-9.78%) observed in flake samples suggests promising shelf life stability. This moisture content value aligns closely with the findings reported by Sumczynski et al. (2015) for commercial flake samples (9.2%). The ability to maintain the low moisture content in flake samples contributes significantly to their shelf life stability by inhibiting microbial activity and chemical reactions that may compromise food quality and stability (Okolie et al., 2022). An increase in the percentage inclusion of mango peel flour led to a significant ($p<0.05$) decrease in the percentages of flake's protein and fat. Furthermore, an increase in the percentage inclusion of soybean flour caused a significant increment in the percentage of protein, fat, and energy value. The same significant increase in protein, fat, and energy by the inclusion of soybean has been severally reported to be due to the high protein and fat content in soybean (Akinjayeju et al., 2019; Okwunodulu et al., 2020). In addition, a gradual increase in protein and ash is attributable to the rise in the percentage of yellow maize used, with a significant ($p<0.05$) increase when the percentage of yellow maize used was increased from 50 to 100%.

The protein content of flake samples ranged from 8.05 to 35.37%, with the highest percentage of protein content obtained from using 50% soybean and 50% yellow maize flour. The lowest protein content was from a composite formulation containing 50% yellow maize and 50% mango peel flour. The highest protein content (35.37) obtained in this work is more than double of the highest (14.4%) reported for wheat-mango flakes by Juniour et al. (2022), while it is more than triple of the highest protein content (10.50%) reported by Adebanjo et al., (2020) for extruded flakes from pearl millet (*Pennisetum glaucum*)-carrot (*Daucus carota*) blended flours. However, the highest protein content (48%) reported for flakes by Olorunsogo & Adejumo (2023) was higher than that obtained in this work but within the range of the optimized sample protein content of 38% obtained from 30.5 % corn flour, 11.2 % millet flour, 18.3 % soybean meal

The fat content of flake samples ranged from 3.13% to 13.74%, with the highest fat content obtained from flake produced from 50% soybean and 50% yellow maize flour, while the lowest fat content was from 75% yellow maize and 25% mango peel flour. The work of Sumczynski et al. (2015) reported the lowest fat amount in commercial flakes (2.1%) and the highest amount was red wheat flakes (3.3%). The comparatively high fat content obtained in this study is due to the high percentage of soybean inclusion in flake production. Fats are nutritionally significant and possess physiological importance, serving as both an energy source and a crucial supplier of fatty acids (De Carvalho & Caramujo, 2018). However, they also impact food quality, as evidenced by their potential to induce off-flavors in stored flakes and flours (Yang et al., 2021).

Table 2. Result of proximate composition of flakes

Run	A:Yellow maize	B:Soybean	C:Mango peel	Moisture	Protein	Fat	Ash	Crude fibre	Carbohydrate	Energy
	%	%	%	%	%	%	%	%	%	kCal
1	50	50	0	7.06^{k}	35.37^{a}	13.74^{a}	2.72^{e}	1.71^{i}	39.41^{f}	422.71^{a}
2	75	0	25	7.12^{k}	8.84^{gh}	4.50 ^{g}	5.60^{ab}	3.79^{c}	70.15^{a}	356.49^{g}
3	50	50	0	7.35^{J}	35.02^{a}	13.64^{a}	3.16^{de}	1.75^{hi}	39.09^{f}	419.22^{b}
4	50	25	25	8.32^{f}	13.48^{d}	8.56^{e}	4.22^{c}	2.79^{e}	62.63^{c}	381.51^{e}
5	83.33	8.33	8.33	8.92^{e}	9.89^{f}	5.85^{f}	3.97^{cd}	2.08^{g}	69.28^{a}	369.38^{f}
6	58.33	33.33	8.33	9.49^{c}	22.15^{c}	11.83^{b}	4.77^{bc}	2.35^{f}	49.42^{d}	392.73^{c}
7	58.33	8.33	33.33	9.65^{b}	8.23^{h}	5.44 ^{f}	5.67^{ab}	4.42^{b}	66.59^{b}	348.21^{h}
8	100.00	0.00	0.00	7.70^{i}	10.86 ^{e}	3.21^{h}	5.72^{a}	1.83^{h}	70.69^{a}	355.03^{g}
9	66.67	16.67	16.67	8.15^{g}	9.63^{fg}	9.50^{d}	5.82^{a}	2.41^{f}	64.49^{bc}	381.98^{e}
10	75	25	0	8.93^{e}	29.68^{b}	10.50^{c}	5.56^{ab}	2.16^{g}	43.18^{e}	385.92^{d}
11	50	0	50	9.29^{d}	8.05^{h}	3.14^{h}	2.97^{e}	5.60^{a}	70.94^{a}	344.25^{i}
12	100	0	0	7.80^{h}	11.21^{e}	3.43^{h}	5.88^{a}	1.71^{i}	69.97^{a}	355.58^{g}
13	75	0	25	7.03^{k}	8.75^{gh}	4.00^{gh}	5.71^{a}	3.70^{d}	70.80^{a}	354.22^{g}
14	50	0	50	9.78^{a}	8.05^{h}	3.13^{h}	3.11^{de}	5.55^{a}	70.38^{a}	341.95^{i}

Values are in mean +SD of duplicate determination. Means differently superscripted along the vertical columns are significantly different ($P<0.05$).

Particularly vital in the diets of infants and young children, fat fulfils various essential functions, including the provision of necessary fatty acids, facilitation of fat-soluble vitamin absorption, augmentation of dietary energy density, and prevention of undesirable weight gain in infants (Ravisankar et al., 2015).

The carbohydrate content of the flake sample, which ranged from 39.41 to 70.94%, decreased significantly ($p<0.05$) with an increase in the percentage of soybean and gradually increased the percentage of yellow maize flour. However, the carbohydrate content of this study is higher than that reported (33.5 - 63.2%) for flakes from the combination of corn flour, millet flour, and soybean meal by Olorunsogo & Adejumo (2023). The relatively high percentage of carbohydrates in flake samples from the yellow maize, soybean, and mango peel flour composite indicates that the product will provide the energy required for optimum growth and development (Tanyitiku & Petcheu, 2022).

The ash content of flakes ranged from 2.72 to 5.88%. It was observed that an increase in the inclusion of mango peel flour and soybean flour in the flake samples increased the ash content. The interaction between maize and mango peel had a decreasing effect on the ash content, while the interaction between mango peel and soybean flour increased the ash content. The range of ash content obtained in this work is higher than that obtained for wheat-mango peel flakes Nur Azura et al. (2019) but similar to the flakes' ash content (0.99-5%) produced from corn flour, millet flour, and soybean meal by (Olorunsogo & Adejumo, 2023). This study's crude fibre of flake samples ranged from 1.75-5.55%. Mango peel flour had the highest contribution of crude fibre to flakes. The sample with the highest percentage of mango peel flour had the highest crude fibre. The crude fibre content of flakes obtained in this work is higher than the crude fibre content of the flakes (0.92-2.3) reported for wheat-mango flakes by Juniour et al. (2022).

The energy value of flakes ranged from 341.95-422.71 kCal, and the most energy value contributing mixture component was an equal volume of maize and soybean. The energy value of flakes significantly increases with increase in the inclusion of soybean flour. The highest energy value obtained for the flake sample (422.71 kCal) was obtained from the highest percentage (50%) inclusion of soybean flour. Concerning the energy value of flakes, the variation of yellow maize flour inclusion also significantly influenced the energy value of flake samples.

3.3. Antioxidant Composition of Flake Samples

Presented in Table 3 is the result of the antioxidant composition of the breakfast cereal flakes samples. The anti-oxidative parameters obtained were TPC (19.24-25.14 mgGAE/g), TFC (1.79-4.40 mgQuarcetin/g), FRAP (2.59-4.40 mg/g), TAC (0.89-2.61 mgGAE/g) and DPPH (30.91-80.52%). The result shows that flaked sample produced from 50% yellow maize and 50% soybean had the lowest total phenolic content (19.24 mgGAE/g) and the flaked sample from 58.33% yellow maize, 8.33% soybean and 33.33% mango peel flour had the highest total phenolic content (25.14 mgGAE/g) which is higher compared to the range of 3.96 – 8.86 mgGAE/g reported for commercial and non-traditional wheat flakes by Sumczynski et al., (2015). This finding indicates that mango peel possesses a significant concentration of antioxidant compounds, sparking considerable interest in the potential health benefits of consuming a diet abundant in antioxidants. Such dietary practices may potentially mitigate the risk of various prevalent chronic ailments, including cancer, cardiovascular disease, and chronic inflammatory conditions (Ademosun et al., 2023).

Generally, flakes' antioxidant properties, including FRAP, TPC, and DPPH, were significantly influenced by the increment in mango peel flour inclusion for flake production. More specifically, the highest

percentage (50%) of inclusion of mango peel flour resulted in flakes with the highest values of FRAP (4.40 mg/g), TPC (25.12 mgGAE/g), and DPPH (80%). Except for the composite flour mixture of 58.33% (yellow maize), 8.33% (soybean), and 33.33% (mango peel) that gave the highest TFC (4.40) and TAC (2.61 mg/g), total flavonoids increased with an increase in the percentage of mango peel flour. Concerning TFC and TAC, there was no defined increase or decrease pattern in flakes regarding mango peel flour. This could be because the interaction of composite mixtures and processing had a combined greater influence on the TFC and TAC.

Table 3. Results showing the antioxidant properties of flake samples

S/N	Yellow maize	Soybean	Mango peel	FRAP	TFC	TPC	TAC	DPPH
				mg/g	mgQuarcetin/g	mgGAE/g	mgGAE/g	%
1	50	50	0	3.05^{c}	1.78^{c}	19.24^{f}	1.46^{e}	30.93^{j}
2	75	0	25	4.20^{d}	2.85^{b}	25.10^{a}	2.15^{b}	55.80^{f}
3	50	25	25	4.26^{a}	1.91^{bc}	25.12^{a}	2.26^{b}	78.94^{b}
4	83.33	8.33	8.33	3.44^{b}	1.91^{bc}	24.36^{b}	1.58^{de}	68.74^{d}
5	58.33	33.33	8.33	3.26^{bc}	2.35^{b}	23.94^{c}	1.78^{c}	37.17^{i}
6	50	50	0	3.07^{c}	1.79^{c}	19.21^{f}	1.44^{e}	30.91^{j}
7	58.33	8.33	33.33	4.39^{a}	4.40^{a}	25.14^{a}	2.61^{a}	69.90^{c}
8	100	0	0	3.43^{b}	1.86^{bc}	23.42^{d}	0.91^{f}	56.75^{e}
9	50	0	50	4.39^{a}	3.14^{a}	25.10^{a}	1.72^{cd}	80.51^{a}
10	66.67	16.67	16.67	3.40^{b}	2.52^{bc}	24.18^{bc}	1.54^{e}	47.13^{g}
11	75	25	0	2.59^{d}	1.89^{bc}	22.48^{e}	0.89^{f}	44.66^{h}
12	50	0	50	4.40^{a}	3.16^{a}	25.12^{a}	1.73^{cd}	80.52^{a}
13	75	0	25	4.23^{d}	2.83^{b}	25.13^{a}	2.14^{b}	55.81^{f}
14	100	0	0	3.40^{b}	1.87^{bc}	24.18^{bc}	0.92^{f}	57.13^{e}

Values are in mean +SD of duplicate determination. Means differently superscripted along the vertical columns are significantly different ($P<0.05$).

Yellow maize and soybean percentage variation in flake formulation did not significantly influence the antioxidant properties of the flake samples. Also, the DPPH of flake samples was the only antioxidant property that was significantly influenced by variation in composite flour formulation. However, there was no defined trend for both yellow maize and soybean flour. This could be linked to the comparatively low antioxidant properties of yellow maize and soybean flour when compared with mango peel flour

3.4. Colour Properties of flour and flakes

Colour is one of the fundamental criteria for the visual assessment of flakes. The result of lightness (L), redness (a), and yellowness (b) is presented in Table 4. Directly after the breakfast cereal flakes sample production, the L parameter that determined the colour brightness fluctuated between 22.34 and 48.21. In comparison, the redness (a) and yellowness varied from 3.71-8.72 and 6.76 to 20.62, respectively. The addition of mango peel flour causes a decrease in the yellow colouration of the sample. The highest yellow colouration was found in a flaked sample produced from 100%

yellow maize flour (20.62), which is a natural consequence of the colour of the maize.

Table 4. Results of the colour analysis of flakes

Sample	Yellow maize	Soybean	Mango peel	L	a	b	ΔL	Δa	Δb	ΔE
1	50	50	0	42.42^{b}	4.78^{b}	17.33^{b}	24.53^{b}	1.22^{b}	15.65^{b}	29.10^{b}
2	75	0	25	25.79^{h}	3.80^{cde}	8.62^{f}	7.84^{h}	0.22^{cde}	6.95^{f}	10.50^{h}
3	50	25	25	28.51^{g}	4.93^{b}	9.49^{e}	10.58^{g}	1.36^{b}	7.81^{e}	13.22^{g}
4	83.33	8.33	8.33	36.35^{c}	3.85^{cde}	13.77^{c}	18.42^{c}	0.29^{cd}	12.08^{c}	22.03^{c}
5	58.33	33.33	8.33	29.47^{f}	4.92^{b}	10.26^{d}	11.54^{f}	1.39^{b}	8.58^{d}	14.44^{f}
6	50	50	0	42.41^{b}	4.79^{b}	17.35^{b}	24.52^{b}	1.21^{b}	15.67^{b}	29.12^{b}
7	58.33	8.33	33.33	25.16^{h}	4.03^{cd}	8.15^{g}	7.23^{h}	0.46^{cd}	6.47^{g}	9.72^{i}
8	100	0	0	48.21^{a}	3.48^{e}	20.62^{a}	30.27^{a}	-0.08^{e}	18.94^{a}	35.72^{a}
9	50	0	50	22.33^{i}	3.71^{de}	6.74^{h}	4.4^{i}	0.12^{de}	5.07^{h}	6.71^{j}
10	75	0	25	25.78^{h}	3.81^{cde}	8.64^{f}	7.86^{h}	0.23^{cde}	6.94^{f}	10.51^{h}
11	66.67	16.67	16.67	30.42^{e}	4.09^{c}	10.44^{d}	12.49^{e}	0.51^{c}	8.76^{e}	15.26^{e}
12	75	25	0	33.34^{d}	8.72^{a}	13.66^{c}	15.42^{d}	5.15^{a}	11.98^{c}	20.20^{d}
13	50	0	50	22.34^{i}	3.70^{de}	6.76^{h}	4.41^{i}	0.13^{de}	5.08^{h}	6.72^{j}
14	100	0	0	48.2^{a}	3.47^{e}	20.61^{a}	30.28^{a}	-0.09^{e}	18.93^{a}	35.71^{a}

Values are mean +SD of duplicate determination. Means differently superscripted along the vertical columns are significantly different ($P<0.05$).

Including mango peel flour in flake formulation significantly reduced the lightness, redness, and yellowness of flake samples. However, the redness (a=3.48) for the control sample (100% maize flour) flakes was not significantly different from flakes produced with 0-50% inclusion of mango peel flour. Elevated redness (a) properties could be linked to brown colour caused by non–enzymatic browning during toasting and enzymatic browning due to oxidation of mango peel (Nur Azura et al., 2019). The colour difference (ΔE) between two samples determines colour perception by a human observer. In the examined flakes, the lowest colour difference was recorded in the flake sample from 50% yellow maize and 50% mango peel, while the highest colour difference was obtained from the flake sample from 100% yellow maize. The inclusion of mango peel flour caused a significant decrease in the colour intensity (ΔE) and other colour parameters determined in this study. A similar change was reported when mango peel flour was included in the production of noodles ((Nur Azura et al., 2019)

3.5. Sensory Evaluation of Flaked Sample

The sensory evaluation rating of breakfast cereals produced from the composite of yellow maize, soybean, and mango peel flour is presented in Table 5. The analysis showed that panellists' sensory ratings for all the samples were significantly different ($p>0.05$). The sensory appearance rating ranged from 6.04 to 7.69, with a high proportion inclusion of mango peel causing a reduction in the appearance rating by the panellist. Texturally, panellist rates ranged from 5.86 to 7.17, with the sample from 100% yellow maize having the highest rating. This result is similar to that of flakes

from banana pulp and maize (Tay et al., 2021), which have the highest texture rating from yellow maize flakes (7.19).

Table 5. Results of the sensory analysis of flakes

Sample	Yellow maize	soybean	Mango peel	Appearance	Texture	Mouthfeel	Taste	Aroma	Overall acceptability
1	50	50	0	7.68^{a}	6.48^{abc}	6.40^{ab}	6.51^{abc}	6.57^{ab}	7.64^{a}
2	75	0	25	7.14^{abcd}	6.71^{abc}	6.49^{ab}	6.38^{bc}	6.69^{ab}	7.37^{ab}
3	50	25	25	6.35^{de}	5.86^{c}	5.97^{b}	6.10^{bc}	6.21^{ab}	7^{ab}
4	83.33	8.33	8.33	6.52^{cde}	6.52^{abc}	6.31^{ab}	7^{ab}	6.62^{ab}	7^{ab}
5	58.33	33.33	8.33	6.62^{bcde}	6.21^{bc}	6.41^{ab}	6.69^{abc}	6.66^{ab}	7^{ab}
6	50	50	0	7.69^{a}	6.47^{abc}	6.41^{ab}	6.51^{abc}	6.58^{ab}	7.65^{a}
7	58.33	8.33	33.33	6.76^{bcde}	6.28^{abc}	6.69^{ab}	6.58^{abc}	6.27^{ab}	7^{ab}
8	50	0	50	6.04^{e}	5.86^{c}	5.96^{b}	5.71^{c}	5.82^{b}	6.71^{b}
9	66.67	16.67	16.67	7.45^{ab}	7.17^{a}	7.21^{a}	7^{ab}	6.86^{ab}	7.24^{ab}
10	100	0	0	6.78^{bcde}	6.54^{abc}	5.97^{b}	6.45^{abc}	5.9^{b}	7.28^{ab}
11	75	0	25	7.13^{abcd}	6.72^{abc}	6.48^{ab}	6.37^{bc}	6.67^{ab}	7.36^{ab}
12	75	25	0	7.35^{abc}	7.07^{ab}	6.86^{ab}	7.41^{a}	7.24^{a}	7.59^{ab}
13	50	0	50	6.03^{e}	5.85^{c}	5.95^{b}	5.71^{c}	5.81^{b}	6.71^{b}
14	100	0	0	6.79^{bcde}	6.55^{abc}	5.96^{b}	6.44^{abc}	5.91^{b}	7.26^{ab}

Values are in mean +SD of duplicate determination. Means differently superscripted along the vertical columns are significantly different ($P<0.05$).

In the inclusion of mango peel flour, up to 33.33% had sensory (appearance, texture, mouthfeel, aroma, and overall acceptability) ratings that were not significantly different from the control sample (100% maize flour) flake. Regarding overall acceptability, flakes produced from 50% yellow maize and 50 % soybean flours were the most acceptable by panellists. Whereas the least acceptable by panellists was made from 50% yellow maize and 50% mango peel flour. The inclusion of the combination of soybean and mango peel in yellow maize flour increased the sensory acceptability of the flakes produced.

3.6. Modeling and Optimization

3.6.1. Modeling of Proximate Composition of Flakes

The estimated coefficient of the model and analysis of variance showing the effect of raw materials on the flake's proximate composition is shown in Table 6 and the response surface plate is presented in Figure 2 a-g.

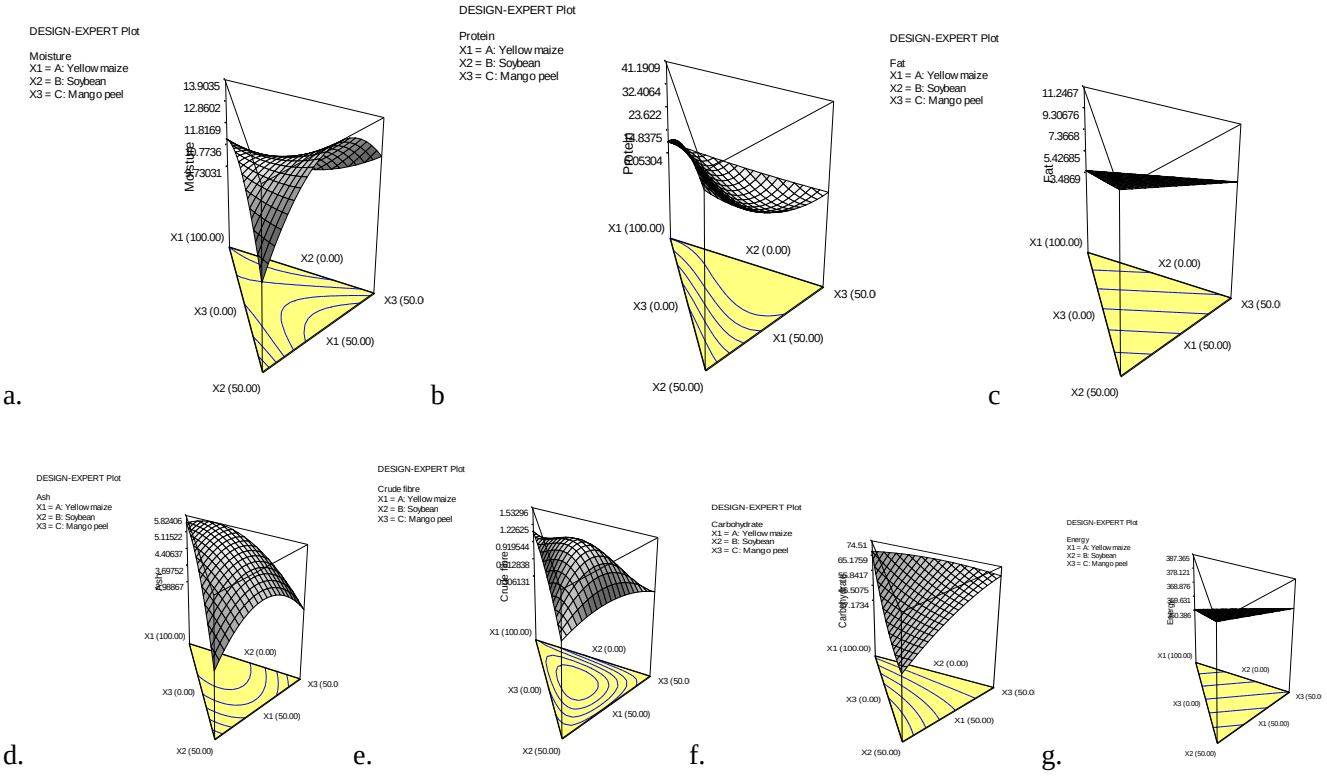

Figure 1. Response surface plot showing the effect of raw materials on flakes' proximate composition.

Table 6. Estimated coefficient of the model showing the effect of raw materials on flakes' proximate composition

Component	Moisture	Protein	Fat	Ash	Crude fibre	Carbo-hydrate	Energy
A-Yellow maize	7.75	10.67	3.19	5.53	1.72	70.94	358.19
B-Soybean	7.24	35.21	13.72	2.99	1.77	38.79	419.69
C-Mango peel	9.58	8.37	3.05	3.20	5.65	69.94	343.30
AB	7.77	24.22	9.21	3.28	0.96	-32.42	-
AC	-5.31	-3.07	4.58	4.65	0.04	6.24	-
BC	2.03	-30.56	2.07	6.09	-3.38	36.76	-
ABC	-	-204.07	-	-	-	70.94	-
R-Squared	0.76	0.99	0.99	0.76	0.98	0.97	0.97
Adj R-Squared	0.62	0.99	0.98	0.60	0.97	0.94	0.96
Pred R-Squared	0.08	0.89	0.97	0.37	0.95	0.87	0.96
Adeq Precision	5.73	34.45	30.85	5.46	23.34	17.00	32.43
p-value	0.0204	<0.0001	<0.0001	<0.0001	0.0233	<0.0001	<0.0001

The coefficient of determination (R^2) for fat (0.99), Protein (0.99), crude fibre (0.98), carbohydrate (0.97) and energy (0.97) are on the very high side for response surface and

indicated that the fitted models accounted for over 95% of the variance in the experimental data, which were highly significant. Moreover, achieving a high precision greater than 4 for all proximate components indicates that the model can effectively navigate the design space (Osunrinade & Akinoso, 2020).

The model coefficient in Table 6 showed that an increase in soybean flour caused the highest increase in protein, fat, and energy level followed by yellow maize. Moisture content had the highest increment as mango peel flour inclusion increased. Also, the interaction of yellow maize, soybean, and mango peel caused a significant decrease in the protein content of flake samples, while the same interaction caused a significant increase in the carbohydrate content of the flake samples. However, the interaction of yellow maize and mango peel significantly reduced the moisture and protein content of flake samples.

The linear model proved significant ($p<0.05$) for the energy content of breakfast cereal flaked samples made from yellow maize, soybean, and mango peel flour. Conversely, the quadratic model was deemed suitable for modelling moisture, fat, ash, and crude fibre content. Meanwhile, the estimated coefficient of the special cubic model demonstrated adequacy in predicting the protein and carbohydrate content of flake samples. However, it's noteworthy that the quadratic model fitted for flake samples displayed no substantial agreement in the predicted values of R^2 and adjusted R^2. This suggests that apart from the raw materials used (such as yellow maize, soybean, and mango peel flours), processing temperatures could also play a crucial role in determining the moisture content of the final flake product.

3.6.2. Modeling of Antioxidant Properties of Flake

Response surface modelling was applied to examine the effect of raw material on flakes' antioxidant properties, and the result is shown in Table 7 while the response surface plots are shown in Figure 3 a-e. The result obtained confirmed the fitting of the model ($R^2>0.50$) for all antioxidant properties, which denoted that at least 50% of the predicted values could be matched with the actual values. The model coefficient indicated that an increase in mango peel led to a rise in total flavonoid content, total phenolic content, DPPH, FRAP, and TAC. The combination of soybean and mango peels increased the flake samples' antioxidant activities.

Table 7. Estimated coefficient of the model showing the effect of raw materials on flakes' antioxidant properties

Component	**TFC**	**TPC**	**DPPH**	**FRAP**	**TAC**
A-Yellow maize	2.36	23.72	59.21	3.45	1.56
B-Soybean	1.66	19.38	29.15	3.08	1.47
C-Mango peel	3.37	24.92	79.53	4.34	1.83
AB	-	4.73	-6.66	-3.02	-3.11
AC	-	1.27	-52.84	1.19	1.60
BC	-	12.21	69.37	1.97	2.44
R^2	0.528	0.962	0.854	0.977	0.652
Adj R^2	0.442	0.938	0.764	0.962	0.434
Pred R^2	0.357	0.904	0.591	0.934	0.343

Adeq Precision	6.603	18.569	9.255	24.394	6.430
p-value	0.016	0.000	0.003	0.000	0.082

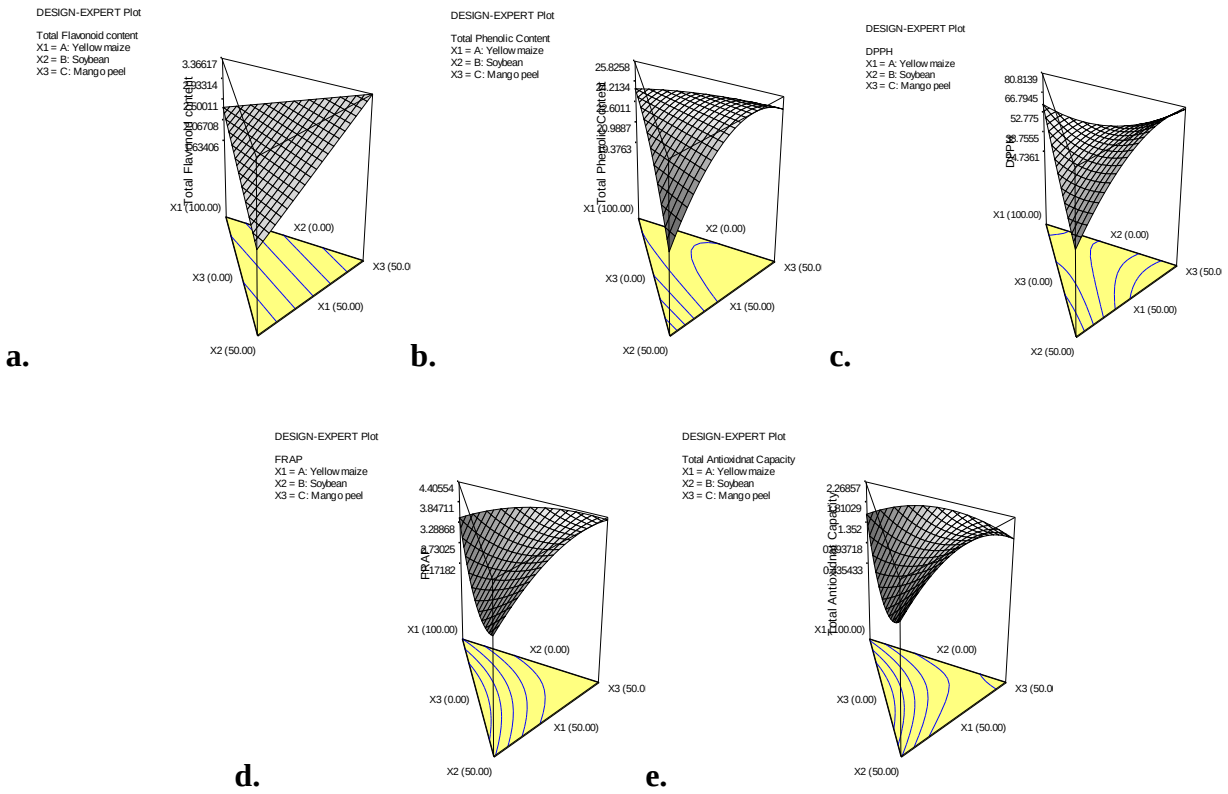

Figure 3. Response surface plot showing the effect of raw materials on flakes' antioxidant properties

The adequate precision values exceeding 4 for TFC, TPC, DPPH, FRAP, and TAC indicate the suitability of the design model. The estimated coefficients of the quadratic model were found to be adequate and significant ($p<0.05$) in depicting TPC, DPPH, and FRAP for flaked samples, while the linear component of the model significantly ($p<0.05$) sufficed to predict the TFC of flake samples. However, it's worth noting that the model fitted for TAC was not significant and this could be that there could be other factors such as the processing condition that determine the variation in the TAC of flake samples.

3.6.3. Modeling of Colour Attributes of Flakes

The estimated coefficient and analysis of variance of colour parameters presented in Table 8 indicated that the linear model was significant ($p<0.05$) for Lightness (L), yellowness (b), and colour intensity (ΔE). In contrast, the cubic model was significant for the redness (a) colour properties.

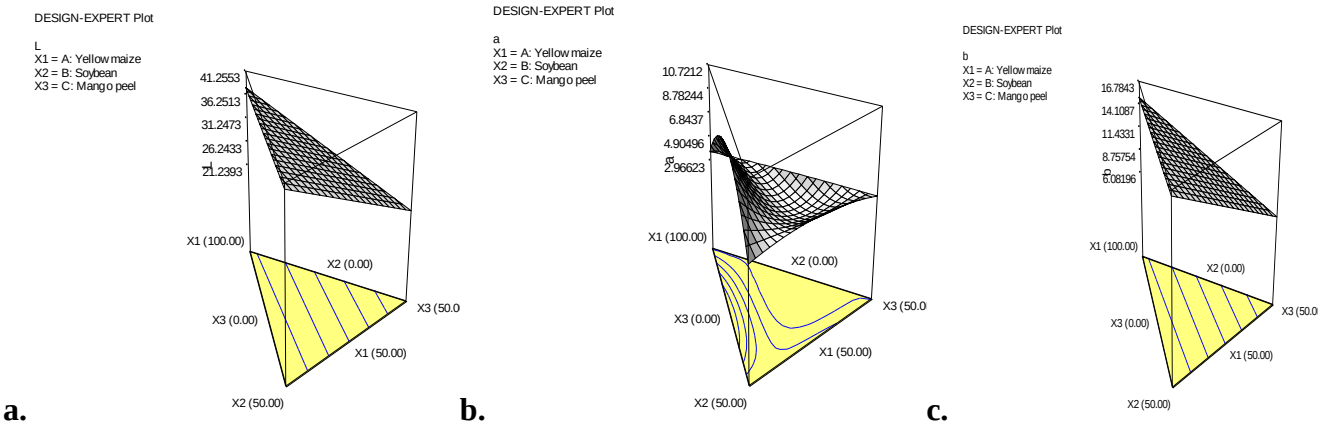

Figure 4. Response surface plot showing the effect of raw materials on flakes' colour properties

Table 8. Estimated coefficient of the model showing the effect of raw materials on flakes' colour properties

Component	**L**	**a**	**b**	**ΔL**	**Δa**	**Δb**	**ΔE**
A-Yellow maize	37.87	3.69	14.98	19.94	0.12	13.29	24.06
B-Soybean	40.18	4.71	16.21	22.27	1.14	14.53	26.70
C-Mango peel	21.24	3.84	6.08	3.31	0.27	4.40	5.36
AB	-	17.02	-	-	17.06	-	-
AC	-	0.32	-	-	0.31	-	-
BC	-	3.26	-	-	3.28	-	-
ABC	-	-79.99	-	-	-79.76	-	-
R^2	0.514	0.949	0.507	0.514	0.950	0.507	0.515
Adj R^2	0.426	0.905	0.417	0.426	0.907	0.418	0.427
Pred R^2	0.253	0.325	0.228	0.253	0.341	0.229	0.249
Adeq Precision	5.980	17.501	5.891	5.983	17.693	5.894	5.997
p-value	0.019	0.000	0.020	0.019	0.000	0.020	0.019

The response surface model presented in Table 8 indicated that a significantly increased yellow maize and soybean ($p<0.05$) increased the flakes' lightness. The decrease in the lightness is attributable to the inclusion of mango peel (Jahurul et al., 2015). The variation in the response surface plot shapes presented in Figure 4 a-e is an indication of the significant effect of the raw materials on the colour attributes of flake samples.

The regression model developed for the redness has the coefficient of determination R^2 of 0.949 and a p-value of 0.000, indicating that the special cubic model is adequate and significant to depict the effect of yellow maize, soybean, and mango peel on the redness of its flakes. The interactive effect of yellow maize and soybean caused the highest increase in the redness of flake samples. The response surface model shows that an increase in mango peel led

to a decrease in the yellowness of the flakes, whereas yellow maize and soybean increased the flakes' samples' yellowness.

3.6.4 Optimisation for Protein, Energy, and Antioxidant rich flake

Considering the importance of protein content, energy value, sensory acceptability, and the antioxidant component of food, the optimization criteria for protein content, energy value, overall sensory acceptability, and all the antioxidant properties were set at maximum. In contrast, other flake parameters determined in this work were set to be in range to determine the optimum mixing ratio of yellow maize, soybean, and mango peel flours. The result presented in Table 9 indicated the highest desirability of 0.57 for the mixture of 50% yellow maize, 28.45% soybean, and 21.55% mango peel that gave the optimum property of protein (16.15%), energy (386.76kCal), overall acceptability (6.85), total phenolic content (24.76 mgGAE/g), total flavonoid Content (2.39 mg/g), total antioxidant capacity (2.22mgGAE/g), ferric reducing antioxidant power (4.11 mg/g) and DPPH (67.88%).

Table 9. Result of Optimisation for Flake Samples

Number	1	2	3	4
Yellow maize (%)	50	50	50	58.02
Soybean (%)	28.45	29.28	33.58	0
Mango peel (%)	21.55	20.72	16.42	41.98
Appearance	6.28	6.31	6.46	6.75
Texture	5.76	5.76	5.82	6.39
Mouthfeel	6.24	6.24	6.22	6.49
Taste	6.34	6.36	6.48	5.76
Aroma	6.34	6.34	6.37	6.18
Overall acceptability	6.85	6.87	7.00	7.29
Moisture (%)	8.75	8.70	8.46	8.57
Protein (%)	16.15	16.68	19.66	8.33
Fat (%)	9.63	9.80	10.67	3.69
Ash (%)	4.57	4.56	4.40	4.20
Crude fibre (%)	2.61	2.56	2.30	5.02
Carbohydrate (%)	61.24	60.62	57.13	70.94
Energy (kCal)	386.76	388.04	394.60	345.69
Total Flavonoid content (mgQuarcetin/g)	2.39	2.37	2.22	3.20
Total Phenolic Content (mgGAE/g)	24.76	24.64	23.89	24.90
DPPH (%)	67.88	66.85	61.00	69.15
FRAP (mg/g)	4.11	4.08	3.93	4.36
Total Antioxidant Capacity (mgGAE/g)	2.22	2.21	2.12	2.00
Desirability	0.546	0.545	0.533	0.306
	Selected			

4.Conclusion

This study utilized the composite of yellow maize, soybean, and mango peel flour to produce breakfast cereal flakes that are dense in protein, energy, and antioxidants. It was observed that an increase in the percentage inclusion of soybeans caused a significant increment in the percentage of protein, fat, and energy values. Further, the inclusion of mango peel increased the flake sample's carbohydrate and antioxidant properties. In contrast, yellow maize increased the flake samples' yellowness, lightness, and sensory acceptability. However, including the combination of soybean and mango peel with yellow maize flour increased the sensory acceptability of the flakes produced. The optimization result indicated that the mixture of 50% yellow maize, 28.45% soybean, and 21.45% mango peel gave the optimum protein, energy, and antioxidant properties.

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Funding

No external funding was received for this research.

Authors` Contributions

Oludolapo A. Osunrinade: Conceptualization**,** Writing – original draft, Writing– review & editing, Visualization, Software, Project administration, Methodology, Investigation, Data curation, formal analysis. **John O. Babalola:** Visualization, Validation, Manuscript write up-review and editing**.** **Oluwole A. Adeoti:** Visualization, Validation, Manuscript write up-review and editing. **Akeem O. Raji:** Visualization, Validation, Manuscript write up-review and editing.

Conflicts of Interest

The authors declare no known conflict of interest.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

CHEMICAL CHARACTERISTICS AND COMPOSITIONS OF PRICKLY PEAR SEEDS OILS EXTRACTED BY TWO DIFFERENT METHODS

Samia Motri[1,3]✉, Rabeb Lassoued[2,3],Amira Touil[1],Fethi Zagrouba[1]

[1] Environmental Science and Technology Research Laboratory Technopole BorjCedria, 1003 HammamLif, Tunisia

[2] Laboratory of Materials Molecules and Applications, IPEST, BP 51, 2070 La Marsa, Tunisia

[3]Higher Institute of Technological Studies of Zaghouan, , 1121 Zaghouan, Tunisia

✉*motrisamia@gmail.com*

https://doi.org/10.34302/crpjfst/2024.16.3.4

Article history:

Received:
January 14th, 2023

Accepted:
August 22nd, 2024

Keywords:
Extraction;
Prickly pear seeds;
Fatty acid;
Sterols;
Triglycerides.

ABSTRACT

The present work was undertaken to compare the physico-chemical characteristics, fatty acid and sterol compositions as well as the triglyceride composition of *Opuntia ficus indica* seed oils extracted using two different methods: cold pressing and Soxhlet extraction. The results showed that the prickly pear seeds (PPS) were (on a dry weight basis) : water 6.63%, ash 1.1%, oil 8.64%, and protein 9.18%. PPS were also a good source of K, and Mg. Solvent extraction had a significantly ($p < 0.05$) higher oil yield compared to cold pressing. The main fatty acids in PPS oils were linoleic (58.04% , 57.90 %) and oleic (26.29 % , 25.96 % in solvent-extracted and cold pressed oil, respectively. Fatty acid and sterol composition were not affected by the extraction method. The peroxide index and free acidity of the solvent-extracted oil was significantly higher ($p < 0.05$) than that of the pressed oil.

1.Introduction

The prickly pear (*Opuntia ficus-indica L.*) belongs to the Cactaceae family and has spread widely throughout the world. It was introduced into North Africa around the end of the 16 th century (Arba, 2009). About 1500 species of cacti belong to the genus Opuntia and are distributed mainly in South America, Australia, Africa, including the Mediterranean area (Ammar *et al.*, 2014). Prickly pear cactus (*Opuntia ficus-indica*) is extensively cultivated in Tunisia. Actually, about 100.000 ha have been devoted to prickly pear cultivation in Tunisia, spread over Sahel, Kasserine and Kairouan regions (Motri*et al.*, 2022). Currently, the prickly pear is of great interest not only for its ecological roles but also for its nutrtional, pharmaceutical and cosmetic potential (Barba *et al.*, 2017).The literature reports promising information concerning biology, chemical composition, cultivation and applications of the different parts of this plant (Moudene*t al.*, 2016; Paiva *et al.*, 2019; El Azizi *et al.*, 2019; Jouini*et al.*, 2021). The main studies on Opuntia fruits have been the chemical analysis of pulp, skin and seeds and use of the pulp in juice production (Albergamo*et al.*, 2022). The seeds make up about 10-15% of edible pulp and are usually discarded as waste after pulp extraction. Prickly pear seeds showed to contain polysaccharides, cellulose and hemicelluloses (Al-Naqeb*et al.*, 2021). The extracted oil constitutes 0.5-15.5 % of the total seed weight (Regalado et al., 2018). The composition of prickly pear seed oil and its chemical characteristics have been studied (Albergamo*et al.*, 2022;Al-Naqeb*et al.*, 2021). Cactus pear seed oil has been viewed as an important vegetable oil, it is rich in polyunsaturated fatty acids, linoleic acid was established as a major fatty acid in seed oils. Significant levels of carotenoids and γ-tocopherols, which can be used as antioxidants

(Loizzo et al., 2019) and sterols were also found in this oil.

Two different methods are commonly used for the production of oils : pressing and solvent (Soxhlet method). The Soxhlet method generally gives the highest oil yield but can have adverse effects on oil quality (Mechqoqa et al., 2021). The cold pressing procedure is desired because it does not use heat or chemical treatments to obtain natural edible petroleum products. However, Regalado-Rentería et al. (2018) reported that this approach has some disadvantages, such as low productivity and difficulty generating a consistent quality result. There is a scarcity of information on comparative studies of *Opuntia ficus-indica* oils extracted using both cold pressing and solvent extraction methods. The aim of this study was to examine the physicochemical properties of *Opuntia ficus-indica* seeds extracted using two different methods: cold pressing and the Soxhlet extraction system. The physicochemical characteristics assessed included oil yield, fatty acid composition, refractive index, density, peroxide value, free fatty acids, triglyceride composition, as well as sterols.

2.Methods

2.1.Plant material

The fruits of *Opuntia ficus indica*species were harvested at maturity in the region of Knais, (Sousse, Tunisia) at 35.68 m altitude. The fruits are washed under running water and air-dried in the dark and peeled by hand. The seeds are separated from the pulp using a manual cooking grinder then they are washed several times with distilled water and dried in an oven in the dark at 35°C.

2.2.Weight and size

The weight of the seeds was evaluated, randomly, on one hundred seeds. Ten differents samples were used for the size determination of PPS. With the Vernier, the length and diameter were measured and expressed in centimeters (Lassoued et al., 2021).

2.3.Proximate chemical analysis

The moisture content of prickly pear seeds was evaluated using AOAC (2000) techniques. The ash content was determined according to the AOAC(1990) method. Crude protein content was estimated by Kjeldahl (N × 6.25).

2.4.Mineral contents

Ca, K, Mg, and Na were quantified using atomic absorption spectrophotometry. Sample were incinerated and then dissolved with two milliliters of H_2O_2. Concentration standards of 0.5 – 25.0 μgmL^{-1} were used to determine the minerals quantitatively (AOAC, 1984).

2.5.Extraction techniques of prickly pear seed oils

The oil was extracted with two extraction methods:

- Solvent extraction : The seeds were ground in a grinder (Fritsch International, Germany). The oil was extracted in a Soxhlet with hexane at 68°C for 8 hours. A vacuum rotary evaporator was used to remove the solvent with little heating (40°C). The recovered oil was kept at a temperature of - 20°C.. The recovered oil was stored at -20°C.
- Extraction by cold pressing : a SMIR screw press was used. The recovered oil was decanted and then stored at -20°C.

2.6.Oil yield

The oil yield of each extraction method was calculated as follows :

$$Yield(\%) = \frac{Mass\ of\ oil\ extracted\ (g)}{Mass\ of\ PPS\ (g)} \times 100 (1)$$

2.7.Physicochemical parameters

An Abbe refractometer with a temperature adjustment was used to determine the refractive index of oil samples (model G manufactured by Carl-Zeiss, Germany). A densimeter PAAR DMA 45 was used to measure density at 20 °C (Chempro, Paar, Graz, Austria). Official procedures were used to establish the value of peroxide and to quantify free fatty acids (AOAC, 1997).

2.8.Fatty acid composition

The total fatty acid composition of the PPSoils, determined by conversion oil to fatty acid methyl esters (FAME), was achieved according to the NF T60-233, and NF T60-234 method. 30 mg of lipids were diluted in 3 ml of sodium methoxide (0.05 M) containing three or four drops of phenolphthalein. After a 10-minute reflux, 3 mL of methanol/acetyl chloride (1:1, v/v) were added to the mixture. The mixture was then cooled at room temperature after another 10 minutes of refluxing.10 ml of ultrapure water and 6 ml of hexane were added. The upper organic phase containing FAMEs was finally recovered. The methyl esters were then analyzed by gas chromatography (Agilent, HP 6890 series) with a flame ionization detector equipped with a capillary column packed with a stationary phase: Agilent CP-Sil 8850, 50 m × 0.25 mm internal diameter × 0.2 mm film thickness). Operating conditions were as follows: helium as the carrier gas was at a flow rate of 1.5 mL/min and injection volume was 1 mL.The injector temperature was kept at 250°C, while the oven temperature was kept at 165°C and gradually increased to 210°C at a rate of 2°C/min. By comparing the peaks area and retention times of the fatty acid methyl esters to those of the pure standard FAME, the peaks were classified and quantified. The percentage of total detected fatty acids (g/100 g total fatty acid) was used to calculate the results.

2.9.Triglyceride Composition

The triacylglycerol (TAG) profile was determined using reverse-phase high-performance liquid chromatography equipped with a refractive index detector. The separation of triglycerides was carried out using a hypersil ODS column (250 × 4 mm) with a particle size of 5 μm and were eluted from the column with an acetonitrile-acetone mixture (65/35, v/v) at a flow rate of 0.8 mL/min. The peaks were identified by comparison with standard TAG peak retention times and retention times observed in chromatographs of other vegetable oils such as rapeseed oil, soybean oil, and olive oil. The peak areas produced by the data integrator were used for quantification.

2.10.Sterols analysis

Separation of sterol was performed according to the NF ISO 12228 method. After adding 1 mg of FLUKA cholesterol as an internal standard and a few antibumping granules, 250 mg of PPS oil was refluxed for 15 minutes with 5 mL ethanolic KOH solution (3 %, w/v). 5 mL ethanol was added to the mixture right away. At a flow rate of 2 mL/min, the unsaponifiable component was eluted through a glass column packed with a slurry of 260 aluminum oxide (Scharlau) in ethanol (1:2, w/v) with 5 mL ethanol and 30 mL diethyl ether. The extract was evaporated at 40°C under reduced pressure in a rotary evaporator, and the ether was entirely evaporated under nitrogen steam. A FLUKA silica gel F254 plate was created in the solvent solution n-hexane–diethyl ether (1:1, v/v) for sterol characterisation. The thin-layer plate was sprayed with methanol to detect sterols; the sterol bands were scraped off the plate and recovered using diethyl ether extraction. After that, the extract was evaporated in a rotary evaporator and nitrogen was added. The trimethylsilyl ether sterols (TMS) derivatives were prepared by adding 100 μl of a silylant reagent N-methyl-N-(trimethylsilyl) rifluoroacetamide/pyridine (1:10, v/v) in a capped glass vial and heated to 105 °C for 15 min. Standard solution preparation: derivatization was used to create a combination of sterol standard solutions (cholesterol, sitosterol, stigmasterol, betulin, ergosterol, and campesterol). A Hewlett Packard 6890 (Agilent) gas chromatograph with a FID detector and a capillary column HP5 (5 percent phenylmethylsiloxane, 30 m 0.32 mm; internal diameter, film thickness 0.25 m) was used to analyze sterols by gas chromatography. Injector temperature was 320°C, column temperature was a 4°/min gradient from 240°C to 255°C (65 min), and the carrier gas was helium at a flow rate of 1 mL/min. The ISO 12228 reference method was used to identify the sterol peak, which was then validated by mass spectrometry using the NIST 2002 database.

2.11.Statistical analysis

The values of different parameters were expressed as the mean ± standard deviation ($\bar{x}$ ± SD) of three independent experiments. one-way analysis of variance (ANOVA) was used for statistical analysis where the method of extraction is the only factor taken into account. The comparison between the means was carried out with the Student–Newman–Keuls test and the differences between individual means and each single used mean were deemed to be significant at $p < 0.05$.

3.Results

3.1.Size and weight

Few studies have examined the size and morphological description of prickly pear seeds. Morphological characterization showed that *Opuntia ficus indica* seeds are very hard, flat in shape, more or less lenticular. The seed size was 0.52 cm for the length and 0.28 cm for the width (Table 1).

Table 1. Size and weight of PPS

Length (cm)	width (cm)	weigh of 100 seeds (g)
0.52 ± 0.05	0.28 ± 0.04	1.65 ± 0.08

Values are means ± Standard Deviations (SD) of three determinations.

These values are in agreement with those reported by Barabraet al. (1995). The mass of 100 seeds was 1.65 g. This result is higher than that obtained by Ennouriet al. (2005) (1.38 g), and Tliliet al. (2011) (0.89 g to 1.42 g). The variation in prickly pear seed mass and size certainly attributed to botanical origin; it is influenced by differences in growing conditions (agronomic and climatic).

3.2.Proximate chemical analysis

The chemical composition of prickly pear seeds is shown in Table 2. The moisture content was low (8.71%), which was beneficial in extending the shelf life of prickly pear seeds because higher moisture content may cause decomposition by micro organism. The oil yield value (8.64%) obtained for PPSwas lower than that of Nassar (2008) containing 10.43% oil. But they are close to those reported by Habibi (2004) and Tliliet al. (2011) who estimate this content at 9%, and 6.85% respectively. Crude protein, and total ash were 9.18%, and 1.1%, respectively, for prickly pear seeds on a dry weight basis. The protein content was higher than that found by Nebbache et al. (2009) while it is close to the result obtained by Tlili et al. (2011), (8.10%). The ash content of PPSharvested in Tunisia was consistent with those reported by Ennouriet al. (2005) and Habibi (2004) who estimate this content at: 1.1% and 1.3%.respectively. Differences in chemical composition of prickly pear seeds could be attributed to growing climates, ripening stages, extraction methods used and plant varieties (Ettalibi et al., 2021).

Table 2. Chemical composition (% dry matter) of PPS

Moisture	8.71±0.2
Ash	1.1±0.13
Protein	9.18±0.2
Crude oil	8.64±0.62

Values are means ± Standard Deviations (SD) of three determinations.

3.3.Mineral contents

The mineral content of prickly pear seeds is shown in Fig. 1. The most abundant mineral in prickly pear seeds was potassium (167 mg/100 g), while the calcium content was the lowest (18.2 mg/100 g). These results are in agreement with those of Mostafaet al. (2014) for the Moroccan PPS. However, The calcium and magnesium contents are lower than those found by Ozcan and Al Juhaimi (2011) for the turkishPPS. Prickly pear seeds are a good source of minerals, especially potassium, from a nutritional aspect. This mineral contributes to alkalinization and helps to maintain the electrolyte balance of body fluids.

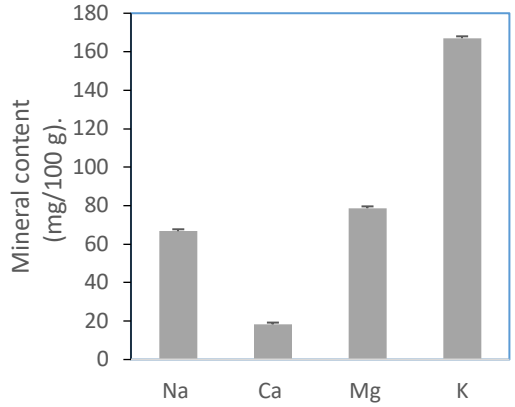

Figure 1. Mineral content of PPS (mg/kg).

3.4.Oil yield

The oil yields of PPSfrom cold pressing extraction are significantly lower ($p < 0.05$) than that obtained by solvent extraction (Fig. 2). The oil yield of *Opuntia ficus indica* seeds was 4.68 % and 8.64% for cold pressing extraction and solvent extraction, respectively. It was also found by Regalado-Rentería et al. (2018), Loizzo et al. (2019)and De Wit et al. (2021) that the oil yield of prickly pear seeds was affected by the extraction technique. Karabagiaset al. (2020) reported that the most important factors on the oil yield of PPS are the variety and the extraction process. Generally, the majority of scientific work is on solvent-extracted oils which give better oil yield (Al Naqeb et al., 2021).

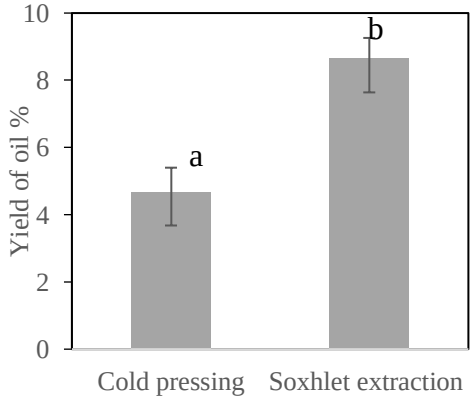

Figure 2. Oil yields obtained by different recovery techniques (Values with different letters are significantly different $p < 0.05$)

3.5.Physicochemical parameters

The different physico-chemical indices of PPSoil are presented in Table 3. The peroxide index is a useful measure for predicting the onset of oxidation since it quantifies the amount of hydroperoxide in the oil (Martin-Polvillo et al., 2004). The peroxide index of the solvent-extracted oil was significantly higher ($p<0.05$) than that of the pressed oil (Table 3), 5.13 meqO_2 kg^{-1} and 2.14 meq O_2 kg^{-1} for solvent-extracted oil and pressed oil, respectively. These peroxide index values are lower than the 10 meq O_2/kg oil values found in most conventional oils(Codex, 2009). Peroxide index values of chemically extracted prickly pear seed oils were above the recommended limit, De wit et al.(2017) reported that it ranged between 9.5 and 23.30 meq O_2 kg^{-1}. A peroxide index value of 12 meq O_2/kg has been reported for Algerian *Opuntia ficus-indica* cold-pressed seed oils (Koshak et al., 2020). Peroxide value is affected by oil extraction method, cultivars, growth conditions, soil geography, harvesting routines, as well as handling and storage, according to Koubaa et al. (2016).

Lipids are one of the most alterable compounds, the presence of water or air can respectively lead to hydrolysis and oxidation phenomena (Ollé, 2000). Free fatty acids is an important indicator of oil quality by measuring the level of free fatty acids in oils. Lipid hydrolysis is affected by chemical or enzymatic processes, resulting in the presence of free fatty acids (Sharma and Jain, 2015). As shown in Table 3, free fatty acids content of oil cold pressed (4.16%) was significantly ($p < 0.05$) lower than these obtained by soxhlet method (9.09 %). This is due to highly polar solvents induce triglycerides hydrolysis and saponification processes in vegetable oils, allowing for the generation of free fatty acids (Gharby et al., 2015). These results were compared to previous study obtained by De Wit et al. (2017) where free fatty acids ranged between 2.49 % and 5.08% in chemically extracted oil and less than 2 % in cold-pressed oil. The free fatty acid concentration of cold-pressed PPS oil was lower than that found by Brahmi et al. (2020) (21.2% for Algerian cold-pressed PPS oil) but higher than those found by Ozcan and Al Juhaimi (2011) (1.41% for Turkish cold-pressed PPS oil). A combination

of catalysts, such as enzymes, and residues generated after extraction may be responsible for the high levels of free fatty acids. Furthermore, free fatty acids could be present since the oil was not refined.

As indicated in Table 3, there was no significant difference in refraction index, extracts by solvent and by oil press ($p < 0.05$). According to the Table 3 refraction index was 1.4721 for solvent extracted oil and pressed oil. This result is similar to those reported by Gharbyet al.(2011) (1.464) and De Wit et al. (2017) (1.4658 and 1.4676), but it was lower than that reported by Ennouriet al. (2005) (1.4831).

The density of the PPS oil at 20°C was established as 0.901 and 0.907 (Table 3) for cold pressing extraction and solvent extraction, respectively. The density of both samples was generally determined within the range of published cactus seed oil density values(Ennouri, 2005; Zine et al., 2013)]. The results of the one-way ANOVA study revealed no significant differences between PPS oil extracted by pressing and PPS oil extracted by solvent.

Table 3. Comparison of physic-chemical properties of Soxhelt extracted and cold pressed PPS oils

Parameter	**Soxhlet extracted**	**Cold pressed**
Refractive Index	$1,4721^{a}\pm 0.01$	$1,4712^{a}\pm 0.0$
Density	$0,901^{a}\pm 0.03$	$0,907^{a}\pm 0.01$
Free acidity (%)	$9,09^{b}\pm 0.20$	$4,16^{a}\pm 0.05$
Peroxide value (meq O_2 kg^{-1})	$5,13^{b} \pm 0.2$	$2,14^{a} \pm 0.1$

Values with different letters in the same row are significantly different $p < 0.05$. Values are means ± Standard Deviations of three determinations.

3.6.Fatty acid composition

Fatty acid composition of PPS oils is shown in Table 4. Eight kinds of fatty acids were detected in PPS oils samples. Analysis of the fatty acid composition of the lipid fractions obtained with differents extraction methods (cold-pressing and soxhlet) did not reveal significant difference ($p < 0.05$) in the fatty acid profile. These results were similar to those previously reported for red pepper seed oils, which found no significant variations in fatty acid composition between cold pressing and soxhlet extracted seed oils (Chouaibi et al., 2019). By comparing the results of the two extraction methods, it can be seen that the PPS oil produced by cold pressing is also rich in unsaturated fatty acids (UFA) (C18:1 =25.96% and C18:2 = 57.90 %) than that extracted by the Soxhlet method (C18:1 = 26.29 and C18:1 = 58.04%). Polyunsaturated fatty acids (PUFAs) were the most prominent fatty acid with rate, of total fatty acids, greater than 58%. They are mainly represented by linoleic acid with a higher content than in sesame oil (42.10%) (Kurt, 2018) and a much higher content than in *Pistacia lentiscus* seeds oil (<24%) (Ait Mouhamed et al., 2020). This fatty acid may have nutritional and physiological benefits in the prevention of coronary heart disease and cancer (Oomah et al., 2000). Adulteration of cactus oil with other oils rich in linolenic acid, such as rapeseed oil and soybean oil, can be detected by measuring the modest amount of linolenic acid (0.4 and 0.65 percent) (Taoufik et al., 2015) PUFAs are followed by monounsaturated fatty acids (MUFA) making up more than 25% of total fatty acids. In addition of his nutritional value, the presence of a significant amount of oleic acid ensures the oil's stability and suitability for industrial use.Total saturated fatty acid (SFA) contents were 14.51% and 14,97% for PPS oil extracted by solvent and pressing, respectively. Palmitic acid is the most abundant SFA which offers the appropriate amount of plasticity for cosmetics applications. Furthermore, it should be noted that PPS oil is highly unsaturated with unsaturation rate UFA/SFA greater than 4.5%. The high unsaturated/saturated ratio may give PPS oil its nutritional, dietetic virtues and curative properties. On the other hand, high ratio signifies high potential for oxidation reactions of the oils with relation to the presence of double bonds. The richness of omega-6 of this oil makes it as an alternative source of this essential fatty acid. Results

revealed that fatty acid compositions of the studied PPS oils were consistent with the results obtained by Ozcan and Al Juhaimi (2011) where content of palmitic acid (12.23%), oleic acid (25.52%) and linoleic acid (61.01%). Nonetheless, other studies have reported lower levels of oleic acid (18.88%) and higher levels of linoleic acid (63.46%) (Ettalibi et al., 2021). Some investigations observed differences in linoleic acid content, which could be attributed to fruit genotype, growth environment, or maturation stage. PPS oil, based on our results, could be a valuable supply of essential fatty acids, which could be beneficial to the cosmeceutical and pharmaceutical industries.

Table 4. Fatty Acid Composition (g/100 g) of Soxhelt extracted and cold pressed PPS oils

	Soxhletextracted	**Cold pressed**
Palmitic C16 :0	11.30 ± 0.30 [a]	11.40 ± 0.10 [a]
PalmitoléicC16 :1	0.40 ± 0.20 [a]	0.55 ± 0.05 [a]
Stearic C18 :0	2.94 ± 0.30 [a]	3.30 ± 0.01 [a]
Oleic C18 :1n9	26.29 ± 1.05 [a]	25.96 ± 1.50 [a]
Linoleic C18 :2	58.04 ± 2.10 [a]	57.90 ± 2.20 [a]
LinolénicC18 :3n3	0.40 ± 0.05 [a]	0.65 ± 0.01 [a]
Arachidic C20 :1	0.13 ± 0.01 [a]	0.14 ± 0.01 [a]
Arachidic C20 :0	0.27 ± 0.02 [a]	0.27 ± 0.05 [a]
SFA	14.51 ± 0.62 [a]	14.97 ± 0.16 [a]
PUFA	58.44 ± 2.15 [a]	58.55 ± 2.21 [a]
MUFA	26.82 ± 1.26 [a]	26.65 ± 1.56 [a]
UFA/SFA	4.58	4.55

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids;
Values with different letters in the same row are significantly different $p < 0.05$.
Values are means ± Standard Deviations (SD) of three determinations.

3.7.Triglyceride Composition

Determining the type and amounts of TAG species present in oil can be used to understand their physical and functional properties. According to the Table 5, prickly pear seed oils contain 12 TAGs, the predominant component is LLL (22.79% – 21.99%), followed by OLL (21.36%–21.56%) for the oil extracted by solvent and cold press, respectively. PLL and PLLn triglycerides are found in trace amounts. This result is in agreement with that reported by Ettalibiet al. (2021).

Table 5. Triacylglycerol (TAG) composition ofSoxhelt extracted and cold pressed PPS oils

	Relative composition (%)			**Relative composition (%)**	
	Soxhlet extracted	**Cold pressed**		**Soxhlet extracted**	**Cold pressed**
LLLn	22.79^{b} ±0.02	21.99^{a} ±0.01	**OOO**	1.86^{a} ±0.00	2.32^{b} ±0.01
OLL	21.36^{a} ±0.02	21.56^{b} ±0.03	**PLLn**	0.29^{a} ±0.02	1.13^{b} ±0.04
POL	14.03^{a} ± 0.01	15.06^{b} ± 0.01	**POP**	2.65^{b} ±0.05	2.21^{a} ±0.02
LOO	13.22^{a} ±0.05	13.29^{a} ±0.03	**ALO**	2.41^{a}±0.00	2.99^{b}±0.01
POO	1.99^{a} ±0.02	1.95^{a} ±0.03	**SOO**	9.61^{b}±0.01	6.93^{a}±0.02
PLL	0.31^{a} ±0.01	0.30^{a} ±0.02	**OPS**	3.78^{b}±0.02	2.99^{a}±0.03

Values with different letters in the same row are significantly different $p < 0.05$.
Values are means ± Standard Deviations (SD) of three determinations.

In addition, the triglyceride profile obtained for cold press oil extraction in this study are close to those described by Zineet al. (2013). When the triglyceride content of oils extracted using two different procedures was compared, significant differences (p0.05) were found for LLLn, OLL, POL, OOO, PLLn, POP, ALO, SOO, and OPS. Therefore, the extraction method should affect triglyceride levels.

3.8.Sterols analysis

The sterol composition of PPS oil is presented in Table 6. Six kinds of sterols were identified in PPS oils and quantified by corresponding sterol standards. Sterolic fraction was composed by β-sitosterol: 71.56% and 70.26%, campesterol: 11.26% and 10.68%, Δ5-avenastérol: 8.68% and 9.45%, Δ7- avenastérol: 3.75% and 3.84%, stigmasterol: 3.44% and 3.71% and cholestérol: 1.12% and 1.05% for PPS oil extracted by pressing and solvent methode, respectively. These results are in good agreement with prior studie' finding (Taoufik et al., 2015). The one-way ANOVA analysis did not show a significant ($p < 0.05$) difference between PPS oil extracted by pressing and solvent methode. β-sitosterol was reported as the main sterol identified in seed oils with about 70% of total sterols. Furthermore, campesteroland Δ5-avenasterol were found in concentrations that exceeded 10% and 9% of the total sterol content, respectively. The presence of low levels of stigmasterol, cholesterol, and 7-avenasterol was also discovered.El Mannoubi*et al.* (2009) and Zine et al. (2013) were published similar results where β-sitosterol was the sterol marker with 72% and 75.3% respectively.The obtained results for β-sitosterol were lower than those reported for PPS oil (80.82% and ≥ 77%) from Morocco (Taoufik et al., 2015; Gharby et al., 2020). However, in our study, the cholesterol values were higher than those found in literature. The mature stage of seeds may be responsible for differences in sterol content. In addition to their antioxidant activity, sterols are interesting because they affect the human body by preventing cholesterol absorption from the intestine and lowering blood levels of the low density lipoprotein cholesterol component. Phytosterols have been proven to have a variety of nutritional impacts (Tapieroet al., 2003).

Table 6. Sterol composition (Weight Percentages) of Soxhelt extracted and cold pressed PPS oils

	Soxhlet extracted	**Cold pressed**
Cholesterol	1.12 ± 0.01[a]	1.05 ± 0.10 [a]
Campesterol	11.26 ± 0.05 [a]	10.68 ± 0.60 [a]
Stigmasterol	3.44 ± 0.02 [a]	3.71 ± 0.01 [a]
β-Sitosterol	71.56 ± 1.05 [a]	70.26 ± 2.00 [a]
Δ5-avenasterol	8.68 ± 0.50 [a]	9.45 ± 0.01 [a]
Δ7- avenasterol	3.75 ± 0.01 [a]	3.84 ± 0.01 [a]

Values with different letters in the same row are significantly different $p < 0.05$. Values are means ± SD of three determinations.

4.Conclusion

Comparative study of physicochemical, fatty acid, triglyceride and sterols compositions of PPS oil according to its extraction method had been examined. Refractive index and density did not change significantly with the used technique, while free acidity and peroxide value increased significantly when organic solvent extractionwas used. This study revealed that fatty acid and sterols composition were not affected by the extraction technique, however extraction with hexane greatly affect triglyceride levels.

Highlighting, the good chemical composition and the richness in PUFA of PPS oil permitted to establish its potential applications in nutritional or pharmaceutical industry. PPS oil is also a suitable ingredient in the fields of cosmetic and phytotherapy industry.Other biological activities should be evaluated, as well as the active components responsible for the oil's antioxidant activity.

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CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

STUDY ON THE AERODYNAMIC AND DIMENSIONAL PROPERTIES OF CORN USED TO OBTAIN BIOFUEL

Ioan Radu Sugar[1], Ferenc Gaspar[2]✉, Olivian Chiver[1]

[1]Technical University of Cluj-Napoca, Faculty of Engineering, Romania
[2]Technical University of Cluj-Napoca, Faculty of Automotive, Mechatronics and Mechanical Engineering, Romania
[3]Technical University of Cluj-Napoca, Faculty of Science, Romania
✉*ferenc.gaspar@auto.utcluj.ro*

https://doi.org/10.34302/crpjfst/2024.16.3.5

Article history:
Received: April 15th, 2024
Accepted: September15th, 2024

Keywords:
Maize seed;
Pioneer P991;
Seed properties;
Terminal velocity.

ABSTRACT

The study focuses on the determination and evaluation of aerodynamic and dimensional characteristics of Pioneer P9911 maize seeds, important for post-harvest operations. Measurements of 320 seeds from eight maize ears were used to calculate geometrical mean dimensions and aerodynamic features. At a moisture content of 14% the thousand seed weight was 360 g and hectolitre mass (HLM) of 79-84 kg/hl. Seeds average dimensions for length was 13.08 mm, width of 8.61 mm, and thickness of 5.18 mm. The results show 98.30% of seed dimensions fell within three standard deviations (STD) of the mean interval. The calculated terminal velocity of 14.53 m/s and experimental value of 16.5 m/s are above literature references. These findings offer valuable insights for configuring machinery in grain conveying, sorting, and cleaning processes.

1. Introduction

Concerns on a global level for saving fuels and finding alternatives for them, were triggered by the embargo on oil in 1973.

Thus, in the current geopolitical conditions regarding the war in Ukraine, the conflict in the Middle East, and the restrictions regarding the environment, automobile manufacturing companies are looking for new solutions. If in the last century the cars equipped with internal combustion engines prevailed over the electric ones thanks to the innovative ideas of Henry Ford in 1913, after a century the electric cars will take their revenge.

However, the researchers still have some problems to solve. From the point of view of pollution for the production of the batteries needed for electric cars, this reaches 80% of the pollution of a classic car equipped with a spark ignition engine. (Stan 2024)

Another unsolved problem is the recycling of these batteries, which, as they are not standardized and have elements that are not very friendly to the environment, require additional costs. At the level of current technology, hybrid cars seem to be the optimal solution. Biofuels have also been and will remain an alternative in countries such as Brazil, the USA or the EU. Although the cost price is sometimes higher than the price of classic fuels in periods of geopolitical crises and possible embargoes, their use can become an objective necessity. For the production of biofuels, pneumatic transport and raw material cleaning play an important role in obtaining good quality fuels.

Cereals have been vital for human sustenance since ancient times, with maize standing out as Europe's second most cultivated grain, crucial for the food industry.

The quest for high-quality maize begins with selecting appropriate varieties suited to specific cultivation regions and climates. Post-harvest processes, including cleaning, sorting,

transportation, drying, and storage, are pivotal for maintaining raw material quality. Machinery used in these primary processes relies heavily on the geometrical and aerodynamic characteristics of grains to ensure high-quality end products. Understanding cereal grain properties is crucial not only for post-harvest operations but also for analyzing processes like sowing (Findura 2018, Csizmazia 2008,) and sorting in harvesting combines (Gheres 2020).

Knowledge of the physical characteristics of the seeds is also required in post-harvest machinery design processes (Liu 2022, Panwar 2023).

The physical properties (Atere 2016, Brar 2017) of grain seeds, such as dimension, humidity content, weight, bulk density, shape, depends primarily on the type of variety (Beral 2020, Darfour 2022, Markowski 2013), climatic and geographical and conditions (Mandea 2018).

The geometric dimensions of the grain define their shape which is mathematically defined by the sphericity factor (Kaliniewicz 2015). The shape of the seeds is also important influencing the aerodynamical behaviour.

The aerodynamic properties of grains and seeds affected by moisture was analysed by Chavoshgoli 2014, Gierz 2018 developed a method for aerodynamic properties measurements of crop seeds. Kumar 2020 studied the characteristics of Indian wheat crop. Aerodynamic characteristic of different cereal seeds was also studied by Matouk 2005, Nalbandi 2010 (wheat and triticale), Shahbazi 2014, Shahbazi 2015a (lentil seeds), Shahbazi 2015b (mung bean seeds). These data are important in pneumatic-based post harvesting processes (Ghafori 2011).

The terminal velocity of seeds is drag coefficient dependent. Many theoretical and experimental studies were developed on drag coefficient of seeds (Bagheri 2016, Chavoshgoli 2014, Haider 1989, Poleak 2016, Tran-Cong 2004).

For corn grown in Romania, some of the physical characteristics are presented by Stefanescu 2003: the dimensions are (5.5 – 13.5 mm) for length, width of (5.0 – 11.5 mm), thickness between (2.5 – 8.0 mm), the thousand seed weight of 130 – 380 g, bulk density of 0.68-0.82 kg/dm^3, true density of 1.3 – 1.4 kg/dm^3 and, porosity of 35-55%, and a terminal velocity of 12.5 – 14 m/s.

2. Materials and methods

The analysed maize seed was the PIONEER P9911, a semi-divided maize hybrid belonging to the Optimum AQUA max group, with excellent yield capacity. The most used hybrid by processors for the brewing industry. It reacts very well to intensive technology. It is recommended for plain and hilly areas from the south, west and east of the country of Romania (*** Catalog 2018). The thousand seed weight is 330-360 g, respectively a hectolitre mass (HLM) of 79-84 kg/hl.

To perform the statistical calculations, N=320 seeds from the studied material were measured with a digital calliper having accuracy of 0.01 mm. Three series of values for length (L), width (W) and Thickness (Th) resulted, being delimited between minimum (L_{min}, W_{min}, Th_{min}) and maximum (L_{max}, W_{max}, Th_{max}) values. The values resulting from the measurements were grouped into 10 classes and statistically analysed based on the methodology presented by Sugar et al. 2021.

The study of aerodynamical behaviour of seeds is important in the cleaning, sorting and pneumatical transportation processes.

The mathematical relation used for theoretical calculation of terminal velocity (Mujumdar 2015) is shown below:

$$v_T = \sqrt[2]{\frac{2 \cdot m_s \cdot g \cdot (\rho_g - \rho_a)}{C_d \cdot A_s \cdot \rho_s \cdot \rho_a}} \text{ , (m/s)} \qquad (1)$$

Where $m_s=372 \cdot 10^{-6}$ kg represents the mass of a single maize seed, g=9,81 m/s^2, the density of seed ρ_s in kg/m^3 was determined with relation (2), ρ_a=1.2047 kg/m^3 is air density at 20 °C.

$$\rho_s = \frac{m_s}{V_s} \text{ ,(kg/m}^3\text{)} \qquad (2)$$

The surface area of the seed cross section (A_s) normal to the airflow, and the seed

theoretical volume (V_s) is had been calculated from equations (3) and (4).

Where the equivalent diameter of the maize (d_s) seed was calculated based on relation equation (5) considering the average values for length (M_L=13.8 · 10^{-3} m), width (M_W=8.61 · 10^{-3} m), respectively the thickness (M_{Th}=5.18 · 10^{-3} m).

$$A_s = \frac{\pi \cdot d_{gs}^2}{4}, (m^2) \qquad (3)$$

$$V_s = \frac{4}{3} \cdot \pi \cdot \left(\frac{d_s}{2}\right)^3, (m^3) \qquad (4)$$

$$d_s = \sqrt[3]{M_L \cdot \frac{(M_W - M_{Th})^2}{4}}, (m) \qquad (5)$$

The aerodynamic coefficient c_d can be calculated theoretically (Bagheri 2016). For this case the value of 0.5 was chosen, commonly used in specialized literature (Mujumdar 2015).

The experimental setup used for terminal velocity measurements is shown in Figure 1.

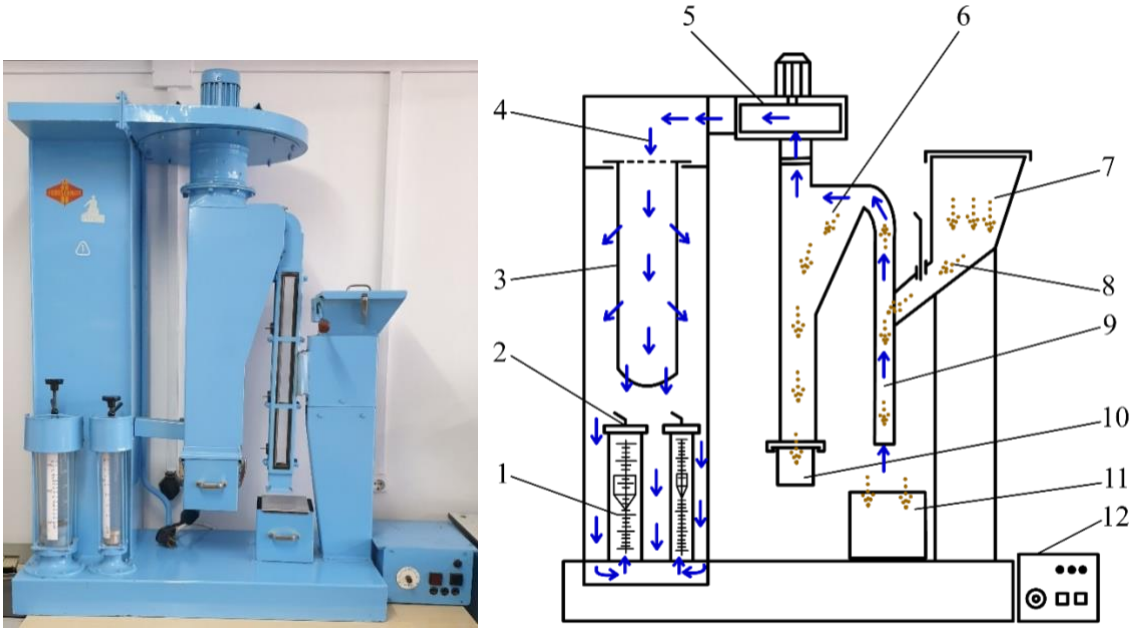


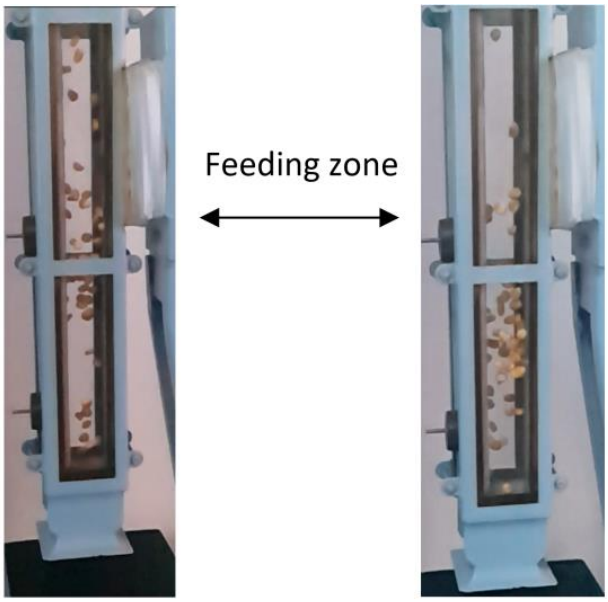
Figure 1. The experimental setup used for terminal velocity measurements:
1 – flowmeter, 2 – flow valve, 3 – bag filter, 4 – air flow, 5 – air pump, 6 – air – grain separator zone, 7 – feed hopper, 8 – grain flow, 9 – observation column, 10 and 11 – grain collectors, 12 – control box.

Maize seeds fed into feed hopper are dosed into the observation column. The air flow in the installation is provided by the centrifugal fan through the observation column, then passes through the separation zone, through the bag filter and then out through the flow meter calibrated to the air speed in the observation column. The air flow is adjusted at the flow valve.

Depending on the air velocity in the observation column, the maize seeds are lowered into seeds collector or are lifted into the separation zone. Determining the terminal velocity is possible by following the distribution of the grains in the observation column and correlating the position of the float device in the flow meter calibrated with the air speed in the observation column.

During the experimental measurements, it was found that the maize seeds start to float at the air speed of 17.5 m/s (Figure 2.b), at 19.1 m/s, a balance was observed for most of the seeds in the air tunnel (Figure 2.a).

At an air speed over 20 m/s in the observation tunnel, the effect of pneumatical transport took place.



a. b.

Figure 2. Maize seeds behaviour during the experiment.

3. Results and discussions

The extreme, and average values of dimensions, standard deviations (STD), theoretical, and experimental terminal velocity of Pioneer P9911 maize seeds are shown in Table 1.

The theoretical determined terminal velocity (for Cd=0.5) was 14.53 m/s and the empirically determined terminal velocity was 16.5 m/s with a deviation of 12%.

The average values of dimensions found for the studied Pioneer P9911 seeds correspond to the data given in the literature (Stefanescu 2003), but the maximum values are found above the limits of the range.

The average dimensions resulted were length of 13.8 mm, width 8.61 mm, respectively the average thickness found was 5.18 mm.

In the case of length size, the STD indicate a greater dispersion of values, while the values for thickness are more closely clustered around the mean. The one STD of the mean (M-σ, M+σ), two STD (M-2σ, M+2σ), and three STD of the mean (M-3σ, M+3σ) are ploted in table 2 corelated to the frequency occurrence for each dimension.

The STD of the mean show a goodcorrelation with the 68 – 95 - 99.7 rule in the case of width dimension.

Table 1. Statistical evaluation of measurements, and terminal velocity results

Dimension	D_{Max} (mm)	D_{Min} (mm)	$D_{Average}$ (mm)	STD	Terminal velocity (m/s)	
					Calculated	Experimental
Length	15.94	7.84	13.08	1.23	14.53	16.5
Width	11.02	5.56	8.61	0.90		
Thickness	8.47	3.80	5.18	0.85		

Table 2. STD of the mean and frequency occurrence of geometrical values

Dimension	(M-σ, M+σ)	Frequency (%)	(M-2σ, M+2σ)	Frequency (%)	(M-3σ, M+3σ)	Frequency (%)
Length	(13.07, 14.29)	53.77	(10.64, 15.51)	93.44	(9.42, 16.72)	98.36
Width	(7.71, 9.51)	69.51	(6.81, 10.41)	95.41	(5.91, 11.31)	99.02
Thickness	(4.33, 6.03)	79.67	(3.48, 6.88)	94.09	(2.63, 7.73)	98.36

For length dimensions, the one STD of the mean shows unexpected frequency, lower occurrence and in the case of the three STD of the mean the values are close to the 99.7%rule.

The frequency occurrence for geometrical dimension values plotted against the size range for ten dimension classes are shown in figure 3 (for length), figure 4 (for witdh) and figure 5 (for thickness).

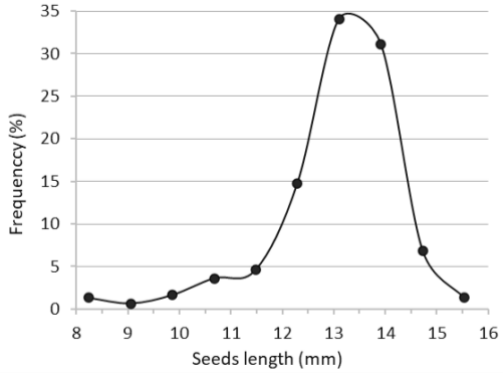


Figure 3. The distribution of lenght values across dimension classes within the size range.

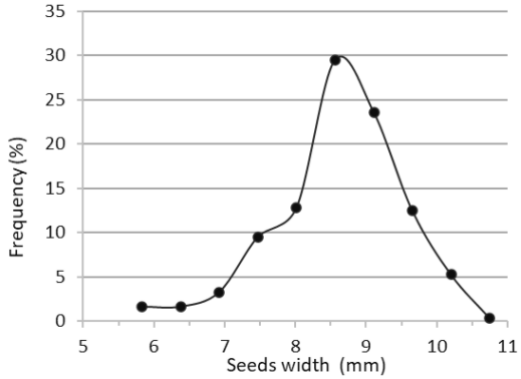


Figure 4. The distribution of width values across dimension classes within the size range

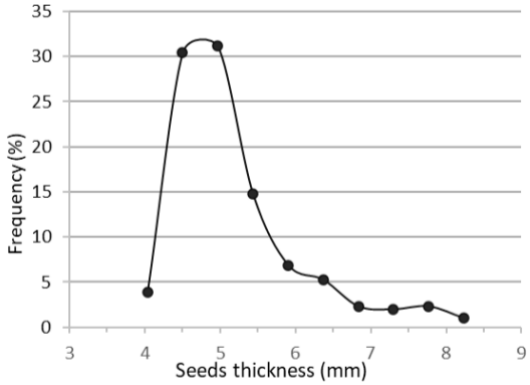


Figure 5. The distribution of thickness values across dimension classes within the size range.

In case of length values the highest occurrence of 34% is in class 7 with interval limits of (12.7, 13.51), for width dimensions the highest occurrence of 29.5% is in class 6with interval limits of (8.29, 8.84), and thickness values highest occurrence of 31% are in calss 3 having interval size limits of (4.73, 5.20).

4. Conclusions

In this study, the aerodynamic and dimensional properties of Pioneer P9911 maize seed were analysed as in pneumatic conveying, sorting, and cleaning processes the terminal velocity of seeds is an essential parameter. Average dimensions and STD values are also important in mechanical cleaning and sorting of grains in the case of choosing the size of the sieve openings.

The average value for length size was 13.08 mm with a STD of 1.23, for width the average value was 8.61 mm having a STD of 0.9, and the average thickness of 5.18 mm with a STD of 0.85 was found.

The empirical value of terminal velocity (16.5 m/s) for the studied maize seeds are closely related to the theoretically calculated (14.53 m/s), having a deviation of 12%. The Pioneer P9911 maize seeds terminal velocity is above of the given interval of (12.5, 14 m/s) found in the literature.

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*** Catalog Pioneer 2018

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

EFFECT OF MODIFIED STARCH/ NON-STARCH THICKENER COMBINATION ON CONSISTENCY, STABILITY AND RHEOLOGICAL PROPERTIES OF TOMATO KETCHUP

Mina Dzhivoderova-Zarcheva[1]✉, Kremena Nikovska[2] and Eva Dimitrova[3]

[1]Department of Technology of Tobacco, Sugar and Plant Essential Oils, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria
[2]Department of Nutrition and Tourism, Economic Faculty, University of Food Technologies, Plovdiv, Bulgaria
[3]Department of Mathematics, Physics and Informatics, University of Food Technologies, Plovdiv, Bulgaria
✉*mina_dj@abv.bg*

https://doi.org/10.34302/crpjfst/2024.16.3.6

Article history:
Received:
January 14th, 2023
Accepted:
August 22nd, 2024

Keywords:
Modified starches;
Hydrocolloids;
Tomato ketchup;
Rheological properties;
Consistency;
Syneresis.

ABSTRACT

Ketchup is one of the most popular tomato products on the world market and requires limited equipment and simple processing. Thickeners are used in the manufacturing process due to their ability to act on the viscosity, affect the consistency and prevent the ketchup from delaminating.

The effect of two modified starches in combination with a non-starch thickener (guar gum, xanthan gum and carrageenan) was investigated on the consistency, stability and rheological properties of tomato ketchup. A two-way ANOVA was performed to evaluate the effects of starch and non-starch thickener on structural mechanical properties and Bostwick consistency of ketchup.

All samples appeared to be non-Newtonian fluids and their viscosity and variation were close. Ketchup samples showed the highest shear stress values with 0.2% carrageenan with 3.4% modified potato starch, while the lowest were shown for samples with 0.1% guar gum. The highest consistency values determined by the Bostwick method of ketchups were reported for the combination of 3.4% modified potato starch and 0.1% guar gum, and the lowest for 3.8% modified waxy corn starch and 0 .2% carrageenan. During the analysis of the obtained samples, the serum-separated liquid was detected in ketchup with only modified potato starch, in combination with guar gum, in an amount of 0.1%. Based on these results, the combination of modified waxy corn starch and 0.2% carrageenan was the most suitable to be used for the production of tomato ketchup, with the aim of creating a more sustainable product.

1. Introduction

The production of tomatoes (Lycopersicon esculentum) is one of the first in the world among vegetables. Tomatoes can be eaten raw, but due to their perishable nature, they are processed (Quinet *et al.*, 2019). Much of the world's tomato crop is processed into tomato paste, which is subsequently used as an ingredient in many food products, mainly soups, sauces and ketchup (Cammarano *et al.*, 2022; Roccotiello *et al.*, 2022). Tomato ketchup is an easy-to-use and low-calorie product made from concentrated tomato paste with spices, salt, sugar and vinegar with or without starch, onion and garlic and contains no less than 12% tomato solids (Alqahtani, 2020; Mohamed *et al.*, 2020). Tomatoes have been used to modify the taste and/or aroma of certain foods and culinary preparations, with consistency and colour widely appreciated by consumers (Ahouagi *et*

al., 2020). Although tomato ketchup is the most commonly used snack condiment in homes and restaurants, its nutritional value and biofunctional properties are limited to the nutrients and bioactive compounds present in tomatoes and their stability during and after processing (Prakash *et al.,* 2016; Ahouagi *et al.,* 2021; Szabo, 2022). Tomatoes, as the main ingredient in ketchup, are recognized as a source of carotenoids (lycopene), a very important class of bioactive compounds, particularly known for their anti-inflammatory properties and supporting prostate health (Salehi *et al.,* 2019; Przybylska, 2020; Coelho *et al.,* 2023). There are many types of ketchup in the market, such as baby ketchup, fine, spicy, ketchup with different types of flavours, etc. These ketchup differ mainly in the content of the main ingredient, i.e. the tomatoes and spices used, as well as stabilizers (modified starch, pectin), which are often widely used (Fritsch *et al.,* 2017; Himashree *et al.,* 2022). Important characteristics of this type of product are its stability, consistency and rheological properties. Ketchup is a thin liquid with a yield point. It also exhibits thixotropic and viscoelastic properties (Torbica *et al.,* 2016; Li *et al.,* 2017; Shokraneh *et al.,* 2023).

The rheological properties of ketchup are mainly influenced by the rheological characteristics of tomato concentrate (Anamaria and Giani, 2019; Stanciu *et al.,* 2020; Gao *et al.,* 2021). The volume fraction of solids is the most important parameter affecting the rheological properties of tomato concentrate and ketchup (Wang *et al.,* 2018; Gao *et al.,* 2021). The viscosity is one of the main quality aspects that must be considered to determine the overall quality and consumer acceptability of many tomato products. The degree of ripeness, processing temperature, solids content, particle size and number of particle interactions play a role in determining the viscosity of tomato products (Shatta *et al.,* 2017; Jayathunge *et al.,* 2018). The consistency is related to non-Newtonian or semi-solid liquids (sauces, purees and pastes) with suspended particles and long-chain soluble molecules and is practically measured by product distribution or flow (Kumbar *et al.,* 2019; Pirsa and Hafezi, 2023). The consistency and rheological properties of ketchup depend not only on the amount of tomato paste used and its rheological characteristics but also on the type and amount of thickeners added (Torbica *et al.,* 2016; Diantom *et al.,* 2017; Thanh-Blicharz and Lewandowicz, 2020; Himashree *et al.,* 2022). Starch is a functional and commonly used food component. However, in its natural form, it shows low rheological stability and low resistance to mechanical, thermal and chemical agents. Furthermore, it undergoes retrogradation and syneresis phenomena, which limit the use of natural starch in many food products. To improve certain physicochemical properties of natural starch, it can be modified by chemical, physical and/or enzymatic methods or combinations thereof. The resulting starch preparations exhibit various functional properties and are used as gelling, thickening, stabilizing and fillers in food production (Zia-ud-Din *et al.,* 2017; Liu and Xu, 2019; Cui *et al.,* 2022; Obadi *et al.,* 2023). Much research has been done to obtain the properties required for a particular application using mixtures of starch and hydrocolloids. Previous research has reported that mixtures of starch and hydrocolloids have been used as thickeners and/or stabilizers to control water mobility, facilitate processing, and improve stability in food systems (Li and Nie, 2016; Mahmood *et al.* 2017; Cai *et al.,* 2020). The most commonly used thickeners are polysaccharide hydrocolloids such as: guar gum, xanthan, tragacanth, pectins and sodium alginate (Li and Nie, 2016; Pirsa and Hafezi, 2023).

This work aimed to evaluate the effect of two types of commercial modified starches of different origins in combination with different types of hydrocolloids on the consistency, stability and rheological properties of ketchup. Rheological properties of tomato ketchup such as viscosity and flow curves were analyzed for 24 tomato ketchup combinations.

2. Materials and methods

2.1. Materials

The following modified starches and hydrocolloids were used: Acetylated distarch adipate from waxy maize starch (Resistamyl 341), Tate & Lyle (R); Chemically modified potato starch (cross-linked esterified distarch adipate) (Adamil 2075 starch), Global Ingredient MSK (A), Gum guar (GG), Biovegan, German, Gum Xanthan (GX), ZoyaBG, Chaina, Carrageenan Iota-type pure semi-refined carrageenan whose composition has natural high polymeric hydrocolloid extracted from red algae (Eucheuma Denticulatum) (TS-200), Tacara SDN BHD, Malaysia (TS) and the other materials were from the local market.

2.2. Methods

2.2.1. Preparation of ketchup

Ketchup was made by mixing all the ingredients: sugar, starch, nonstarch thickener, salt, citric acid, tomato concentrate (36°Brix) and water. The mixture was homogenized and then heated at 75°C for about 7 minutes. Samples (25-26°Brix; pH 3.4-3.8) were allowed to cool and stood for at least 24 hours before analyses. There were made 24 samples with two types of modified starch (R and A) in two concentrations (3.4% and 3.8%) in combination with nonstarch thickeners gum guar (GG), gum xanthan (GX) and carrageenan (TS) in two levels (0.1 and 0.2 %), shown in Table 1.

Table 1. Quantity and type of starch/hydrocolloid combinations used as thickeners in the ketchup composition

Starch/non-starch thickener, %	GG(0.1)	GG(0.2)	GX(0.1)	GX(0.2)	TS(0.1)	TS(0.2)
A(3.4)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
A(3.8)	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
R(3.4)	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18
R(3.8)	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24

2.2.2. Rheological measurements

All measurements were carried out at a constant temperature of 25 °C. Experimental results were modelled using a power law, also known as the Ostwald-de Waele model. Rheological characteristics were determined using a Rheotest-2 rotational viscometer (RHEOTEST Medingen GmbH, Medingen, Germany), within the shear rate range from 0.33 to 145.8 s^{-1}.

The dynamic viscosity (η, Pa.s) was calculated (Rao, 2014) using the formula:

$$\eta=\tau/D \quad (1)$$

Where τ is the shear stress, Pa; D is the shear rate, s^{-1}.

The most used model for complex fluids is the famous power law dedicated principally to the Ostwald-de Waele model (Stanciu *et al.*, 2020), which is simply expressed as follows:

$$\tau=k.D^n \quad (2)$$

Where K is the flow consistency index, Pa.s; n – is the flow behaviour index, D is the shear rate or the velocity gradient perpendicular to the plane of shear, s^{-1} and τ is the shear stress (Pa)

Determination of Bostvik consistency

Flow lengths (after 30 s) of the samples (at 20°C) were measured using a standard Bostwick consistometer (Operating instruction, Bostwick consistometer, Labomat). The results were

obtained as the average values of three parallel measurements (McCarthy and McCarthy, 2009).

2.2.3. Determination of syneresis resistance

Following centrifugation (Hettich Zentrifugen EBA 200) for 15 min at 3000 min^{-1}, the separated liquid was quantified by weight and expressed as a percentage of the sample.

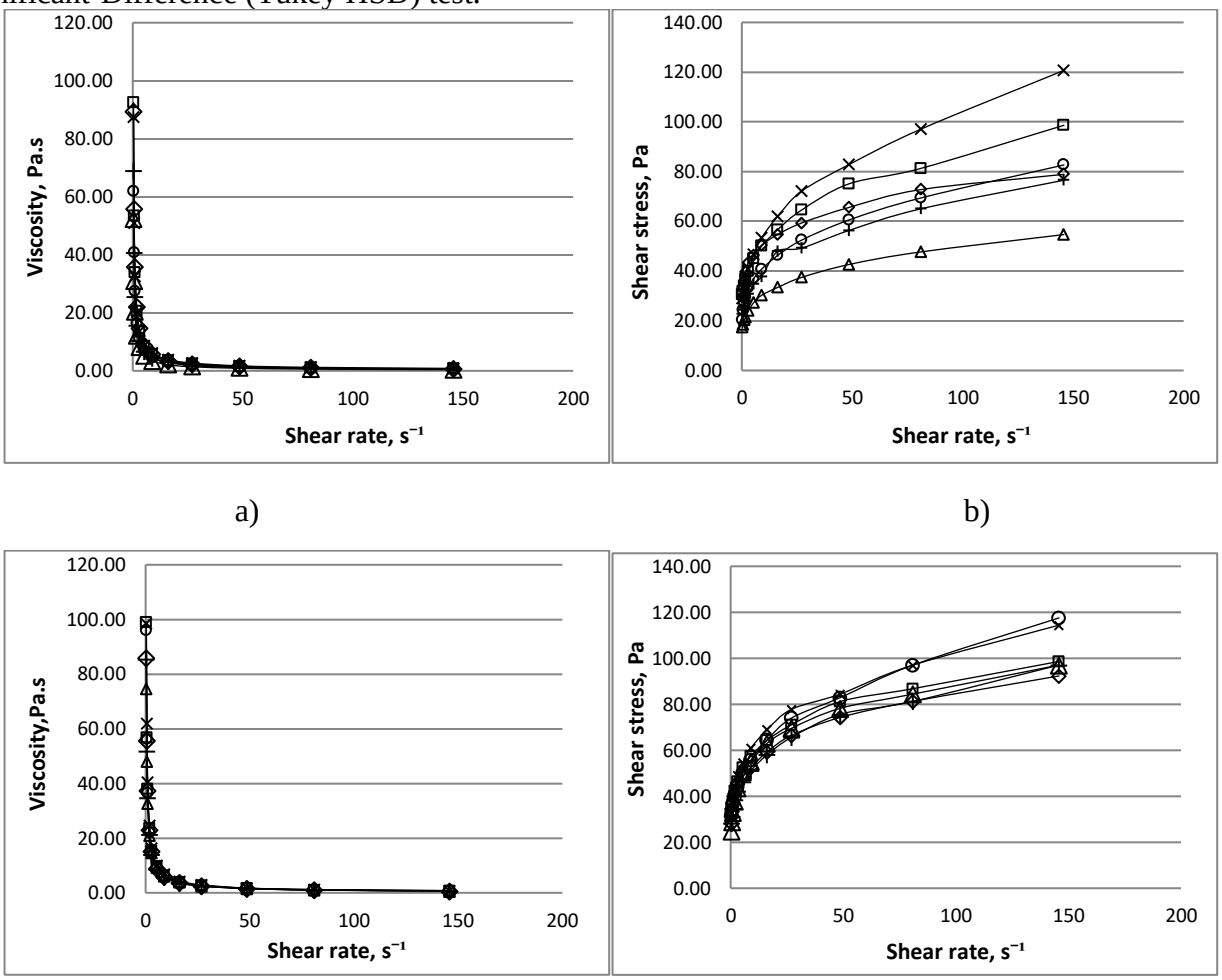
2.2.4. Statistical analysis

A two-way ANOVA was conducted to examine the effects on the ketchup properties of different starch/hydrocolloid combinations used as thickeners. The data are presented as means±standard deviation. The data were submitted for analysis of variance partitioned into components attributable to different sources of variation (starch, hydrocolloid, interaction starch-hydrocolloid). The chosen level of significance was α=0.05. The post-hoc analysis was performed using Tukey's Honestly-Significant-Difference (Tukey HSD) test.

3. Results and discussions

3.1. Rheological properties

The most important factor determining the structural-mechanical properties and quality of tomato ketchup is its viscosity (Kumbár *et al.*, 2019). Flow characteristics are an important parameter for all food products and this is information that is relevant to the economic design of the equipment used and the food processing operations to be selected (Ahmed *et al.*, 2017). Viscosity as a function of the velocity gradient "D" is presented in Fig. 1 (a) and c) for the samples with 3.4% and 3.8% modified starch R, e) and g) for the samples with 3.4% and 3.8% modified starch A) and different types of hydrocolloids in the amount of 0.1% and 0.2%.



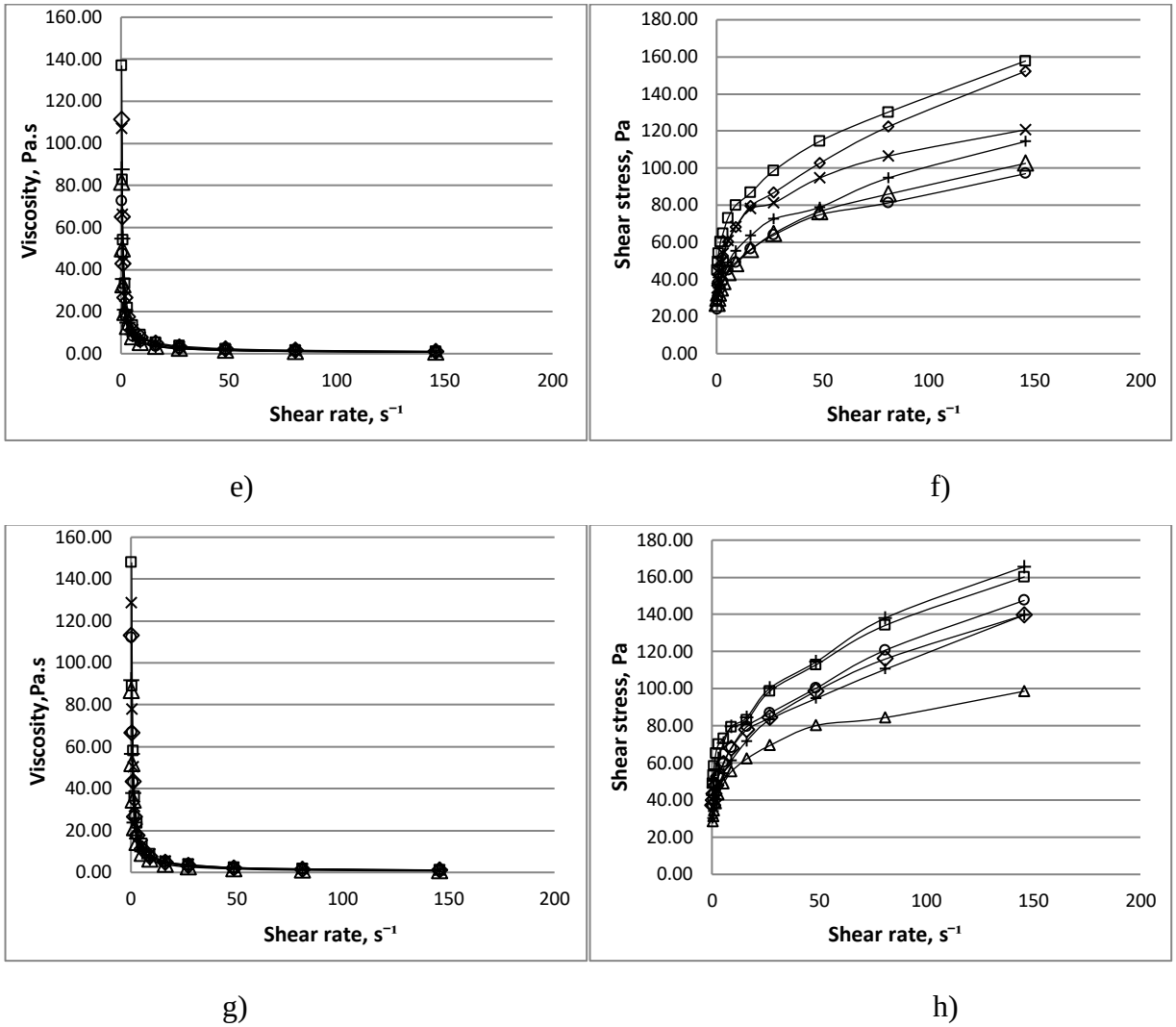


Figure 1. Viscosity and rheograms of ketchup with R 3.4% (a) and b)), R 3.8% (c) and d)) or A 3.4% (e) and f)), A 3.8% (g) and h)) and TS 0.1% (○), TS 0.2% (□), GG 0.1% (Δ),GG 0.2% (◊), GX 0.1% (+), GX 0.2% (X).

After obtaining the results, it was obvious that all the samples were non-Newtonian fluids. The shape of these curves indicates a shear-thinning non-Newtonian flow with a tendency toward yield stress. Non-Newtonian flow behavior of ketchup has also been observed by many authors (Berta *et al.*, 2016, Kumbár *et al.*, 2019). The highest shear stress values are shown for the ketchup sample with A(3.8%)+TS(0.2%), while the lowest is shown for the sample with A(3.4%)+GG(0.1%). These results correlate with consistency values, showing that ketchup thickened with A(3.4%)+GG(0.1%) showed the longest flow length (was the thinnest), while that with A(3.8%)+TS(0.2%) shows one of the shortest jet lengths (it was the thickest). The shear thinning behavior, i.e. a decrease in viscosity with increasing shear rate is a common phenomenon. In the case of ketchup, which is a product with a suspension structure, the shear thinning phenomenon results from the orientation of the tomato paste solids along the flow lines. The other factor affecting the viscosity of ketchup is the presence of swollen and partially gelatinized starch granules or their fragments. At higher shear rates, the individual starch granules may deform.

For samples containing modified waxy maize starch, the highest shear stress values

were reported for samples with R(3.8%)+TS(0.2%), while the lowest were reported for samples with R(3.4%)+ GG(0.1%). The results have a similar dependence to those with modified potato starch.

Fig. 1 also presents the rheograms (b) and d) for the samples with 3.4% and 3.8% modified starch R, f) and h) for the samples with 3.4% and 3.8% modified starch A) are presented in Fig. 1. All samples show similar rheological behavior. From Fig. 1, the graphical correlation shows that in rheological terms the analyzed samples are non-ideal plastic bodies. The rheological behavior of the emulsions is typical of the Ostwald-de Waele models, which is evident from the coefficients of determination (R^2) obtained, ranging from 0.9651±0.0567 to 0.9994±0.0450. This model is widely used in the analysis of various food systems.

The consistency factor (k) and flow behavior index (n) obtained by fitting the power law and Ostwald–de Waele models to the experimental data for shear stress and shear rate as a function of temperature are shown in Table 2.

Table 2. Parameters of Ostwald–de Waele models for flow curves of the ketchup with modified starch/nonstarch thickener.

Sample	**K**	**n**	**R^2**	**K**	**N**	**R^2**
	Upward curve			**Downward curve**		
Sample 1	20.08±1.52	0.193±0.02	0.9932±0.0356	17.03±0.85a	0.230±0.12	0.9994±0.0450
Sample 2	34.14±2.34a	0.196±0.12	0.9809±0.0484	30.23±1.23bc	0.234±0.04	0.9975±0.0352
Sample 3	25.85±1.42b	0.204±0.03	0.9823±0.0235	23.04±2.35d	0.234±0.03	0.9947±0.0052
Sample 4	33.11±1.65ac	0.239±0.05	0.9827±0.1361	31.10±1.54bc	0.272±0.11	0.9989±0.0126
Sample 5	26.22±1.12b	0.218±0.03	0.9943±0.0089	19.47±3.25ae	0.275±0.06	0.9923±0.0225
Sample 6	35.71±2.03ad	0.158±0.11	0.9984±0.1230	22.54±1.25de	0.249±0.04	0.9991±0.0231
Sample 7	33.03±3.25ac	0.223±0.02	0.9953±0.0232	30.25±2.56bc	0.228±0.01	0.9969±0.0064
Sample 8	38.39±2.42dc	0.187±0.08	0.9973±0.0335	32.85±3.12b	0.217±0.05	0.9978±0.0356
Sample 9	34.15±2.36a	0.200±0.02	0.9958±0.0125	28.37±1.24cf	0.250±0.10	0.9984±0.0036
Sample 10	40.09±3.55e	0.200±0.05	0.9962±0.1136	33.97±2.13bg	0.242±0.02	0.9968±0.0187
Sample 11	36.14±2.32ad	0.185±0.10	0.9961±0.0356	26.28±1.25f	0.247±0.03	0.9983±0.0256
Sample 12	36.60±1.54ad	0.216±0.03	0.9851±0.0635	31.32±2.01bc	0.261±0.01	0.9851±0.0458
Sample 13	35.009±2.56ad	0.222±0.02	0.9903±0.0089	27.953±4.56cf	0.267±0.02	0.9929±0.0154
Sample 14	43.745±1.23f	0.200±0.02	0.9983±0.0023	36.381±3.24gh	0.223±0.06	0.9934±0.0025
Sample 15	30.847±2.41	0.224±0.01	0.9980±0.0129	26.060±2.63df	0.257±0.01	0.9975±0.0032
Sample 16	42.806±3.25f	0.231±0.03	0.9841±0.0069	37.009±2.75gh	0.259±0.02	0.9849±0.0254
Sample 17	31.943±1.26c	0.219±0.06	0.9868±0.0682	27.557±2.45cf	0.261±0.04	0.9989±0.0356
Sample 18	53.522±6.45g	0.198±0.01	0.9876±0.0856	40.364±4.23ij	0.251±0.08	0.9877±0.0568

Sample 19	34.715±2.36ad	0.208±0.11	0.9978±0.0024	29.439±3.25c	0.233±0.02	0.9981±0.0364
Sample 20	43.372±1.25f	0.216±0.06	0.9867±0.0264	38.482±1.30ih	0.241±0.04	0.9892±0.0256
Sample 21	37.450±1.63de	0.245±0.03	0.9923±0.0036	33.041±2.34b	0.272±0.11	0.9926±0.0085
Sample 22	50.378±2.46g	0.219±0.08	0.9848±0.0256	42.204±1.32j	0.254±0.012	0.9881±0.0253
Sample 23	43.264±2.39f	0.225±0.06	0.9846±0.0368	36.876±2.65gh	0.256±0.05	0.9873±0.0785
Sample 24	56.762±2.68	0.182±0.01	0.9651±0.0567	41.977±3.45j	0.245±0.07	0.9881±0.0365

***** *In a column means followed by the same lowercase letters do not differ significantly by the two-way ANOVA and Tukey HSD test p<0.005.*

A two-way ANOVA was performed to evaluate the effects of starch and non-starch thickeners on k and n. For k of the upward curve, there was a significant main effect for the starch ($p < 0.001$); no significant main effect for the non-starch thickener ($p > 0.05$) and a significant interaction between starch and non-starch thickeners ($p < 0.001$). For the downward curve k the results were the same: a significant main effect for the starch ($p < 0.001$); no significant main effect for the non-starch thickener ($p > 0.05$) and a significant interaction between starch and non-starch thickeners ($p < 0.001$). For the flow behavior index n factors did not have any significant influences, neither together nor separately. The results from the two-way ANOVA and the Tukey HSD post hoc test are presented in Table 2. Means in a column followed by the same lower-case letters do not differ significantly.

The consistency factor k from the Ostwald-de Waele model can also be used as a viscosity criterion. In terms of this coefficient, all samples thickened with modified potato starch have high viscosity, with the highest being Sample 12 with A(3.8%)+TS(0.2%), which is the most viscous. The lowest value of the coefficient k is the sample with the lowest viscosity Sample 1 with A(3.4%)+GG(0.1%). The results reported for the modified waxy cornstarch samples (Samples 13 to 24) show similar results and the highest k value (highest viscosity) is sample 22 (R(3.8%)+GX(0.2%)). The lowest values were reported for samples 15 and 17 (R(3.4%)+GX(0.1%)/TS(0.1%)). When comparing the values between the ascending and descending curves, it is evident that the samples thickened with A(3.4%)+TS(0.2%), A(3.8%)+TS(0.1%), R(3.4%)+GG(0.2%), R(3.4%) have the greatest changes in combination with TS(0.2%) and R(3.8%)+TS(0.1%). With the smallest deviations, it is evident that they are the samples with Adamil 3.4% with GX 0.1% and 0.2%. The power law equation was an adequate model to describe the flow behavior of the samples in this study.

The flow behavior index, n, informs the deviation of the Newtonian flow for which n = 1. This parameter for all samples was below 1 point, indicative of the pseudoplastic (shear-thinning) nature of tomato ketchup (Kumbár et al., 2019). The flow indices (n) of the modified potato starch samples were between 0.158±0.11 (Sample 6 with A(3.4%)+TS(0.2%)) and 0.275±0.06 (Sample 5 with A(3.4%)+TS(0.1%)). For the samples with modified starch from the wax maze, the lowest value of the coefficient is for sample 21 (R(3.4%)+GX(0.1%)), and the highest is Sample 24 (R(3.8%)+TS(0.2%)).

3.2. Bostvik consistency

The Bostwick consistometer is commonly used in ketchup quality control, measuring the flow length (in centimeters) of a product sample

in 30 s. The means and standard deviations for Bostwick consistency of the ketchup are presented in Table 3.

Table 3. Bostwick consistency of the ketchup

Ketchup with starch and non-starch thickener, %	GG (0.1)	GG (0.2)	GX (0.1)	GX (0.2)	TS (0.1)	TS (0.2)
A (3.4)	7.27±0.25	5.50±0.00ab*	5.37±0.06bc	4.57±0.15de	6.00±0.20	4.30±0.00f
A (3.8)	6.57±0.06	4.87±0.06g	4.53±0.06de	4.03±0.06h	4.67±0.15d	4.03±0.06h
R (3.4)	5.67±0.12ai	4.40±0.10ef	5.27±0.06cj	5.17±0.06j	5.80±0.26i	3.43±0.06
R (3.8)	5.60±0.10a	3.93±0.06h	4.90±0.10g	2.87±0.06	4.53±0.06de	2.37±0.06

**Means followed by the same lower case letters do not differ significantly by the two way ANOVA and Tukey HSD test ($p<0.05$).A two-factor analysis was performed on all values of starch and thickener to determine the factor influence.*

A two-way ANOVA was performed to evaluate the effects of starch and non-starch thickeners on the ketchup Bostwick consistency. The results indicated a significant main effect for the starch, ($p < 0.001$); a significant main effect for the non-starch thickener ($p < 0.001$) and a significant interaction between starch and non-starch thickener ($p < 0.001$). In Table 3. the means followed by the same lower-case letters do not differ significantly according to the Tukey HSD test.

Since the ketchup model systems contain the same amount of starch, the observed differences (Table 3) may be due to the different botanical origin and/or modification pattern of the starch preparations. In general, corn starch showed lower Bostwick values than potato starch, which is also indicative of the higher viscosity. The same dependence was observed in the analysis of the obtained ketchup samples. The samples with cross-linked esterified distarch adipate from potato starch showed a thinner consistency than the acetylated distarch adipate from waxy maize starch. With the highest value is the sample with A(3.4%)+GG(0.1%) (7.27±0.25 cm) , and with the lowest value R(3.8%)+TS(0.2%) - 2.37±0.06 cm. It is observed that with an increase in the amount of starch, as well as rubber, the viscosity also increases, correspondingly, the Bostwick consistency values decrease. The lowest values were recorded for the combinations with 0.2% TS.

3.3. Syneresis resistance

Potato starches contain more amylose than maize starches, while amylose is absent in waxy maize (Li and Nie, 2016). Retrogradation involves the formation of a gel-like texture by linking amylose chains and forming a double helix and by linking amylopectin chains into double helices. The retrogradation of amylose chains occurs at a much faster rate than that of amylopectin. This was also observed in the analysis of the ketchup samples obtained with different types of modified starch in combination with hydrocolloids. Despite the evidence presented that guar gum and xanthan gum and their mixtures were most successful in reducing the serum release of tomato ketchup (31), in the analysis of the samples obtained, the serum-released liquid was found in ketchup with only modified potato starch (Adamyl), in combination with guar gum, in an amount of 0.1%. After centrifugation of the samples, separation of separation liquid was detected in samples 1 and 7. In sample 1, the liquid was separated in the amount of 17.999±1.023% (on the 5th day); 20.333±1.113% (on the 10th day) and 26.300±0.897% (on the 20th day), and sample 7 -12.881±1.056% (on the 5th day), 13.767±0.876% (on the 10th day) and

14.088±2.345% (on the 20th day). As a comparison, when increasing the amount of GG to 0.2%, no syneresis fluid is released.

4. Conclusions

Based on the conducted analyses, it can be concluded that when comparing the properties of the used modified starches, tomato ketchup with modified waxy corn starch is more stable. In combination with increasing the amount of non-starch thickeners, this stability is enhanced. The best results in terms of rheological properties as well as consistency are observed with a combination of 3.4% modified starch (Resistamil) + 0.2% carrageenan. The international requirements for the Bostwick index are between 7.5 and 10 cm so the concentration of the thickeners should be reduced. This will help to obtain a more resistant product to mechanical impact and storage.

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CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

THE POTENTIAL OF COFFEE (*COFFEA ARABICA* L.) PULP AND CACAO (*THEOBROMA CACAO* L.) HUSK AS A SOURCE OF PREBIOTICS, ANTIOXIDANTS, AND ANTIMICROBIAL COMPOUNDS

Pacheco-Martínez,[1] C.K., Saucedo-Castañeda, G.,[1] Rodríguez-Durán, L.V., [2] Trejo-Aguilar, G.M., [1] Pérez-Chabela, M.L. [1]✉

[1]Biotechnology Department. Universidad Autónoma Metropolitana Iztapalapa. Av. San Rafael Atlixco 186. Col Vicentina. Alcaldía Iztapalapa. 09340 CDMX.
[2]Universidad Autónoma de Tamaulipas Unidad Académica Multidisciplinaria Mante Blvd. Enrique Cárdenas González No. 1201 Pte. Col. Jardín C.P. 89840
✉lpch@xanum.uam.mx

https://doi.org/10.34302/crpjfst/2024.16.3.7

Article history:
Received:
June 19th, 2023
Accepted:
September 23rd, 2024

Keywords:
Cacao husk;
Coffee pulp;
Dietary fiber:
Prebiotic;
Short-chain fatty acids;
Polyphenols;
Antioxidant activity;
Antimicrobial activity.

ABSTRACT

The objective of this work was to determine the viability of the most important agro-industrial coproducts, such as cacao husk and coffee pulp, as sources of bioactive compounds, as prebiotic fiber, and polyphenols as antioxidants and antimicrobials. Main carbohydrates and prebiotic activity score were determined, as well as the characterization of main polyphenols, antimicrobial and antioxidant capability. The flours presented a higher dietary fiber content (64 % for coffee pulp and 70 % for cacao husk), with a higher glucose content in cacao husk flour. This explained the higher prebiotic activity score obtained, as compared to coffee pulp. During lactic acid fermentation, the SCFA was adequate, with a higher production of lactate and acetate when coffee pulp was employed as a carbon source. Total polyphenols content was higher in cacao husk, and although TEAC was higher with this flour, DPPH, and total antioxidant activity were higher for coffee pulp. Cacao husk flour or coffee pulp can be employed as functional ingredients to improve intestinal health promoting the selective growth of probiotics, as well as inhibiting pathogens microorganisms due to their antimicrobial activity. Both ingredients can be employed as natural antioxidants as well.

1.Introduction

The Agro-industrial co-products are valuable ingredients and employed as a source of important biomolecules that represent an opportunity to impulse circular economics looking for the sustainability in the food industry chains. A circular economy implies systems that reuse and recycle resources in other materials that can be use in the same process or different ones, resulting in cycles to optimize resources and diminish residue production (Sauvé et al., 2016). There have a essential to keep up a cross-sectorial visualization to link the gaps between the co-products management and business opportunities, regarding four main challenges according to Gontard et al. (2018): first, environmental and economic management strategies especially the lack of guidance to manage residues; second, the use of the actual residue converting technologies; third, the energy consumption during co-products recovery and conversion to biomass or bioproducts; and fourth, the integration of these residues in a circular economy context. In this respect, there are six types of circular business models proposed by Donner et al. (2020) related to: 1) biogas production by anaerobic digestion process, 2) environmental refineries to produce biofuels mainly, 3) production of high-value materials by upcycling entrepreneurship, 4)

spatial cluster of companies in an industrial symbiosis in a circular bio-based system, or agro parks, 5) agricultural cooperative as the association of persons involved during all the productive chain, and 6) support structure to coordinate, networking, and enlace different companies to take advance of technological and logistics infrastructures.

These co-products from fruits and vegetables are fiber-rich ingredients that, after dehydration and milling, present a great physiological functionality due to the dietary fiber content, antioxidants, and many nutrients for example lipids, proteins, and glucosides (Garcia-Amezquita et al., 2018). Since the fiber amount is a major concern in processed food today, these agro-industrial co-products can be employed as an inexpensive ingredient to improve nutritional profile of processed foods. Most recent definition imply that the prebiotic must be metabolized by the native intestinal microbiota, must be able to withstand gastric conditions, mammalian enzymes before being absorbed in gastrointestinal tract, also can be used by the intestinal microbiota, stimulate selectively the activity of bacteria with propitious effect to the host (Lordan et al., 2020). Additionally, dietary fiber include compounds as: non digestible oligosaccharide, polysaccharide, and starch, and non-glucosides compounds or associated substances; since early definitions of fiber referred plant constituents (lignin, cellulose, hemicellulose), expanded later to the carbohydrate chains with the or more carbons, undigestible and non-absorbed in human intestine (Rezende et al., 2021). These ingredients, besides the digestive effect, can also present prebiotic properties, in addition to the naturally occurring polyphenol compounds, with antioxidant and antimicrobial properties.

Coffee and cacao are two economically important productive chains in developing countries, that also generate an important number of co-products. On one hand, coffee processing implies the transformation of the harvested fresh coffee cherries to low moisture (around 12%) green coffee beans, involving pulping, fermentation, and washing, where the cherries are left with parchment and mucilage before drying process. From this wet processing method, coffee pulp and pulp can be recovered in conjunction fraction, named coffee pulp, representing around 40 % of the fresh coffee cherries (Esquivel and Jiménez, 2012). Coffee by-products comprise carbohydrates, proteins, fibers and other compounds, represent a good source of phytonutrients, in the pharmaceutical and food industry containing of polyphenols, hydroxycinnamic acids, flavanols and condensed tannins (Esquivel and Jiménez, 2012). Otherwise, cacao pod husk is a economic source of co-product as pectin, antioxidants, dietary fiber and minerals, being an fountain of organic constituents with nutraceutical characteristics, besides aroma compounds or food texturizing agents (Campos-Vega et al., 2018). Cacao husks present a larger quantity of phenolic compounds, proving to be an inexpensive source of bio-compounds, as soluble phenols, and tannins, with antioxidant activity, besides a considerable amount of soluble dietary fiber (Okiyama et al., 2017).

This work has the objective to determinate the capability of coffee pulp and cacao husk even as a fountain of functional compounds, suchlike as antioxidants, antimicrobial compounds, and prebiotics, to promote the circular economy of these co-products.

2.Materials and methods

2.1.Raw materials

Dry coffee pulp (*Coffea arabica* L.) be collected during the harvest time from January to March 2022. Dry cacao husks (*Theobroma cacao* L.) were collected during October and November 2022 as well. Both co-products were obtained at Angel Albino Corzo locality, at Chiapas, México (15°46’00” N, 92°41’00” W, 365 m.a.s.l.). Both co-products were hacking in a Weston 74-1001-W oven as to 65 °C by12 h, to homogenize moisture content (10-12%). Dry materials were pulverized in a food processor and sieved in a mesh #100 to obtain homogeneous dust named flour and stored in hermetic plastic containers until use.

2.2.Fiber, glucose, reducing sugars and prebiotic activity score.

The percent of fiber was determined by the enzymatic method, AOAC Official Method 991.43 (AOAC, 1996). The carbohydrates content was calculated by difference. Glucose was determined by HPLC, using an isocratic method with sulfuric acid 5 mM as eluant, flux 0.6 mL/min at 60 °C and retention time of 15 min. Samples were analyzed in a Shimadzu Prominence (Shimadzu Corp, Kyoto) with a hydrogen column HPX-87H (300×7.8 mm) (Sluiter et al., 2011).

For prebiotic activity, a set of flours coffee pulp and cacao husk was evaluated based on a report by Huebner et al. (2007). The different probiotic strains (Lactobacillus (L.) *rhamnosus* Rosell, *L. rhamnosus* GG, *L. casei* Shirota, and *L. acidophilus*) and the pathogen *Escherichia coli* were growth in a culture medium employing coffee pulp flour or cacao husk flour (1%) as carbon source (casein peptone 0.5 % along with yeast extract 0.3 %) and glucose was the control (1.0 %) . After 18 h of incubation, the biomass production (calculate as optical density at λ=600 nm), the prebiotic activity score was calculated as:

$$\text{Prebiotic activity score} = \text{LAB}\frac{\Delta N\ \text{Flour}}{\Delta N\ \text{glucose}} - \text{Enteric}\frac{\Delta N\ \text{Flour}}{\Delta N\ \text{glucose}} \quad (1)$$

Where: ΔN is the subtraction of the CFU/mL in the final time and CFU/mL at the initial time for the coffee pulp flour or cacao husk flour and the growing with glucose difference, the value of the pathogen (*E. coli*) from the employed probiotic lactic acid bacteria (LAB).

2.3.Short chain fatty acids (SCFA)

In the quantification of SCFA, acetic, propionic, butyric fatty acids, and lactic acid were determined using the probiotic strains *L. acidophilus*, *L. casei* Shirota, *L. rhamnosus* Rosell and *L. rhamnosus* GG, using gas chromatography on a GC HP5890, with a flame ionizer, AT-1000 column (10 m × 0.25 mm) with a temperature gradient of 90 to 120 °C, increasing 5 °C/min, N2 carrier gas, at a flow of 1 mL/min. The standards were prepared at a concentration of 25-700 ppm and were expressed in mg/mL.

2.4.Polyphenols content, polyphenols composition, and antioxidant properties.

An ethanolic extract (90:10 ethanol:water) from both flours (5% w/v) were obtained by maceration at 60 °C during 30 min subsequently was percolate, and polyphenols content was determined by Folin-Ciocalteu reagent (Singleton et al., 1999). Flavonoids were analyzed based on what was reported by Vega et al. (2017). Condensed tannins (pro anthocyanidins), as insoluble, water soluble, organic solvent soluble, were determined (Shay et al. 2017). And hydroxycinnamic acids were determined by HPLC (Rodriguez-Duran et al., 2014).

Antioxidant properties were determined as Trolox equivalent antioxidant capacity (TEAC), based on what was reported by Re et al., (1999). Antiradical power was determined as inhibition of DPPH in percent, as specified by Randhir and Shetty (2007). And the total antioxidant activity, based on the inhibition of linoleic acid peroxidation by compounds present in extracts (Starzynska-Janiszewska et al., 2008).

2.5.Antimicrobial activity of ethanolic extracts

The antimicrobial activity of coffee pulp and cocoa husk was determined using the methodology of (Bauer et al., 1966). As enteric strains (*Escherichia coli, Salmonella (S.) typhimurium., Pseudomonas (P). fragilis, P. fluorescens, P. putida, Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis and S. sp*), they were plated on Mueller-Hinton agar ($2X10^8$ CFU/mL) and filter paper discs (1.0 cm diameter) containing commercial antibiotics such as erythromycin (NeoPharma, Mexico), chloramphenicol (Pharmacos Exakta, Mexico) and tetracycline (Bioresearch, Mexico) at a concentration of 5 mg/mL were used. The ethanolic extract of coffee pulp flour and cocoa

shell (ethanol:water 90:10) was diluted to 5 mg/mL of polyphenols and finally the inhibition diameters were quantified, reported as the mean of three reproducible assays.

2.6.Experimental design and statistical analysis

The influence of the flour's coffee pulp and cacao husk on antimicrobial activity was determined according to the model:

$$y_{ij}=\mu+\alpha_i+\beta_j+\epsilon \quad (2)$$

Where y_{ij} is the antimicrobial activity for the *i-th* type of antimicrobial compound and the *j-th* of the strain. The μ is the overall mean, α_i is the effect of the type of antimicrobial, and β_j is the effect of the pathogen; and ϵ is the error terms of a presumed normal distribution, N (μ, σ^2) (Der and Everitt, 2001).

3.Results and discussion

3.1.Fiber, glucose, reducing sugars and prebiotic activity score.

The fiber content for both flours was around 60-70% (Table 1). The glucose content in coffee pulp flour was 26 %, and the reported range was from 17 to 33 % (Mawaddh et al., 2022). Cacao husk flour presents higher glucose content (58%). Total sugars content was close to 48% in coffee pulp flour (Pérez-Calvo et al. 2023), in cacao husk the content was higher (75%) (Table 1).

Table 1. Carbohydrates composition and prebiotic activity score for the coffee pulp flour or cacao husk flour

Parameter	**Coffee pulp flour**	**Cacao husk flour**
Main carbohydrates		
Dietary fiber (%)	63.93 ± 0.70	70.60 ± 0.30
Glucose (mg/g sample)	26 ± 7.8	58± 6.54
Total reducing sugars (%)	47.50 ± 1.12	74.50 ± 1.82
Prebiotic activity score		
L. rhamnosus GG	0.12	0.25
L. rhamnosus Rosell	0.08	0.25
L. casei Shirota	0.14	0.29
L. acidophilus	0.13	0.26

The results show that the coffee pulp flour and the cacao husk flour are suitable ingredients that employed to increase the quality of processed nourishment, as well as to carbon source for different fermentation process. By-products can be employed to produce relevant platform chemicals (single cells oil, specific C2-C6 metabolites) and biopolymers (as bacterial cellulose), where bioconversion processes are the most important techno-economic aspect (Koutinas et al., 2014). The coffee by-products singular organic composition made it an ideal substrate for microbial processes to earn betterment products. Likewise, manner, the interesting composition of cacao husk represents a source as well as the production of compounds such as lignocellulosic biomass (Porto de Souza Vandenberghe et al., 2022).

The prebiotic activity score with *E. coli* as pathogen indicator microorganism for the different probiotic strains are show in Table 1, where coffee pulp flour presented higher scores than cacao husk flour. *L. casei* Shirota presented the higher score employing cacao husk as a carbon source, due to the higher sugars content. Inulin, as the prebiotic by antonomasia, presented scores higher than one (Huebner et al., 2007) since inulin composition is higher in soluble fiber (98%). Prebiotic potential is an important characteristic for dietary fiber, since only oligosaccharides, fructooligosaccharides, and inulin are classified as such, so for other

fibers to be prebiotic, demonstrating such an effect is important. (Rezende et al., 2021). The insoluble dietary fiber of cacao fiber contains in glucose, whereby that the most part of polysaccharides is cellulose and pectin, and the insoluble dietary fiber fraction in cacao fiber presents a considerable amount of mannose and galactose, suggesting the presence of galactomannans as monosaccharides part of the pectic substances, as major components of both soluble dietary fiber and insoluble cell wall polysaccharides. For its part, coffee pulp has xylans, and polysaccharide whit cellulose, hemicellulose, and lignin (Machado et al., 2023). Pectin in coffee skin contains uronic acid with different degrees of methyl esterification (Esquivel and Jiménez, 2012). The coffee co-products could exert certain antimicrobial activity in the course the fermentation process in the prebiotic activity determination due to the melanoidins content (Jiménez-Zamora et al., 2015).

The fermentation rate of the prebiotic dietary fibers pivot on solubility and size inters other. The soluble fibers are fermented faster than the insoluble ones, and oligosaccharides than the polysaccharides, so that the fermentation the fiber varied (like the obtained from agro-industrial coproducts, pulp or husks) will be different from the fermentation of individual fibers or a single carbon source (Rezende et al., 2021). For this reason, the determination of the prebiotic activity score is important in these kind of fiber resources, such as coffee pulp and cacao husk, although the inherent and concomitant differences in composition depending on seasonal changes and geographic factors, carbohydrate composition, as soluble or insoluble fiber, represent a nice fountain of prebiotic compounds.

3.2.Short chain fatty acids

For the results, the SCFA, were different depending on the strain employed. The highest concentration of lactic acid for both flours was produced by *L. acidophilus*, as well as highest amount of probiotic. Nonetheless, together butyric, and propionic acids results be similar among probiotic strains for both flours (Table 2).

Table 2. Short-chain fatty acids produced by different probiotic strains with coffee pulp flour or cacao husk flour as carbon sources.

Compound	**Probiotic strain**			
	L. acidophilus	*L. casei* Shirota	*L. rhamnosus* Rosell	*L. rhamnosus* GG
Coffee pulp flour				
Lactic acid	121.5 ± 0.04	84.3 ± 0.04	69.4 ± 0.07	68.6 ± 0.05
Acetic acid	25.7 ± 0.006	18.8 ± 0.01	12.7 ± 0.02	21.0 ± 0.06
Propionic acid	Nd	Nd	2 ± 0.01	Nd
Butyric acid	2.0 ± 0.04	2.1 ± 0.01	2.1 ± 0.01	2.0 ± 0.02
Cacao husk flour				
Lactic acid	129.7 ± 0.05	61.8 ± 0.04	60.7 ± 0.02	70.1 ± 0.06
Acetic acid	41.4 ± 0.03	10.6 ±0.02	11.9 ± 0.03	17.2 ± 0.02
Propionic acid	1.6 ± 0.01	1.7 ± 0.01	2.5 ± 0.01	1.8 ± 0.01
Butyric acid	2.1 ± 0.01	2.1 ± 0.01	3.2 ± 0.02	2.0 ± 0.01

Short chain fatty acids (less than 6 carbons) are produced due to the non-digestible carbohydrates and are related to an indirect way to measure prebiotic capacity (Markowiak-Kopéc and Śliżewska, 2020). This phenomenon has been related to host health affecting the integrity of the gastrointestinal epithelium, and glucose homeostasis (Ashaolu et al., 2021). The intensive metabolic activity of probiotic fermentation of available substrates decreased pH value and increase the production of probiotic fermentation of available substrates

decreased the pH value and increased the yield ok organic acids (Nsor-Atindana et al., 2020), besides SCFA is acetate, propionate and butyrate (Gibson and Roberfroid, 1995). In addition, the fermentation of prebiotic fibers improves the growth of other no-probiotic microorganisms, which employ by-products generated by probiotics. Then, the cross-feeding situation is a dietary regulation of the microbiota results to prebiotic consumption where the nutrients competition and metabolites production with the concomitant pH reduction help to impede the proliferation of other microorganisms, considering the host genetic disposition as well, consumption of required dietary fiber, and/or the constitution of the individual microbiota (Ashaolu et al., 2021).

3.3.Polyphenols content, polyphenols composition, and antioxidant properties.

Total polyphenols content was higher in cacao husk flour, although the flavonoids content was similar for both flours. Condensed tannins content was similar in coffee pulp flour and cacao husk flour, with a higher quantity of insoluble tannins in cacao husk flour. Chlorogenic and caffeic acid were detected in coffee pulp flour, and vanillic acid was detected in cacao husk flour. Ferulic acid was detected in both samples (Table 3). The bio-accessibility and bioavailability of polyphenols are important, since although phenolic compounds decreased during digestion, hydroxycinnamic acids can reach colon and be metabolized by bifidobacteria and lactic acid bacteria (Cañas et al., 2022).

Table 3. Total polyphenol substances contain, polyphenols composition and antioxidant properties of coffee pulp flour or cacao husk flour ethanolic extracts.

Parameter	**Coffee peel flour**	**Cacao husk flour**
Total polyphenols content (Eq. gallic acid mg/g)	3.84±0.21	4.12±0.23
Flavonoids (Eq. catechin mg/g)	1.23±0.05	1.30±0.33
Condensed tannins		
Soluble in organic solvents (mg/g)	0.81±0.09	1.20±0.10
Soluble in water (mg/g)	1.00±0.01	0.85±0.01
Insoluble (mg/g)	0.23±0.01	0.63±0.02
Hydrocinnamic acids		
Chlorogenic acid (mg/g)	0.40±0.02	Nd
Caffeic acid (mg/g)	0.03±0.01	Nd
Ferulic acid (mg/g)	0.03±0.003	0.03±0.001
Vanillic acid (mg/g)		0.07±0.001
Antioxidant properties		
TEAC	13.79±0.90	44.83±0.10
Antiradical power by DPPH	81.67±0.50	72.30±0.67
Total antioxidant activity	80.50±0.60	68.40±0.10

For the Trolox equivalent antioxidant capacity (TEAC), cacao husk flour presented higher values as compared to coffee pulp flour. The antiradical power determined by DDPH was higher for the coffee pulp flour, as well as the total antioxidant activity (Table 3). Antioxidant activity pivot on the amount and the type of polyphenols presents, although the relations among polyphenols content, the antioxidant activity is not directly related since there are several reactive molecules with different response as antioxidant (Alcalde et al., 2019). The different techniques employed measure different reactions and depend on factors such as the concentration of antioxidant-related compounds, extraction method, etcetera

(Yusof et al., 2019). Antioxidant capacity determination the of bio-accessible and the no bio-accessible (or insoluble) fractions of foods after a physiological-resembling digestion (Jiménez-Zamora et al., 2015). The coffee pulp has phenolic content in exerts an important antiradical-reduction effect, and the antioxidant activity could be attributed to the soluble melanoidins fraction (Jiménez-Zamora et al., 2015).

Some polyphenols, such as highly polymerized condensed tannins and phenols associated with proteins and polysaccharides from cell wall are associated whit dietary fiber, and can resist digestive enzymes hydrolysis and although the congregation of the soluble phenolic made-up in cacao fiber was low than the expected, is important to notice that polyphenols present showed antioxidant capacity and free radical scavenging capacities, higher than the reported in other dietary fiber sources, like wheat bran. Minor chemical constituents in coffee pulp have (2-10% of weight) are caffeine, chlorogenic acids, and polyphenols, including flavonoids, being a valuable source of dietary antioxidants supplements, and a source of fiber as well. Coffee growth at different conditions has different antioxidants profile, but there are a useful and cheap source of molecules with biological activity to add to food products (Dorsey and Jones, 2017).

3.4.Antimicrobial activity of ethanolic extracts

A significantly higher antimicrobial activity was observed with erythromycin, followed by tetracycline and coffee pulp flour extract ($P>0.05$). The lower antimicrobial activity was observed in chloramphenicol and cacao husk flour extract. In the same manner, *P. fragi*lis was the significant ($P>0.05$) most sensitive strain to the employed antimicrobials, followed by *L. monocytogenes* and *B. subtilis*, and *Salmonella* sp. The rest of the strains were less sensitive to these antimicrobials (*E. coli*, *P. fluorescens*, *P. putida*, *S. aureus*, and *S. typhimurium*) (Table 4). These results indicate that the coffee pulp flour ethanolic extract presented an antimicrobial activity as good as tetracycline, and cacao husk flour ethanolic extract has the inhibitory pathogens capacity like chloramphenicol.

This is important since extracts from these agro-industrial co-products can be employed as antimicrobials, or in conjunction with drugs to reduce the risk of the antimicrobial resistance. A specific extraction of compounds of interest could be made to increase the antimicrobial capacity. Melanoidins present in coffee by-products present antimicrobial activity related to their chelating capacity (Jiménez-Zamora et al., 2015). The cacao pod husk extracts, Kayaputri et al. (2020) ensure the caffeine, as the leading alkaloid identified in chloroformic fraction, presented a bacteriostatic effect on Gram negative *P. aeruginosa*, and although theobromine has been reported as the main alkaloid in cacao, the synergic effects of different alkaloids are responsible of the antimicrobial effect. Gram-positive bacteria are most receptive to polyphenols and tannins, as hydrophobic compounds, since the cell membrane of Gram-negative bacteria, made-up of phospholipids, hydrophobic compounds are not absorbed. Polyphenols present antimicrobial activity because of the disruption of the perviousness of the cell membrane (Duangjai et al., 2016), and by a metal chelating effect of melanoidins (Rufián-Henares and de la Cueva, 2009). Changes in cell wall integrity due interactions with phenolic compounds resulted in the alteration of various intracellular functions, by hydrogen bonding enzymes, provoking an irreversible damage in cell cytoplasmic membrane, with the concomitantly cease of intracellular enzymes activity, and death (Khochapong et al., 2021).

Table 4. Antibacterial activity by disk diffusion method of ethanolic extract of coffee pulp flour or cacao husk flour as compared to commercial antibiotics against the different pathogen strains.

Pathogen	Diameter of inhibition zones (cm)				
	Chloramphenicol	Tetracycline	Erythromycin	Coffee pulp flour	Cacao husk flour
E. coli	0.45±0.07 C, d	0.63±0.14 B, d	1.00±0.07 A, d	0.67±0.05 B, d	0.48±0.04 C, d
Salmonella sp.	0.53±0.07 C, c	1.43±0.07 B, c	0.75±0.06 A, c	0.39±0.07 B, c	0.43±0.05 C, c
P. fragilis	1.43±0.56 C, a	0.48±0.15 B, a	0.58±0.04 A, a	0.26±0.05 B, a	0.35±0.03 C, a
P. fluorescens	0.48±0.07 C, d	0.50±0.14 B, d	0.95±0.07 A, d	0.34±0.02 B, d	0.38±0.02 C, d
P. putida	0.45±0.07 C, d	0.40±0.07 B, d	0.75±0.02 A, d	0.30±0.07 B, a	0.52±0.04 C, d
L. monocytogenes	0.60±0.06 C, b	0.50±0.06 B, b	1.03±0.14 A, b	0.39±0.05 B, b	0.45±0.05 C, b
S. aureus	0.55±0.05 C, d	0.90±0.14 B, d	0.80±0.07 A, d	0.30±0.03 B, d	0.47±0.06 C, d
B. subtilis	0.60±0.05 C, b	1.63±0.07 B, b	0.65±0.28 A, b	0.27±0.08 B, b	0.45±0.02 C, b
S. typhimurium	0.43±0.07 C, d	0.38±0.07 B, d	0.88±0.14 A, d	0.33±0.05 B, d	0.45±0.07 C, d

A, B - Means with the same letter in the same row are not significantly ($P<0.05$) different for antimicrobial.
a, b - Means whit the same letter in the same column is not significantly ($P<0.05$) different for pathogen microorganisms.

4.Conclusion

Coffee pulp flour and cacao husk flour are a sources of functional ingredients, such as fiber and polyphenols, where the relatively high concentration of fiber present in these two by-products make them a functional ingredient that could improve intestinal health promoting the selective growth of probiotics, as well to inhibit pathogens microorganisms due to their antimicrobial activity.

The demonstrated ability to serve as a prebiotic carbon source and antimicrobial, in addition to the antioxidant capability of the polyphenols present in both cacao husk flour and coffee pulp flour, stands out the importance of these byproducts as a fountain of important bioactive substances, so that be employed as meal ingredients by enhance the nutritional value of processed foods. Cacao and coffee are commodities whit important economic importance in several countries, implying as well small factories that can use these ingredients in a circular economy, looking for a sustainable agricultural chain.

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CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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NUTRITIONAL AND TECHNOLOGICAL QUALITY OF GLUTEN-FREE BREADS FORMULATED WITH NON-CONVENTIONAL FUNCTIONAL FLOURS/POWDERS/EXTRACTS- A REVIEW

Lale ACAR[2], Semin Ozge KESKIN[1,2]✉

[1]Kocaeli University, Vocational School of Izmit, Food Technology, Kocaeli, Turkey
[2]Kocaeli University, Polymer Science and Technology, Kocaeli, Turkey
✉seminozgekeskin@gmail.com

https://doi.org/10.34302/crpjfst/2024.16.3.8

Article history:
Received:
October 15th, 2023
Accepted:
August 23rd, 2024

Keywords:
Bread;
Functional ingredient;
Gluten-free;
Nutritional quality;
Technological quality.

ABSTRACT

Production of high quality and nutrient-rich gluten-free (GF) bread remains a challenge for food scientists. Incorporation of new functional ingredients is one of the main approaches to improve the acceptability of GF bread by consumers. This review highlights recent studies (2016-to present), adopted to improve the nutritional and technological quality of GF bread with the help of non-conventional functional ingredients, edible insect powder/flour, microalgae and seaweed powders, green mussel, bee pollen, anchovy flour, coffee/cocoa by-products, and leaf powder/extract. The scientific studies reviewed in this paper demonstrated that those non-conventional ingredients provided nutritional and technological functionality to the GF bread in different manners. They acted as coloring, flavoring, antioxidant, texturizing, or anti-staling agents in GF bread formulations. These non-conventional functional ingredients have the potential to produce nutrient-rich GF bakery products with improved quality.

1. Introduction

Gluten-containing grains have been an important item of human diet for more than 10,000 years (Cabanillas, 2019) and are also expected to remain important in human diet for many years. Despite its ubiquity, gluten intake causes disorders in humans in the prevalence of 1-13% of the population, such as wheat allergy (allergic), celiac disease (auto-immune), dermatitis herpetiformis (auto-immune), gluten ataxia (auto-immune) and gluten sensitivity (immune-mediated) (Gao *et al.*, 2018). The most effective treatment known to reduce or prevent the side effects of gluten-related disorders is a lifelong gluten-free (GF) diet. GF bakery products are part of the GF diet, and their nutritive value and quality are significant concerns. Among the bakery products, the production of high quality and nutritious GF bread is still a challenge (da Rosa Machado and Thys, 2019). The main challenges related to GF bread can be divided into technological and nutritional issues related to the absence of gluten and insufficient nutrient-rich ingredients in GF bread formulations.

Gluten is a functional component in bread formulations in terms of technological quality, due to its unique structure-forming properties during processing (El Khoury *et al.*, 2018). Since gluten is responsible for the elastic properties of bread, its porous structure and volume; absence of gluten in bread formulations induces GF breads with low quality, such as low volume, crumbling texture, dense and dry structure, high crumb hardness, lighter crumb and crust color, poor flavour and mouthfeel, and rapid staling (Naqash *et al.*, 2017). Furthermore, since GF breads prepared with conventional ingredients usually have high

caloric value and glycemic index and unsatisfactory nutritive value, development of healthy GF breads with balanced nutrients is still a challenge (Javaria *et al*., 2016). Recipe and technology-based approaches have been applied to cope with these common problems (Naqash *et al*., 2017).

Various conventional and non-conventional GF ingredients have been incorporated in GF breads to mimic gluten network and improve their nutritional quality. Conventional ingredients used in GF breads are GF cereal and pseudocereal flours, legume, seed and nut flours, starches and proteins, protein isolates and concentrates from different GF sources. Edible insect powder/flour, microalgae and seaweed powders, green mussel, bee pollen, anchovy flour, coffee/cocoa by-products, and leaf powder/extracts are the recently investigated non-conventional functional ingredients used in GF bread formulations.

Edible insects are cheap, environmentally friendly protein-rich materials with biological activity (e.g., cricket powder has ability to reduce inflammation, and support intestinal microflora) (Kowalczewski *et al*., 2021). GF bread has been one of the application areas of edible insects to produce value-added products (da Rosa Machado and Thys, 2019; Kowalczewski *et al*., 2019, 2021; Nissen *et al*., 2020). Eukaryotic microalgae and prokaryotic cyanobacteria; both commonly referred as microalgae and macro-forms such as seaweeds are photosynthetic organisms in the group of algae with functional bioactive compounds (Lee, 2008; Renuka *et al.*, 2018). The microalgae genera, *Spirulina*, *Botryococcus*, *Chlorella*, *Dunaliella*, *Haematococcus*, and *Nostoc* have ability to produce bioactive compounds providing antioxidant, antimicrobial and anti-inflammatory activities (Morais *et al*., 2015). Those functional properties make microalgae one of the promising sources of functional food ingredients (Gouveia *et al*., 2008; Buono *et al*., 2014). Besides their nutritional functionality in food products, microalgae can be used as texturizing ingredients. The structural biopolymers of photoautotrophic microalgae, such as proteins, storage polysaccharides and cell wall related polysaccharides have ability to alter textural properties of the fortified food products (Bernaerts *et al*., 2019). One of the promising application areas of microalgae to produce functional foods is GF bakery products (Diprat *et al*., 2020; Khemiri *et al*., 2020; Nunes *et al*., 2020). Seaweeds are marine macroalgae that contain excellent sources of bioactive components like dietary fibers, carotenoids, essential fatty acids, proteins, minerals, and vitamins (Barba, 2017; Lopez-Santamarina *et al.*, 2020; El-Sheekh *et al*., 2021). Seaweeds have been added to GF formulations to benefit those functional compounds and improve nutritional quality of GF breads (Rózylo *et al*., 2017). Since green mussel is a functional ingredient that contains bioactive components and proteins (Vijaykrishnaraj *et al*., 2015); bee pollen is also a natural functional food and food ingredient due to its nutritional properties and health benefits (Thakur and Nanda, 2020) and anchovy flour consists of valuable components, such as essential fatty acids and amino acids, and minerals (Yılmaz and Koca, 2020), they are potential ingredients to be used in GF bread formulations to improve nutritive value. Addition of coffee by-products provide high fiber and antioxidant contents, which have positive impacts on colonic health, and make them functional (Gemechu, 2020). Coffee parchment is a by-product of coffee wet processing, used in food formulations not only for its antioxidant capacity and dietary fiber content but also for its antifungal activity (Klingel *et al*., 2020; Littardi *et al*., 2021). Cocoa bean shells are by-products of cocoa processing with nutritional components. They are rich in dietary fiber, polyphenols, and antioxidants (Okiyama *et al*., 2017). Besides their nutri-functional properties, they have also been added to bakery products for their techno-functional characteristics, such as their role as fat replacers, texturizing, or anti-staling agents (Collar *et al*., 2009). Fruit, vegetable-based by-products are considered as low-cost potential

sources of valuable ingredients, such as proteins, vitamins, and minerals as well as dietary fibre and bioactive compounds (Bedrníček *et al*., 2020; Sedlar *et al*., 2021; Krupa-Kozak *et al*., 2021; Vacca *et al*., 2023). In addition, fruit, vegetable, and leaf powders have been found to be effective to control retrogradation of starchy foods in literature (Salehi and Aghajanzadeh, 2020; Park *et al*., 2021).

2. Functional Non-Conventional Ingredients

2.1. Edible insect powder/flour

Edible insects have been added to GF bread formulations to produce value-added protein-rich products (da Rosa Machado and Thys, 2019; Kowalczewski *et al*., 2019, 2021; Nissen *et al*., 2020) (Table 1). Addition of cricket powder (*Gryllus assimilis*) to the formulation increased protein and lipid contents of GF breads and provided darker crumb and crust color (da Rosa Machado and Thys, 2019). The results indicated that incorporation of 20% cricket powder doubled the protein content of GF bread. The high water and oil holding capacities of cricket powder provided acceptable technological properties, such as increased porosity and cell density, to GF breads, which is related to high protein and lipid contents of insect powders (da Rosa Machado and Thys, 2019) (Table 1). On the other hand, cricket powder fortified GF breads presented crumbs with higher hardness and chewiness values than the control GF bread. However, removal of canola oil from the cricket powder fortified GF bread formulation decreased hardness and chewiness and increased cohesiveness values providing similar characteristics to the control sample. The emulsifying properties of cricket proteins ensured reduction in hardness and chewiness values and increase in crumb consistency and cohesiveness values to GF bread, reflecting internal cohesion of the crumb (Kowalczewski *et al*., 2019). Nissen *et al.* (2020) incorporated cricket flour to sour dough bread formulation as protein source. They determined the volatile compounds, protein profile and antioxidant activity of GF sourdough and bread. The results showed that cricket containing dough had similar fermentation process, typical flavoring profile and significantly higher antioxidant activity with the standard dough. Moreover, cricket GF bread obtained by LAB fermentation (equal mix of *L. sanfrancisciensis* and *L. plantarum*) had proteins with high nutritional value and enhanced antioxidant activity (Nissen *et al*., 2020). Kowalczewski *et al.* (2021) investigated the nutritional value and biological activity of GF bread enriched with cricket powder. A two, four and seven-fold increase in protein content; 23%, 59%, and 105% increase in fat content was observed for GF breads formulated with 2%, 6% and 10% cricket powder, respectively. Additionally, they stated that replacement of starch with 10% cricket powder resulted in about three-fold and five-fold increase in the content of insoluble dietary fiber and the total polyphenolic compounds, respectively. The undesirable activity of β-glucuronidase was reduced by 65.9% in the small intestine and up to 78.9% in the large intestine with the use of cricket powder at 10%.

2.2. Microalgae and seaweed powders

Microalgae have high protein, lipid and bioactive content and texturizing ability in GF bakery products. Diprat *et al.* (2020) added *Chlorella sorokiniana* biomass powder at the concentrations of 2.5 and 5.0g per 100g of the blend of rice flour and corn starch in substitution of pea flour to increase the nutritional quality of GF bread. Fortification of GF bread with microalgae provided greenish color, higher protein, carotenoids and polyunsaturated fatty acids content without changing specific volume and textural characteristics of them. It has been stated by Khemiri *et al.* (2020) that the addition of two green microalgae species (*Nannochloropsis gaditana* L2 and *Chlamydomonas sp.* EL5) significantly increased the protein, lipid and ash contents of GF bread. A particular increase in linolenic acid (18:3, ω3) and a decrease in ω3/ω6 ratio had been observed. The pigments

in microalgae significantly changed the color of dough, bread crust and crumbs. The high protein content of green microalgae ensured formation of strong texture (decreased crumbliness) by increasing firmness and adhesiveness parameters of GF bread (Khemiri *et al*., 2020). Nunes *et al.* (2020) fortified GF bread with *Tetraselmis chuii* at concentrations of 1%, 2% and 4%. Low contents of *T. chuii* in the formulation destabilized the network formed by starch and HPMC, resulting in low bread volume, high firmness and more compact crumb. Addition of microalgae at the highest concentration (4%) increased the capacity of bread dough to retain the gas bubbles providing higher bread volume and softening effect which competes with the structure of control GF bread. Moreover, the improved bioactivity (higher total phenolic content and antioxidant capacity) of GF breads fortified with 4% *T. chuii* make them healthy GF food alternatives with interesting taste and colour (Nunes *et al.*, 2020).

Seaweeds have been added to GF formulations to improve nutritional and technological quality of GF breads (Rózylo *et al*., 2017). Rózylo *et al.* (2017) added brown algae (*Ascophyllum nodosum*) powder in the amounts of 2%, 4%, 6%, 8%, and 10% of the total flour (white rice, corn and millet flour) content to determine physical, antioxidant, and sensory properties of fortified GF bread. Brown algae fortification increased volume, elasticity, antioxidant activity and antiradical potential and decreased firmness and crumb staling degree of GF breads. The decrease in firmness and crumb staling degree of GF breads could be due to the interaction of algae components with starch in GF flours and decreasing the starch chain interactions. Since over-addition of brown algae powder resulted in an unpleasant taste, the researchers suggested addition of 2% or 4% algae powder to obtain acceptable GF bread (Rózylo *et al*., 2017).

2.3. Green mussel

Green mussel is known to have functional ingredients, such as bioactive components and proteins that make it applicable to be used in food formulations (Vijaykrishnaraj *et al*., 2015). Vijaykrishnaraj *et al.* (2016) added green mussel (*Perna canaliculus*) protein hydrolysates to optimized GF bread formulation (70:20:10 (buckwheat flour: rice flour: chickpea flour)) at the concentrations of 5%, 10%, 15%, 20% on flour weight basis and the quality of fortified GF bread and the peptides responsible for mussel flavour were characterized. Volume of all GF breads was lower, and firmness of them were higher than that of wheat bread (control 1). The reduction in volume was mainly related to absence of gluten in GF formulations. On the other hand, the volume of fortified GF bread up to 20% level was higher than that of GF control bread (control 2, GF bread without green mussel protein hydrolysate), which is related to the interactions between protein and starch components. Addition of mussel protein hydrolysates improved bread swelling and gelling properties and crumb structure significantly (Table 1) with the help of starch-protein interactions. According to the sensory evaluation, GF breads formulated with green mussel protein hydrolates had a mussel flavour, and after taste, it was also noticeable, which was accepted by the sensory panelists. Since protein hydrolysates from green mussel do not contain allergenic proteins, they can be used as alternative natural flavouring agents in GF food systems (Vijaykrishnaraj *et al*., 2016).

2.4. Bee pollen

Bee pollen is known as a natural functional food and food ingredient due to its nutritional properties and health benefits (Thakur and Nanda, 2020). Conte *et al.* (2018, 2020) added bee pollen to GF bread formulations to improve nutri- and techno-functional properties and aroma of GF bread (Table 1). The results obtained by Conte *et al.* (2018) indicated that bee pollen supplementation from 1% to 5% did not significantly change either dough machinability or gassing power ability during fermentation and provided a well-leavened dough system. Bee pollen addition to bread

formulation at medium-high levels (3-4%) improved the technological quality of GF bread providing higher specific volume, darker color, softer, more cohesive and resilient crumb, more homogeneous crumb grain structure, and lower and slower staling kinetics compared to the GF control bread. Bee pollen-fortified samples at medium-high levels showed the highest values of dough area expansion during the proofing process, resulting in GF breads with the highest specific volume. The dark color observed for pollen-fortified GF breads was related to non-enzymatic browning reactions occurred during baking, in which the components of pollen, such as reducing sugars act as substrates in those reactions. Furthermore, the flavonoids and carotenoid pigments found in pollen may have also been contributed to the color of crust. Lower hardness values and the slower firming rate observed in all the fortified breads (up to 4%) was related to the presence of natural emulsifiers (especially monoglycerides) and amylases in the pollen (Conte *et al*., 2018). The interaction of emulsifiers with starch prevents starch retrogradation and contributes to lower crumb firming. Amylases, the starch-modifying enzymes decreases starch-chain length providing less starch-chain interactions and lower firming rate. The overall acceptability of GF breads fortified with bee pollen at medium-high levels was found to be higher than for the control (Conte *et al*., 2018). Conte *et al*. (2020) found that incorporation of bee pollen at the highest levels (4-5%) to the formulation increased proteins, the minerals; K and Ca, soluble and bioaccessible polyphenols, total carotenoids, antioxidant activity and improved aroma composition of GF breads, significantly. Although 48 volatile compounds were found in the fortified breads, only 5, namely pyrazinamide, 5-methyl-2-furaldehyde, 2-acetylfuran, furfural, 2-pentyl-furan, were related to bee polen supplementation by the authors (Conte *et al*., 2020).

2.5. Anchovy flour

Anchovy flour consists of valuable components, such as essential fatty acids and amino acids, and minerals (Yılmaz and Koca, 2020). Yılmaz and Koca (2020) fortified GF corn bread with anchovy (*Engraulis encrasicolus*) flour at the levels of 10, 20, 30 and 40% (corn flour basis) (Table 1). Anchovy flour addition improved nutritional properties of GF corn bread in terms of essential fatty acids (especially ω-3 fatty acids, EPA and DHA), aminoacids and minerals due to its nutritious nature. In terms of technological quality, anchovy flour-fortified GF breads exhibited lower lightness values for crust and crumb and lower hardness and chewiness values, related to their fat-rich composition. The high protein and fat content of anchovy flour contributed to improved chewing properties, desirable bread structure and shape, color, and taste, resulting in highly acceptable sensory properties (Yılmaz and Koca, 2020).

2.6. Coffee/cocoa by-products

Coffee/cocoa by-products are also regarded as promising functional ingredients to improve nutritional and technological quality of GF breads (Guglielmetti *et al*., 2019; Rinaldi *et al*., 2020; Rios *et al*., 2020; Littardi *et al*., 2021). Addition of coffee by-products provide high fiber and antioxidant contents, which have positive impacts on colonic health, and make them functional (Gemechu *et al*., 2020). Coffee silverskin (rich in dietary fiber and present high antioxidant activity) and coffee husk (rich in dietary fiber and phytochemicals) extracts improved nutritional properties, such as dietary fiber content, antioxidant capacity, of GF breads (Guglielmetti *et al*., 2019). Moreover, the authors considered coffee silverskin and husk extracts as natural colorants for GF bread providing the typical appearance of wholemeal bread. Addition of isolated coffee cascara dietary fiber improved nutritional and physicochemical properties of GF bread and provided good sensory profile (Rios *et al*., 2020).

Table 1. Improvements achieved in the nutritional and technological quality of GF breads with the help of functional non-conventional ingredients -Recent studies (2016-to present)

Functional Ingredient / Concentration	Other ingredients	Main improvements	Reference
Cricket powder (*Gryllus assimilis*) / (10, 20%)	Rice flour, corn starch, xanthan gum, CMC	higher protein and lipid contents, darker crumb and crust color, higher porosity and cell density	da Rosa Machado and Thys, 2019
Cricket powder / (2%, 6% and 10%)	Corn starch, potato starch, guar gum, pectin	lower hardness and chewiness values, higher crumb consistency and cohesiveness values	Kowalczewski *et al*., 2019
Cricket powder / (2%, 6% and 10%)	Corn starch, potato starch, guar gum, pectin	higher protein, polyunsaturated fatty acids, insoluble dietary fiber and mineral contents; increased antioxidant activity and decreased β-glucuronidase activity	Kowalczewski *et al*., 2021
Cricket flour	Corn flour, rice flour, HPMC	higher protein content and antioxidant properties	Nissen *et al*., 2020
Chlorella sorokiniana / (2.5 and 5.0g/100g rice flour and corn starch basis in substitution of pea flour)	Rice flour, corn starch, pea flour, xanthan gum, CMC	higher protein, carotenoid and polyunsaturated fatty acid contents	Diprat *et al*., 2020
Nannochloropsis gaditana L2 and *Chlamydomonas sp*. EL5 / (1.0 and 3.0 g/100 g of rice, buckwheat flour and potato starch)	Rice flour, buckwheat flour potato starch, HPMC	higher protein, lipid and ash contents, darker crumb and crust color, improved structural properties (strong texture)	Khemiri *et al*., 2020
Tetraselmis chuii / (1, 2 and 4%)	Rice flour, buckwheat flour, potato starch, HPMC	higher bioactivity (total phenolic content and antioxidant capacity), innovative green appearance at 4% level.	Nunes *et al*., 2020
Brown algae (*Ascophyllum nodosum*) powder / (2, 4, 6, 8 and 10%)	White rice, corn and millet flour	higher volume, elasticity, lower firmness, lower crumb staling degree, antioxidant activity and antiradical potential	Rózylo *et al*., 2017
Green mussel (*Perna canaliculus*) protein hydrolysates / (5, 10, 15, 20%)	Buckwheat flour, rice flour, chickpea flour	higher volume and mussel flavour in GF breads	Vijaykrishnaraj *et al*., 2016
Bee pollen / (1, 2, 3, 4, 5%)	Rice flour, corn starch, guar gum and Psyllium fiber	higher specific volume, darker color, softer, more cohesive and resilient crumb, more homogeneous crumb grain structure, and lower and slower staling kinetics with the incorporation of medium-high levels (3-5%) of bee pollen	Conte *et al*., 2018
Multifloral bee pollen / (1, 2, 3, 4, 5%)	Rice flour, corn starch, guar gum and Psyllium fiber	higher protein, K and Ca contents, improved bioactive properties and aroma composition	Conte *et al*., 2020
Anchovy flour / (10, 20, 30, 40% on corn flour basis)	Corn flour, xathan gum, vegetable mix (chard, leek, onion)	higher protein, fat, essential amino acids (lysine and tryptophan), Omega-3 (EPA + DHA), Ca, Fe, K, P, Zn and Se contents, highly acceptable sensory and textural properties	Yılmaz and Koca, 2020

Abbreviations: CMC, carboxy methyl cellulose; HPMC, hydroxy propyl methyl cellulose; EPA, Eikosapentaenoic acid; DHA, Docosahexaenoic acid

Table 1. Improvements achieved in nutritional and technological quality of GF breads with the help of functional non-conventional ingredients -Recent studies (2016-to present) (CONTINUED)

Functional Ingredient / Concentration	**Other ingredients**	**Main improvements**	**Reference**
Coffee silverskin extract, coffee husk extract / (2.5% d.m.)	Commercial premix (mainly composed of corn starch), inulin, rice protein	higher dietary fiber content and antioxidant capacity	Guglielmetti *et al*., 2019
Isolated coffee cascara dietary fiber / (3.0, 4.5%)	Commercial premix, rice protein	higher dietary fiber and protein contents, higher dough yield and crumb moisture, higher color intensity and crumb elasticity, lower crumb firmness and estimated calorie values, good sensory profile	Rios *et al*., 2020
Green coffee parchment / (2%)	Commercial GF bread mixture (corn starch, rice flour, vegetable fibers (psyllium, bamboo), whole rice flour, lentil flour, HPMC)	higher antioxidant capacity, oxidative stability, darker color and lower presence of HMF	Littardi *et al.*, 2021
Cocoa bean shell added at three different dimensional fractions (F1 (1.00-1.99mm), F2 (0.50-0.99mm) and F3 (0.355-0.490mm)) / (4%)	Commercial GF bread mixture (corn, rice cream soup, tapioca starch, vegetable fibers, guar flour and HPMC)	pleasant darker color, antistaling effect (for F1 fraction)	Rinaldi *et al*., 2020
Moringa oleifera leaf powder / (2.5, 5.0, 7.5, 10.0%)	Rice/field bean semolina	higher total phenolics content and antioxidant activity	Bourekoua *et al*., 2018
Fried onion (FO), dried onion (DO) and onion peel (OP) powders / (5%)	unhusked white buckwheat flour, corn flour, rice flour, linseed flour	higher flavonol and total phenolic contents and in vitro antioxidant activity	Bedrníček *et al*., 2020
Broccoli leaf powder / (5%)	Corn starch, potato starch, pectin	higher protein and mineral contents, improved specific volume and bake loss, improvement of antioxidant potential and anti-glycation end-products activity	Krupa-Kozak *et al*., 2021
Control dough with (YB-AE) and without (YB) artichoke leaf extract and Sour dough with (SB-AE) and without (SB) artichoke leaf extract / (6%)	Rice flour	highest antioxidant activity and highest scores of hydrocinnamic acid and cyclohexanecarboxylic acids for SB-AE containing GF breads	Vacca *et al*., 2023

Abbreviations: HPMC, hydroxypropyl methyl cellulose; HMF, hydroxymethyl furfural

Coffee cascara dietary fiber-fortified breads exhibited significantly higher dietary fiber and protein contents than the control bread. The addition of isolated coffee cascara dietary fiber increased dough yield and crumb moisture, related to water absorption ability of dietary fibers. Moreover, increase in color intensity and crumb elasticity and decrease in crumb firmness had been observed for fortified breads. Estimated calorie values of dietary fiber-fortified breads were found to be lower than control breads. According to sensory analysis, it was observed that the flavor "cereal" significantly masked in breads formulated with isolated coffee cascara dietary fiber by its "toasted (crust)", "bitterness", "sourness" attributes (Rios et al., 2020). Coffee parchment is a by-product of coffee wet processing, used in food formulations not only for its antioxidant capacity and dietary fiber content but also for its antifungal activity (Klingel et al., 2020; Littardi et al., 2021). Littardi et al. (2021) fortified GF breads with 2% green coffee parchment to improve nutritional and technological characteristics of them. Addition of green coffee parchment provided high antioxidant capacity, oxidative stability, and lower presence of hydroxymethylfurfural (HMF) to GF breads. Fortified breads had similar sensory characteristics, volume, moisture content, water activity, hardness, cohesiveness values and staling degree, but darker color compared to the control breads. Besides their nutri-functional properties, cocoa bean shells have been added to bakery products for their techno-functional characteristics, such as acting as fat replacers, texturizing agents, or anti-staling agents (Collar *et al*., 2009). Rinaldi et al. (2020) added cocoa bean shell at different dimensional fractions (F1, 1.00-1.99 mm; F2, 0.50-0.99 mm; F3, 0.355-0.49 mm) to produce functional GF bread. The water binding capacity, water absorption index, water holding capacity, water solubility index and proximate composition of cocoa bean shell fractions were found to be different which affects the functions of them on GF bread quality. Incorporation of cocoa bean shell negatively affected the crumb grain, specific volume, and moisture content of GF breads, but the results indicated that cocoa been shell fortified GF breads had pleasant dark color. Furthermore, F1 gave the lowest hardness value 3 days after storage, demonstrating an antistaling effect probably due to its high-water absorption index value, which could help to prevent the retrogradation of amylopectin. Authors suggested the use of cocoa bean shell fraction F1, in GF bread formulations, as a functional ingredient with limited impacts on fresh and stored bread quality.

2.7. Leaf powder/extract

In recent years, the nutri- and techno-functional aspects of vegetable by-products have made them alternative components of GF bread formulations (Bourekoua *et al*., 2018; Bedrníček, *et al*, 2020; Krupa-Kozak *et al*., 2021; Vacca *et al*., 2023).

Moringa *oleifera* leaf powder is one of the nutri-functional ingredients that has been added to GF bread formulations. Addition of *Moringa oleifera* leaf powder at the amounts of 2.5, 5.0, 7.5 and 10% significantly improved nutritional quality of GF breads by increasing total phenolic contents and antioxidant activity of them, related to their phytonutrients (Bourekoua *et al*., 2018). GF breads fortified with *Moringa oleifera* leaf powder at 2.5% considered the most acceptable GF bread with improved nutritional quality, exhibiting acceptable darker crumb and crust color, lower hardness and chewiness values compared to control GF bread and similar sensory characteristics with control GF bread. On the other hand, incorporation of leaf powder higher than 2.5% level resulted in low specific volume, compact texture, and low overall acceptability (related to taste, aroma, and appearance) compared to control GF bread.

In another study by Bedrníček, *et al*. (2020), powders of three onion waste fractions (fried onion (FO), dried onion (DO) and onion peel (OP)) were used for the preparation of GF bread with improved health benefits. The authors reported that all kinds of onion waste

significantly increased the content of flavonols, total phenolic content and the in vitro antioxidant activity of GF breads. Among the onion wastes, 5% onion peel powder addition provided the highest flavonol and total polyphenol content and antioxidant activity in GF bread without any sensory changes. Moreover, as an indicator of good bioavailability of flavonols, the antioxidant activity in consumers' blood significantly increased with the consumption of OP-bread (Bedrníček, *et al*., 2020).

Broccoli (*Brassica oleracea* var. *italica*) leaves are characterized by a high content of nutrients (proteins, vitamin C, minerals, and trace elements) and bioactive compounds (glucosinolates, phenolic acids, and flavonoids), making them one of the promising functional GF components (Krupa-Kozak *et al*., 2021). Krupa-Kozak *et al*. (2021) investigated the suitability and functionality of broccoli leaf powder (BLP) as a GF component in GF bread. Incorporation of broccoli leaf powder at the level of 5% provided higher protein and mineral contents, higher specific volume, lower bake-loss, higher antioxidant potential and anti-glycation end-products activity to fortified breads, but similar hardness values with control GF breads (Krupa-Kozak *et al*., 2021).

The artichoke plant (*Cynara cardunculus* L.) has been considered as a healthy food in literature due to its antioxidant and bioactive properties. Artichoke leaf, one of the by-products of artichoke plant, have also been indicated as phenol-rich materials with antioxidant and anti-inflammatory properties (Vacca *et al*., 2023). In a study by Vacca *et al*. (2023) powder extract of artichoke leaf was combined with sourdough technology to improve nutritional and healthy features of GF bread. They stated that the incorporation of powder extract of artichoke leaf (6%) in GF sourdough bread formulation provided more than 15-fold and10-fold increase in DPPH (2,2-diphenyl-1-picrylidrazyl) and antioxidant activity values, respectively. Moreover, the highest anti-inflammatory effectiveness was found for artichoke extract added sourdough GF breads (Vacca *et al*., 2023).

3. Conclusions and Future Prospects

According to the results, it can be concluded that non-conventional functional ingredients have the potential to produce nutrient-rich GF bakery products with improved quality. Natural bioactive compounds exist in cricket flour, microalgae (*Tetraselmis chuii*), brown seaweed (*Ascophyllum nodosum*) powder, bee pollen, green coffee parchment, coffee silverskin and husk extracts, broccoli and *Moringa oleifera* leaf, and onion peel powders and powder extract of artichoke leaf can enhance the antioxidant properties of GF breads. From the technological point of view, light crumb and crust color of GF bakery products formulated with conventional ingredients is a common quality problem. Natural pigments of non-conventional ingredients have the potential to solve this problem. Cricket powder (*Gryllus assimilis*), microalgae (*Tetraselmis chuii*), brown algae (*Ascophyllum nodosum*) powder, bee pollen, anchovy flour, coffee/cocoa by-products, broccoli, and *Moringa oleifera* leaf powders improved the color of GF bakery products, providing desirable dark crumb and crust colors. One of the other problems related to GF bakery products is their poor flavor. Satisfactory results have been obtained to improve the flavor of GF bread with the incorporation of green mussel (*Perna canaliculus*) protein hydrolysate and bee pollen to the formulation. Furthermore, cricket powder (*Gryllus assimilis*), green microalgae species (*Nannochloropsis gaditana* L2 and *Chlamydomonas sp*. EL5) and brown algae (*Ascophyllum nodosum*) powder have ability to act as texturizing agents in GF formulations. Since crumbly and weak structure is common deficiencies observed in GF bakery products, mainly GF bread, presence of texturizing and hydrocolloidal agents to mimic gluten functionality in GF formulations is very important to obtain quality. Staling is the other common and serious problem for GF breads.

The anti-staling functions of brown algae (*Ascophyllum nodosum*) powder, bee pollen, and cocoa bean shell are valuable and notable to meet the consumer expectations.

As a future trend, genetic engineering approaches such as genetic modification of yeasts and other microorganisms to improve the rheology and nutritive value of GF batter/dough, may be considered in combination with the current or new non-conventional ingredients to achieve more successful results in terms of GF bread quality.

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Acknowledgment

We thank to Dr. Nadide Seyhun for her assistance in proofreading the manuscript.

Author contribution

Semin Ozge Keskin: Conceptualization, investigation, writing-original draft, review and editing; **Lale Acar:** Investigation and writing-original draft.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

EFFECT OF *MORINGA OLEIFERA* LEAVES AQUEOUS EXTRACT ON THE PHYSICOCHEMICAL, COLOR, SHEAR FORCE, AND LIPID OXIDATION OF VARIOUS GOAT MUSCLES

Alzaidi Mohammed Awad[1], Pavan Kumar[1,2], Muideen Adewale Ahmed[1], Muhammad Nizam Hayat[3], Abubakar Ahmed Abubakar[1], Mohammad Rashedi Ismail-Fitry[4,5], Muhamad Faris Ab Aziz[3], Shokri Jusoh[3], Awis Qurni Sazili[3,5]✉

[1] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India

[3]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[4]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[5]Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 UPM Serdang, Selangor, Malaysia

✉awis@upm.edu.my

https://doi.org/10.34302/crpjfst/2024.16.3.9

Article history:
Received:
June 6th, 2023
Accepted:
September 3rd, 2024

Keywords:
Moringa oliefera;
Goat muscles;
Marination;
Lipid oxidation.

ABSTRACT

The present study evaluated the effect of aqueous extract of *Moringa oleifera* leaves (MOLE) on various physicochemical characteristics color, shear force, and lipid oxidation of various goat muscles. Longissimus dorsi m., infraspinatus m., biceps femoris m., and semimembranous m., were marinated with MOLE (0.10, 0.50, and 1.0% w/v) along with positive control containing 0.1% BHT (butylated hydroxytoluene) and negative control (without extract and BHT). The samples were marinated under refrigeration in low-density polyethylene bags for 7 days and assessed for various quality attributes on 1, 3, and 7 days. The water-holding capacity and moisture content of goat muscles were observed to follow a decreasing trend with increasing storage days, and a higher ($p<0.05$) value was recorded for samples on day 1 of storage as compared to day 7 of storage. Lipid oxidation recorded a significant ($p<0.05$) increase with the advancement of storage days, and samples with 1.0% MOLE were observed to show comparable ($p>0.05$) thiobarbituric acid reactive substances (TBRAS) to that of BHT-added samples. Thus, the inclusion of MOLE at a 1.0% concentration demonstrated significant improvement in the physico-chemical quality, and color stability while also inhibiting lipid oxidation similar to that achieved with 0.10% BHT.

1.Introduction

Goat meat is very popular and universally preferred by consumers across various cultures, religions, and societies due to the absence of religious issues. Goat meat is recommended for health-conscious consumers due to its desirable lipid profile, lower calorific value, and lower sodium content (Chaosap *et al.*, 2021; Watkins *et al.*, 2021). Goat meat has low glycolytic potential, thus making it crucial to preserve muscle glycogen levels by mitigating preslaughter stress for a desirable ultimate pH. In addition, the small and lean carcass of the goat is more prone to shortening during aging

(Pophiwa *et al.*, 2020). Further, the higher toughness/stringiness of goat meat due to its production from culled/spent animals, lack of sufficient nutrition, inappropriate pre-slaughter handling, and post-slaughter processing remain significant issues in goat meat production (Kumar *et al.*, 2022) and warrant the immediate attention of meat scientists. Various technological interventions are applied to improve the tenderness of goat meat, such as marination and ultrasound applications (Kumar *et al.*, 2023; Sobri *et al.*, 2023).

Like other meat, goat meat is prone to quality deterioration due to oxidative and microbial changes during storage (Kumar *et al.*, 2013). Various preservatives are used in the meat industry to control the quality deterioration. These preservatives can be categorized into two groups: synthetic preservatives (butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, etc.) and natural preservatives (plant extracts, essential oils, and bioactive compounds). With the increasing awareness and consumer shifts towards minimally processed food and the potential adverse effects of synthetic preservation on consumer health, the meat industry is increasingly using natural preservatives as replacement of synthetic preservatives (Awad *et al.*, 2021, 2022). Various plant extracts prepared by extracting the bioactive compounds from the plant matrix are increasingly used to develop functional meat products with extended shelf life (Mehta *et al*., 2022).

Moringa oleifera/ drumstick has a high amount of polyphenolic and bioactive molecules such as rhamnetin, anthraquinones, kaempferol, saponins, alkaloids, kaempferitrin, isoquercitrin, terpenoids, triterpenoids, and tannins (Singh *et al.*, 2015). Leaves and flowers of *Moringa* are commonly used as food additives, lactagogues, mineral supplements, immune modulators, antimicrobials, antidiabetic and cardioprotective roles (Gopalakrishnan *et al.*, 2016). *Moringa oleifera* leaves are incorporated in powdered or extract form to improve nutritional and technological qualities, oxidative stability, sensory attributes, and shelf-life extension in food products. *Moringa oleifera* leaves extract (MOLE) incorporation in meat products was reported to improve the organoleptic attributes and inhibit lipid oxidation, such as at 0.3% level in goat meat nuggets (Rahman *et al.*, 2020), 2.0 % extract in buffalo meat (Kenawi and El-Hameed, 2018), and at 0.25% extract in mortadella (Francelin *et al.*, 2022).

Several studies reported the application of MOLE in improving oxidative stability, organoleptic attributes, and shelf life of goat meat products, but there is a lack of studies on the application of MOLE on the various quality attributes of various goat muscles. In addition, a comparative analysis regarding the effect of MOLE on individual muscle groups is lacking. Thus, the present study was undertaken to evaluate the effect of the incorporation of MOLE on the quality attributes of various goat muscles.

2. Materials and methods

2.1. Extract preparation

Moringa plants were cultivated at Ladang 15, Faculty of Agriculture, Universiti Putra Malaysia, and produced organically. Fresh *M. oleifera* leaves were harvested approximately after 60 days of plantation The harvested leaves were cleaned, freeze-dried, and ground to fine powder form. The aqueous extract of *Moringa oleifera* leaves was prepared by taking 1: 10 powder: solvent (double distilled water) ratio in a conical flask at ambient temperature (25 ± 2°C) and stirred at 200 rpm for 2 h followed by centrifugation (Anke DL-6000 B; China) at 2500 g for 30 min at 4°C. The supernatant was stored under refrigeration for use in the study.

2.2. Meat sampling and marination

Goat skeletal muscles were obtained by the slaughter of 5 male goats (Boer cross, live weight 18-21 kg, age 5-6 months) by following Halal slaughter as per the standard protocols outlined in the MS 1500:2009 by the Department of Standards Malaysia. After the ventral neck cut and proper removal of blood, goats were hoisted on rails and skinned and dressed manually. The carcasses were kept in the chiller (4±1°C) for 24 h for aging. Four major muscles viz., longissimus

dorsi (LD), biceps femoris (BF), infraspinatus (IS), and semi-membranous (SM), were carefully cut with a clean and sharp knife under hygienic conditions.

Immediately after collecting muscles from the carcass, the muscles were marinated by preparing marinades viz., negative control (clean potable water without MOLE and BHT) and positive control (water added with 0.1% BHA), T1 (0.1% MOLE), T2 (0.5% MOLE), and T3 (1.0% MOLE), thereby resulted in the preparation of a total of 20 samples for the present study (Table 1).

Table 1. Sampling protocol of the experiment

Muscle type	NC	C	T1	T2	T3
LD	LD-	LD+	LDT1	LDT2	LDT3
IS	IS-	IS+	IST1	IST2	IST3
BF	BF-	BF+	BFT1	BFT2	BFT3
SM	SM-	SM+	SMT1	SMT2	SMT3

(NC- negative control without BHT and MOLE, C- control with 0.10% BHT, LD-Longissimus dorsi m.; IS-Infraspinatus m.; SM- semimembranosus m.; BF-Biceps femoris m., BHT-Butylated hydroxytoluene, MOLE-*Moringa oleifera* leaves aqueous extract)

2.3. Physicochemical parameters

The WHC of goat muscle was assessed by per Whiting & Jenkins (1981). About 30 g of mixed meat of each individual type of goat muscle was taken and placed in between two filter papers. The sample was placed on a rigid glass plate, and 40 psi pressure (equivalent to 2.81 kg) was applied for 5 min. After the pressure application, the meat sample was removed, and the weight of the dry meat flake was noted.

$$\text{WHC (\%)} = [(\text{Pre-press weight of flake} - \text{Post-press weight of meat flake}) / (\text{Pre-press weight of flake})] \times 100 \quad (1)$$

The pH of various goat muscle samples was assessed by using a portable pH meter (Mettler Toledo, AG 8603, Switzerland) calibrated at pH 4.0 and pH 7.0. The 0.5 g of pulverized sample was homogenized (Wiggen Hauser® D-500, Germany) along with 5 mM sodium iodoacetate for 30 s in 10 mL of ice-cold distilled water in order to stop further glycolysis.

Moisture levels in various goat muscles were measured by calculating the reduction in weight before and after heating the samples in the hot air oven at (105°C) for 3 h in a moisture cup and transferred to a desiccator to cool.

$$\text{Moisture \%} = (W1-W2) \times 100 \quad (2)$$

where :
W1= weight g before drying
W2=weight g after drying

2.4. Thiobarbituric acid-reacting substances (TBARS)

The lipid oxidation of various goat muscles was assessed by estimation of TBARS (Wang *et al.*, 2002). Five grams of muscle tissue was homogenized for 30 s in a homogenizer with 25 mL of 20% trichloroacetic acid solution and filtered through the Whatman filter paper number. A 2 mL of 0.02 M thiobarbituric acid aqueous solution (3 g/L) was added to the muscle filtrate in a test tube and heated to 100°C for 30 minutes. The sample was cooled under running water followed by measuring absorbance at 532 nm by using UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBARS value was assessed with help from the standard curve and presented as mg malonaldehyde/kg of sample.

2.5. Shear force analysis

The water-bath cooked goat muscle samples (10 min at 78°C internal temperature) were cut parallel to the direction of the muscle fibers in three replicate samples of uniform size of 1 × 1× 2 cm. Each block was set on the base plate of a TA-HD plus texture analyzer (Stable Micro System, Surrey, UK) and sheared perpendicularly in the longitudinal direction of the fibers with the help of attached Volodkevitch biting jaw to the texture analyzer.

2.6. Color profile

The CIE color value (*L**: +lightness/- dark, *a**: +redness/- green, and *b**: yellowness/- blue) of water bath cooked (10 minutes at 78°C internal temperature) goat muscle samples were measured by using ColorFlex® system (5cm aperture size, 10° standard observer, D65 illuminant). Before usage, the instrument was calibrated using white and black reference tiles supplied with the equipment, and the *L**, *a**, and *b** color coordinates were recorded on the muscle's sliced surface.

2.7. Statistical analysis

The mean value and standard error of replicates (N=6) were analyzed on SPSS-20.0 software packages, IBM Corporation, USA. The significant difference between means within a muscle type affected by MOLE concentration and storage was compared by using a two-way analysis of variance (ANOVA) by using Duncan's Multiple Range Test (DMRT). For texture profiles and color analysis on day 3 of storage, one-way ANOVA was used to compare these attributes among muscle samples. The statistical significance was tested at a 5% level ($p<0.05$).

3.Results and discussions

The marination of goat muscle with various concentrations of MOLE (0.1%, 0.5%, 1.0%) significantly affected the physico-chemical and quality characteristics.

3.1. Water holding capacity

The WHC of goat muscles was affected by the MOLE concentration in marinates and storage days (Table 2). With the advancement of storage days, WHC of all muscle samples exhibited a decreasing trend, with all four muscle samples, viz., LD, IS, SM, and BF samples, exhibiting significantly ($p<0.05$) lower values on day 7 as compared to WHC value of their respective samples on day 1 of storage.

Among LD muscle, increasing MOLE concentration was recorded with higher WHC value with LD-02 sample was recorded significantly ($p<0.05$) higher value than LDT-01 and LD+ and comparable to LD-. On the last day of refrigerated storage, LD-01 and LD-03 samples had comparative WHC and a significant ($p<0.05$) lower value than other LD muscle samples. The mean WHC values of IS+ muscle and IS-01 were recorded as comparable and significantly ($p<0.05$) higher than IS-02. IS-03 sample on day 1 of storage. The WHC of the IS-03 sample exhibited comparable values on day 1 and day 3 and on day 7 of storage, the WHC of IS-03 samples was significantly ($p<0.05$) reduced as compared to those on day 1 and day 7.

On day 1, the mean WHC value of SM-03 was significantly ($p<0.05$) higher than all other SM groups, viz., SM-01, SM-02, SM+, and SM-. There was a significant ($p<0.05$) decrease in WHC value for the SM-02 sample upon storage to 3 days, and the WHC values of SM-02 on day 3 and day 5 were comparable ($p>0.05$). Among SM samples on day 7, the highest value was recorded for SM-01, which was comparable ($p>0.05$) to SM-03 and SM-. The mean WHC of SM-03 was recorded as comparable to all other samples on day 3 and day 7. The mean value of WHC among BF groups, BF-03, had recorded the highest value among all other BF muscle samples throughout the storage.

The WHC of goat muscles has shown a decreasing trend with increasing days of storage. This result could possibly be explained by denaturation and proteolysis of muscle protein during refrigeration storage, leading to decreased water binding by the myofibrillar proteins. A similar decrease in the WHC upon increasing storage was observed in ground meat (Ahmed *et al.*, 2015; Das *et al.*, 2011). The higher WHC of goat muscles with MOLE samples could be due to the potential preservative effect of the MOLE.

3.2. pH values

In general, the mean pH value of goat muscle samples showed an increasing trend with the increasing storage days (Table 3). The mean pH value of all muscle samples on day 7 was significantly ($p<0.05$) higher than their corresponding value at the beginning of the study, i.e., day 1. Among various treatments of LD muscle, the incorporation of MOLE and BHT resulted in increasing pH, on day 1, LD-

samples had significantly ($p<0.05$) lower pH values to LD-01 and lower but non-significant ($p>0.05$) difference than other samples viz., LD-02, LD-03, and LD+. On day 7 of storage, LD-02 and LD-03 samples had comparable values, which in turn resulted in significantly ($p<0.05$) lower pH value than the pH value of LD+ and LD- samples.

Table 2. Water holding capacity (WHC) of goat muscles marinated with *Moringa oleifera* leave extract (Mean ±SE)

Treatment	Day 1	Day 3	Day 7
LD			
LD-01	91.63 ± 0.54^{bA}	90.87 ± 0.44^{bA}	80.36 ± 0.05^{aA}
LD-02	98.64 ± 0.11^{cC}	91.85 ± 0.06^{bA}	84.77 ± 2.90^{aB}
LD-03	95.98 ± 2.31^{cB}	89.48 ± 0.22^{bA}	81.55 ± 0.43^{aA}
LD+	95.91 ± 1.32^{bB}	92.55 ± 1.30^{aB}	86.30 ± 3.33^{aB}
LD-	97.38 ± 0.78^{bC}	90.56 ± 1.93^{aA}	90.04 ± 2.43^{aC}
IS			
IS-01	97.92 ± 0.98^{bB}	91.50 ± 0.43^{aA}	92.65 ± 1.54^{aC}
IS-02	94.21 ± 0.32^{bA}	93.96 ± 0.43^{abAB}	91.76 ± 1.43^{aBC}
IS-03	96.29 ± 0.33^{bAB}	94.19 ± 1.26^{bB}	80.66 ± 0.31^{aA}
IS+	99.09 ± 0.88^{cB}	92.95 ± 0.21^{bA}	88.54 ± 1.02^{aB}
IS-	95.94 ± 0.21^{bAB}	92.16 ± 0.98^{aA}	92.85 ± 1.54^{aC}
SM			
SM-01	94.00 ± 1.32^{bA}	93.26 ± 1.43^{bB}	90.08 ± 0.43^{aB}
SM-02	95.92 ± 0.97^{bA}	88.55 ± 3.22^{aA}	85.42 ± 0.44^{aA}
SM-03	97.68 ± 1.43^{cB}	91.25 ± 0.67^{bAB}	87.88 ± 3.90^{aAB}
SM+	94.86 ± 0.54^{cA}	91.09 ± 0.33^{bAB}	84.78 ± 3.06^{aA}
SM-	93.95 ± 1.43^{bA}	93.70 ± 1.88^{bB}	89.54 ± 2.54^{aB}
BF			
BF-01	94.34 ± 1.20^{bAB}	91.01 ± 0.20^{aA}	90.89 ± 1.09^{aA}
BF-02	96.68 ± 0.11^{bC}	95.68 ± 0.65^{bB}	88.12 ± 3.44^{aA}
BF-03	97.93 ± 0.23^{bC}	94.94 ± 0.43^{aB}	93.54 ± 1.31^{aB}
BF+	95.26 ± 1.03^{bB}	94.09 ± 0.67^{bC}	92.35 ± 0.65^{aAB}
BF-	92.66 ± 0.44^{aA}	92 ± 0.99^{aA}	91.87 ± 1.21^{aAB}

N=6, Mean with different superscripts in A, B, C---and a, b, c differed significantly ($P < 0.05$) within a column-wise and row-wise, respectively; LD: longissimus dorsi m.; IS: infraspinatus m.; SM: semimembranous m.; BF: bicep femoris m., Muscle-1: muscle with 0.10% MOLE, Muscle-2: muscle with 0.50% MOLE, Muscle-03: muscle with 1.0% MOLE, Muscle-: muscle without BHT and MOLE, Muscle+: muscle with 0.10% BHT

Table 3. pH values of goat muscles marinated with *Moringa oleifera* leave extract (Mean ±SE)

Treatment	Day 1	Day 3	Day 7
LD			
LD-01	5.99 ± 0.02^{bB}	5.89 ± 0.02^{aAB}	6.03 ± 0.02^{bAB}
LD-02	5.86 ± 0.02^{aAB}	5.91 ± 0.08^{bAB}	5.95 ± 0.04^{bA}
LD-03	5.86 ± 0.02^{aAB}	6.08 ± 0.04^{cB}	5.93 ± 0.03^{bA}
LD+	5.88 ± 0.04^{aAB}	5.84 ± 0.06^{aA}	6.31 ± 0.02^{bC}
LD-	5.78 ± 0.01^{aA}	5.91 ± 0.04^{bAB}	6.11 ± 0.03^{cB}
IS			
IS-01	5.98 ± 0.02^{aC}	6.15 ± 0.08^{bC}	6.19 ± 0.04^{bC}
IS-02	5.85 ± 0.03^{aB}	5.90 ± 0.07^{bA}	5.94 ± 0.08^{bA}
IS-03	5.99 ± 0.04^{aC}	6.03 ± 0.04^{aAB}	6.05 ± 0.02^{aAB}
IS+	6.03 ± 0.04^{aCD}	6.07 ± 0.05^{abB}	6.14 ± 0.03^{bBC}
IS-	5.61 ± 0.06^{aA}	6.08 ± 0.09^{bB}	6.10 ± 0.07^{bB}
SM			
SM-01	6.03 ± 0.04^{a}	6.15 ± 0.09^{bB}	6.28 ± 0.04^{c}
SM-02	6.00 ± 0.07^{a}	5.90 ± 0.09^{aA}	6.30 ± 0.08^{b}
Sm-03	5.99 ± 0.02^{a}	6.16 ± 0.06^{bB}	6.32 ± 0.06^{c}
SM+	$6.05\pm.03^{a}$	$6.06\pm.08^{aA}$	6.33 ± 0.07^{b}
SM-	6.05 ± 0.04^{a}	6.19 ± 0.03^{bB}	6.31 ± 0.04^{c}
BF			
BF-01	5.98 ± 0.07^{bB}	5.85 ± 0.08^{aA}	6.03 ± 0.06^{bA}
BF-02	5.99 ± 0.02^{bB}	5.91 ± 0.05^{aAB}	6.13 ± 0.08^{cB}
BF-03	5.97 ± 0.06^{aB}	5.88 ± 0.07^{aA}	6.28 ± 0.07^{bC}
BF+	6.06 ± 0.01^{bC}	5.87 ± 0.04^{aA}	6.22 ± 0.04^{cC}
BF-	5.84 ± 0.07^{aA}	6.07 ± 0.03^{bB}	6.09 ± 0.06^{bAB}

N=6, Mean with different superscripts in A, B, C---and a, b, c differed significantly ($P < 0.05$) within a column-wise and row-wise, respectively; LD: longissimus dorsi m.; IS: infraspinatus m.; SM: semimembranous m.; BF: bicep femoris m., Muscle-1: muscle with 0.10% MOLE, Muscle-2: muscle with 0.50% MOLE, Muscle-03: muscle with 1.0% MOLE, Muscle-: muscle without BHT and MOLE, Muscle+: muscle with 0.10% BHT

Similar trends of the lowest mean pH value for the IS- sample were recorded at the beginning of the experiment and thereafter exhibited increasing value with the advancement of storage duration. Further, the pH value for IS samples on day 7 was significantly ($p<0.05$) higher than the pH value on day 1 of their respective IS samples, except for IS-03 samples. In SM samples, the mean pH value of all SM samples was recorded non-significantly ($p>0.05$) different on day 1 and day 7 of storage. The pH value of BF- showed an increasing trend with the advancement of storage days, with pH values of days 3 and 7 comparable and were significantly ($p<0.05$) higher than the pH value on day 1. All other samples of BF muscles exhibited a slight decrease in pH on day 3 compared to day 1 except for BF- samples, and again, increased pH values for all samples were recorded on day 7.

The incorporation of MOLE had an impact on the pH value of goat muscles. The pH showed an increasing trend with the advancement of storage days. The lowest pH among all muscle samples was recorded at the end of the storage period. This process increases proteolysis, forming primary amines (Rusman *et al.*, 2023; Yousof *et al.*, 2024). The higher pH of MOLE extract could also contribute to this value (Widiantara *et al.*, 2023).

3.3. Moisture content

The moisture content of all four-goat muscles under study, viz., longissimus dorsi, infraspinatus, semimembranous, and bicep femoris, exhibited decreasing trends with increasing storage days (Table 4). The muscle samples with the highest MOLE concentration (1.0%) had a higher moisture content than all other respective muscle samples except for IS muscle samples.

The moisture content of all treatments of all four muscle types under studies exhibited significantly ($p<0.05$) lower moisture content on day 7 of storage as compared to the moisture content of their respective treatment at the start of the storage experiment, i.e., day 1 of study. Among LD groups on the day 7 storage, moisture content for LD-03 samples was recorded significantly ($p<0.05$) higher than LD-01 and LD-02 and LD- samples, whereas LD-03 and LD+ have non-significant ($p>0.05$) moisture differences. The moisture content of IS-01 sample was recorded highest and significantly ($p<0.05$) different than all other IS samples on day 1 and day 3 of storage. Among SM muscle on day 7 storage, moisture content of SM-03, SM+, and SM-02 was recorded as comparable and significantly ($p<0.05$) higher than SM-01 and SM- samples

Table 4. Moisture content (%) of goat muscles marinated with *Moringa oleifera* leave extract (Mean ±SE)

Treatment	Day 1	Day 3	Day 7
LD			
LD-01	69.48 ± 1.32^{aA}	67.82 ± 3.65^{aA}	67.22 ± 2.40^{aA}
LD-02	68.26 ± 4.76^{aA}	68.04 ± 2.40^{aA}	68.54 ± 2.44^{aA}
LD-03	73.22 ± 3.43^{aB}	70.87 ± 3.21^{aB}	72.65 ± 1.01^{aB}
LD+	72.42 ± 3.21^{bB}	70.90 ± 2.32^{aA}	70.01 ± 0.32^{aAB}
LD-	73.10 ± 3.27^{bB}	71.50 ± 3.55^{bB}	67.47 ± 2.54^{aA}
IS			
IS-01	74.25 ± 2.95^{bC}	73.18 ± 2.49^{bC}	67.62 ± 2.43^{aB}
IF-02	72.91 ± 2.55^{bB}	70.89 ± 1.43^{bB}	69.92 ± 1.43^{aBC}
IS-03	69.73 ± 1.02^{bA}	65.86 ± 1.43^{abA}	63.81 ± 3.21^{aA}
IS+	72.07 ± 3.21^{bAB}	70.75 ± 0.54^{bB}	62.96 ± 3.72^{aA}
IS-	72.03 ± 2.43^{bAB}	71.45 ± 0.87^{aB}	69.77 ± 1.09^{aC}
SM			
SM-01	69.32 ± 0.55^{bA}	66.78 ± 2.54^{bA}	60.17 ± 1.43^{aA}
SM-02	72.64 ± 1.22^{bB}	70.26 ± 0.96^{bB}	70.06 ± 0.65^{aB}
SM-03	71.51 ± 2.54^{bC}	70.85 ± 2.54^{bB}	71.55 ± 0.54^{aB}
SM+	72.16 ± 1.54^{bA}	72.30 ± 0.76^{bB}	70.03 ± 1.40^{aB}
SM-	68.48 ± 2.32^{bB}	68.15 ± 2.44^{bB}	65.79 ± 1.54^{aA}
BF			
BF-01	71.23 ± 2.43^{bAB}	70.18 ± 0.43^{aAB}	69.08 ± 2.43^{aB}
BF-02	69.89 ± 1.55^{bA}	65.53 ± 1.29^{aA}	65.52 ± 1.98^{aA}
BF-03	72.09 ± 3.21^{bB}	71.72 ± 0.32^{aB}	70.48 ± 1.90^{aB}
BF+	72.42 ± 3.21^{bB}	70.80 ± 1.54^{bB}	67.49 ± 1.54^{aAB}
BF-	70.69 ± 3.25^{bAB}	68.28 ± 3.42^{bAB}	67.74 ± 0.32^{aAB}

N=6, Mean with different superscripts in A, B, C---and a, b, c differed significantly ($P < 0.05$) within a column-wise and row-wise, respectively; LD: longissimus dorsi m.; IS: infraspinatus m.; SM: semimembranous m.; BF: bicep femoris m., Muscle-1: muscle with 0.10% MOLE, Muscle-2: muscle with 0.50% MOLE, Muscle-03: muscle with 1.0% MOLE, Muscle-: muscle without BHT and MOLE, Muscle+: muscle with 0.10% BHT

The average moisture of the muscles under various treatments does not vary significantly ($p>0.05$) based on the type of the muscle and the age. The average moisture was recorded at about 70%, which is about the expected percentage of 75% (Babiker *et al.*, 1990). This could be due to proteolytic enzymes and minerals (calcium ions) present in *Moringa oleifera* leaves. The increasing calcium content has also been known to exert a tenderizing effect by increasing the activity of calpains (Khorchid and Ikura, 2002). The comparatively higher value of water holding capacity for 0.5% and 1.0% MOLE marinades and 0.1% BHA could be due to lower proteolysis in these samples due to better storage stability and antioxidant potential

Table 5. TBARS values (mg malondialdehyde/kg) of goat muscles marinated with *Moringa oleifera* leave extract (Mean ±SE)

Treatment	Day 1	Day 3	Day 7
LD			
LD-01	0.139 ± 0.01^{a}	0.431 ± 0.11^{bA}	0.845 ± 0.01^{cB}
LD-02	0.140 ± 0.05^{a}	0.385 ± 0.32^{bA}	0.749 ± 0.43^{cAB}
LD-03	0.138 ± 0.10^{a}	0.322 ± 1.30^{bA}	0.666 ± 1.55^{cA}
LD+	0.143 ± 0.20^{a}	0.321 ± 0.22^{bA}	0.675 ± 1.40^{cA}
LD-	0.143 ± 1.30^{a}	0.6275 ± 0.43^{bB}	1.238 ± 0.79^{cC}
IS			
IS-01	0.138 ± 1.11^{a}	0.428 ± 1.45^{bB}	0.829 ± 0.66^{cB}
IF-02	0.141 ± 1.65^{a}	0.406 ± 1.76^{bB}	0.763 ± 0.34^{cAB}
IS-03	0.138 ± 1.32^{a}	0.327 ± 1.87^{bA}	0.652 ± 1.54^{cA}
IS+	0.139 ± 0.32^{a}	0.322 ± 0.45^{bA}	0.676 ± 1.76^{cA}
IS-	0.144 ± 0.86^{a}	0.631 ± 0.32^{bC}	1.316 ± 0.98^{cC}
SM			
SM-01	0.14 ± 0.65^{a}	0.427 ± 0.44^{bB}	0.814 ± 0.65^{cC}
SM-02	0.139 ± 0.72^{a}	0.386 ± 0.74^{bAB}	0.732 ± 0.88^{cB}
Sm-03	0.139 ± 0.33^{a}	0.318 ± 0.77^{bA}	0.644 ± 0.32^{cA}
SM+	0.143 ± 1.43^{a}	0.351 ± 0.43^{bA}	0.683 ± 0.15^{cA}
SM-	0.143 ± 0.21^{a}	0.611 ± 0.24^{bC}	1.294 ± 0.98^{cD}
BF			
BF-01	0.141 ± 0.3^{a}	0.433 ± 0.41^{bB}	0.819 ± 0.32^{cC}
BF-02	0.1415 ± 0.2^{a}	0.398 ± 0.17^{bAB}	0.754 ± 1.76^{cB}
BF-03	0.1385 ± 1.54^{a}	0.318 ± 0.43^{bA}	0.644 ± 1.90^{cA}
BF+	0.1385 ± 1.22^{a}	0.388 ± 0.16^{bAB}	0.714 ± 0.76^{cAB}
BF-	0.144 ± 0.28^{a}	0.604 ± 0.55^{bC}	1.281 ± 1.87^{cD}

N=6, Mean with different superscripts in A, B, C---and a, b, c differed significantly ($P < 0.05$) within a column-wise and row-wise, respectively; LD: longissimus dorsi m.; IS: infraspinatus m.; SM: semimembranous m.; BF: bicep femoris m., Muscle-1: muscle with 0.10% MOLE, Muscle-2: muscle with 0.50% MOLE, Muscle-03: muscle with 1.0% MOLE, Muscle-: muscle without BHT and MOLE, Muscle+: muscle with 0.10% BHT

3.4. Thiobarbituric acid reacting substances

The mean TBARS value of all treatments of goat muscles under study was recorded to have a significant ($p<0.05$) increase with the advancement of storage days (Table 5). Among the treatments of muscles, the negative control sample showed the highest TBARS value throughout all sampling days, whereas muscle with 1% MOLE and 0.1% BHA exhibited the lowest and comparable TBARS value. At the end of storage days (day 7), the TBARS value of negative control samples (muscle marinated with potable water) had a TBARS value of more than 1.0 in all muscle samples.

On day 7 of storage, LD-03 and LD+ had comparable ($p>0.05$) TBARS values. The TBARS value of LD muscles at the end of storage exhibited the following trends viz., LD-03<LD+<LD-02<LD-01<LD-. Among groups, all treatments of a muscle (5 treatments per muscle viz., Muscle-01, Muscle-02, Muscle-03, Muscle+, and Muscle -) had non-significant ($p>0.05$) differences in TBARS values at day 1 of storage.

The lower TBARS value of positive control samples (0.1% BHA) and samples marinated in a higher MOLE solution could be due to polyphenolic compounds present in MOLE exerting antioxidant activity (Awad *et al.*, 2022; El-Sayed *et al.*, 2017; Shah *et al.*, 2015; Yadav *et al.*, 2022). The higher antioxidant potential of *Moringa oleifera* leaves and its extract was reported in pork patties (Muthukumar *et al.*, 2014), buffalo meat products (Kenawi and El-Hameed, 2018), cooked meat patties (Das *et al.*, 2012) and goat meat nuggets (Rahman *et al.*, 2020).

3.5. Shear force values

Various goat muscle samples were water-bath cooked samples were measured on day 3 of storage. Marination with MOLE had a significant ($p<0.05$) effect on the mean shear force value of LD muscle (Table 6). The mean shear force value of the LD-03 sample was recorded as the lowest and comparable to other MOLE-incorporated samples of LD muscle. The highest shear force value was recorded for the LD- sample.

Table 6. Shear force values (kg) of goat muscles marinated with *Moringa oleifera* leave extract over 3 days of refrigerated storage (Mean ±SE)

Shear force	Day 3
LD	
LD-01	3.558 ± 0.43^{A}
LD-02	3.508 ± 0.01^{A}
LD-03	3.572 ± 0.15^{A}
LD+	3.787 ± 0.76^{B}
LD-	5.201 ± 1.30^{C}
IS	
IS-01	3.107 ± 0.87^{B}
IS-02	2.814 ± 0.22^{A}
IS-03	2.956 ± 0.88^{A}
IS+	4.807 ± 0.52^{C}
IS-	5.508 ± 1.34^{D}
SM	
SM-01	2.786 ± 0.01^{A}
SM-02	2.657 ± 0.32^{A}
SM-03	$2.606\pm.21^{A}$
SM+	2.707 ± 1.5^{A}
SM-	3.979 ± 1.83^{B}
BF	
BF-01	3.978 ± 0.33^{C}
BF-02	3.219 ± 1.40^{B}
BF-03	2.681 ± 0.43^{A}
BF+	5.452 ± 0.09^{D}
BF-	5.597 ± 1.43^{D}

N=6, Mean with different superscripts in A, B, C differed significantly ($P < 0.05$) within a column-wise for a muscle type; LD: longissimus dorsi m.; IS: infraspinatus m.; SM: semimembranous m.; BF: bicep femoris m., Muscle-1: muscle with 0.10% MOLE, Muscle-2: muscle with 0.50% MOLE, Muscle-03: muscle with 1.0% MOLE, Muscle-: muscle without BHT and MOLE, Muscle+: muscle with 0.10% BHT

Among IS muscle samples, the mean value for IS+ was recorded highest and significantly ($p<0.05$) higher shear force value than all other treatment groups of the same muscle. IS-02 and IS-03 samples had comparable ($p>0.05$) shear force values and were significantly ($p<0.05$) lower than the IS-01 sample. There was a non-significant ($p>0.05$) variation in mean shear

force value recorded for SM muscle samples except SM- which exhibited a significantly ($p<0.05$) higher value. The mean shear force value for BF-03 and BF- muscle was recorded as non-significant ($p<0.05$), but significantly ($p<0.05$) higher than all remaining samples of BF muscle.

The tenderness of the meat was measured by shear force value. The goat muscle samples treated with MOLE extract had lower shear force value as compared to positive and negative control samples. The lowest shear force and thus highest tenderness of goat muscle sample with the highest level of MOLE (1.0%) was recorded for all muscle samples as compared to their other respective samples viz., control, positive control, muscle-01, and muscle-02. This could be due to the proteolytic enzymes present in MOLE extract as well as the higher amount of calcium content in *Moringa oleifera* powder (Dania *et al.*, 2014; Singh *et al.*, 2015). A higher calcium content increases the calpain enzyme actions thus improving the tenderness of the meat (Gerelt *et al.*, 2002).

3.6. Colour profile

The color profile of the various water bath-cooked goat muscle samples was assessed on day 3 of storage (Table 7). The lightness (*L**) of all muscle samples having MOLE concentration was recorded at lower values as compared to the positive and negative control samples. The redness (*a**) and yellowness (*b**) values of LD muscle with MOLE extract were reported higher as compared to positive and negative control samples.

Incorporating higher MOLE levels resulted in better retention of color attributes due to the antioxidant activity of MOLE extract owing to polyphenolic substances. The increasing MOLE concentration in the marinades causes an increase in the yellowness value of muscle samples. This result could be due to pigments in the MOLE. Further, the yellowness and redness were also affected by the grinding and drying methods (Ali *et al.*, 2017). The incorporation of MOLE was reported to improve color stability in raw chilled beef (Shah *et al.*, 2015) and in cooked buffalo meat (Hazra *et al.*, 2012).

Table 7: Color values of goat muscles marinated with *Moringa oleifera* leave extract (Mean ±SE)

Treatment	*L** (lightness)	*a**(redness)	*b** (yellowness)
LD			
LD-01	53.92 ± 0.07^{BC}	9.50 ± 0.10^{C}	16.56 ± 0.02^{B}
LD-02	50.86 ± 1.85^{A}	9.21 ± 0.61^{C}	16.78 ± 0.14^{B}
LD-03	52.49 ± 0.34^{AB}	8.22 ± 0.38^{B}	18.96 ± 0.29^{C}
LD+	55.77 ± 0.16^{C}	6.50 ± 0.32^{AB}	14.52 ± 0.10^{A}
LD-	55.69 ± 1.05^{C}	5.79 ± 0.15^{A}	14.87 ± 0.17^{A}
IS			
IS-01	49.52 ± 0.25^{A}	10.50 ± 0.18^{C}	16.12 ± 0.40^{B}
IF-02	54.79 ± 0.15^{C}	7.17 ± 0.13^{A}	18.20 ± 0.29^{C}
IS-03	51.68 ± 0.09^{B}	11.11 ± 0.60^{C}	18.65 ± 0.12^{C}
IS+	55.41 ± 0.20^{C}	6.97 ± 0.05^{A}	15.08 ± 0.28^{AB}
IS-	55.64 ± 0.34^{C}	8.07 ± 0.14^{B}	13.99 ± 0.03^{A}
SM			
SM-01	49.21 ± 1.39^{C}	8.66 ± 0.50^{AB}	13.34 ± 0.54^{AB}
SM-02	44.57 ± 1.24^{B}	12.01 ± 0.15^{D}	16.39 ± 0.73^{C}
SM-03	40.64 ± 0.50^{A}	11.62 ± 0.67^{CD}	15.71 ± 0.39^{C}
SM+	49.39 ± 1.75^{C}	10.00 ± 0.80^{B}	12.58 ± 0.26^{A}
SM-	51.38 ± 0.40^{D}	7.63 ± 0.21^{A}	13.90 ± 0.43^{B}
BF			
BF-01	46.45 ± 0.24^{A}	8.53 ± 0.20^{C}	13.57 ± 0.08^{D}
BF-02	48.31 ± 0.38^{B}	6.08 ± 0.61^{B}	13.73 ± 0.50^{D}
BF-03	56.15 ± 0.92^{C}	9.41 ± 0.27^{C}	12.91 ± 0.09^{CD}
BF+	57.49 ± 0.22^{D}	5.82 ± 0.27^{AB}	11.39 ± 0.24^{B}
BF-	56.29 ± 0.63^{C}	5.26 ± 0.12^{A}	8.17 ± 1.20^{A}

N=6, Mean with different superscripts in A, B, C differed significantly ($P < 0.05$) within a column-wise for a muscle type; LD: longissimus dorsi m.; IS: infraspinatus m.; SM: semimembranous m.; BF: bicep femoris m., Muscle-1: muscle with 0.10% MOLE, Muscle-2: muscle with 0.50% MOLE, Muscle-03: muscle with 1.0% MOLE, Muscle-: muscle without BHT and MOLE, Muscle+: muscle with 0.10% BHT

4. Conclusions

Thus, it can be concluded that goat muscles marinated in a solution having higher levels of aqueous extract of *Moringa oleifera* leaves (1.0%) retained better quality characteristics such as color, decreased shear force, and lower thiobarbituric acid reacting substances during 7 days of storage under refrigeration conditions. *Moringa oleifera* leaves extract, due to its polyphenolic content, could be used as a natural

preservative in improving the oxidative stability of goat meat. The incorporation of aqueous extract 1.0 % Moringa oleifera leaves extract had a comparable preservative effect similar to the synthetic preservative used in the present study (0.1% BHA).

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Acknowledgment

The authors acknowledge the Geran Putra—IPS (Vote No.: 9687700) funded by the Universiti Putra Malaysia (UPM) as the financial grant for the research work of Alzaidi Mohammed Awad.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

MICROWAVE DRYING OF TOMATO SLICES: AN EVALUATION OF ARTIFICIAL NEURAL NETWORK (ANN) AND ADAPTIVE NEURO-FUZZY INFERENCE SYSTEM (ANFIS) MODELS

Hussein J.B.[1], Oke M.O.[2]✉, Ajetunmobi-Adeyeye R.I.[2] and Adegoke A.F.[3]

[1]Department of Food Science and Technology, Modibbo Adama University, Yola, Adamawa State, Nigeria.
[2]Department of Food Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[3]Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
✉*mooke47@lautech.edu.ng*

https://doi.org/10.34302/crpjfst/2024.16.3.10

Article history:
Received:
December 29th, 2023
Accepted:
September 1st, 2024

Keywords:
Microwave Drying;
Tomato Slices;
ANN;
ANFIS;
Modelling.

ABSTRACT

This study used two methodologies to model microwave drying kinetics of tomato slices: artificial neural networks (ANN) and the adaptive neuro-fuzzy inference system (ANFIS). The tomatoes were pre-treated with water blanching (WBP), ascorbic acid (AAP), and sodium metabisulphite (SBP). The tomatoes were then dried in the microwave at 90, 180, and 360 W after being sliced into 4-, 6-, and 8-mm thicknesses. After fitting ANN and ANFIS models to the experimental drying data, the optimal model topology was identified. The predictive accuracy of these models was assessed through these metrics: the coefficient of determination (R^2), mean squared error (MSE), root mean squared error (RMSE), and mean absolute error (MAE), by contrasting the projected results with experimental data. The results showed a range of 0.92 to 3.75 h for drying time, 0.28 to 2.86 x 10^{-8} m^2/s for D_{eff}, and 0.0027 to 0.0063 kWh/kg for SEC. The results indicated a high-performance capacity of ANFIS compared to ANN, with a higher R^2 of 1.0000 and a lower MSE of 0.9999 to 1.0000, an RMSE of 1.45x10^{-11} to 0.00309, and a MAE of 1.15x10^{-11} to 0.00296. Consequently, the ANFIS model demonstrated superior predictive capabilities compared to the ANN model, achieving a strong fit with the observed data.

1. Introduction

Tomatoes (*Solanum lycopersicum* L.var) hold a crucial position in human diets and contribute significantly to a country's economic growth. They are a rich source of essential nutrients and offer various health benefits (Hussein et al., 2019). Nonetheless, their perishable nature, characterized by high moisture content, increased respiration rate, and soft texture, renders them susceptible to spoilage and presents challenges in terms of transportation. To address these issues, drying has become a widely adopted method for extending the shelf life of tomatoes. Traditional drying methods include sun drying and solar drying, which are relatively cost-effective and straightforward. However, these methods come with several drawbacks, such as prolonged drying times, low energy efficiency, and considerable loss of food quality, including colour degradation and nutrient loss. Therefore, there is a need for more advanced and efficient methods to dry and preserve food.

Microwave drying serves as an alternative approach to address these challenges. This method offers advantages such as reduced drying time, enhanced product quality, and decreased energy consumption, as pointed out

by Darvishi et al. (2013) and Hussein et al. (2019). According to Wray and Ramaswamy (2015), this technique uses microwaves to speed up the drying process by creating volumetric heating. But just like traditional drying, microwave drying uses the disparity in water vapour pressure between the material's interior and outside to propel the transfer of moisture. This complexity results in the microwave drying of tomato fruit being an intricate thermal process involving the simultaneous transfer of heat and moisture. As a result, it demands a significant amount of energy and time.

The complexity of microwave drying has given rise to a variety of mathematical models, both empirical and semi-empirical, to predict the drying patterns of tomatoes. Nevertheless, empirical and semi-theoretical models are generally only applicable within the specific temperature, air velocity, and humidity ranges for which they have been developed (Tohidi et al., 2012). Consequently, they cannot serve as universal correlations for a wide range of drying parameters. In contrast, theoretical models are derived from solutions to partial differential equations based on heat and mass balance principles. While theoretically sound, these models often involve complex calculations and rely on assumptions that may not align with real-world drying systems. As a result, there is a clear need for predictive models to better understand and anticipate drying kinetics. These models can greatly improve the efficiency of the drying process and the quality of the final products. With more precise predictions, they can assist in optimizing drying conditions and enhancing the performance of drying systems.

Artificial Neural Network (ANN) is a versatile mathematical model that draws inspiration from human perception. It has proven to be a useful tool for tackling challenging and nonlinear problems, particularly in the field of drying systems (Oke et al., 2017). Its high learning capacity and ability to discern input and output relationships make it well-suited for modelling the intricate, nonlinear dynamics involved in the drying process. Moreover, ANN enables precise management of the drying process in industrial settings. A significant advantage of ANN over conventional mathematical models is its ability to process vast quantities of noisy data from dynamic and nonlinear systems, particularly when the underlying physical relationships are not completely understood (Aghbashlo et al., 2015). Indeed, numerous studies have utilised ANN to predict drying kinetics in different drying processes. For example, Pedren et al. (2005) employed ANN to predict the drying kinetics in microwave-assisted drying. Poonnoy et al. (2007a) used ANN to model the temperature and moisture content of tomato slices during microwave-vacuum drying. Similarly, Poonnoy et al. (2007b) used ANN and regression models to estimate the moisture ratio in mushrooms during microwave-vacuum drying. Sarimeseli et al. (2014) used the ANN model to describe how thyme leaves dry quickly in microwaves. Bai et al. (2018) also used the ANN model to predict how Ginkgo biloba seeds dry quickly in microwaves and how their colour changes during drying. The examples provided underscore the efficiency of ANN in diverse drying applications. They showcase ANN's capability to optimize drying procedures and enhance the quality of the resulting dried products.

The Adaptive Neuro-Fuzzy Inference System (ANFIS) is an integrated modelling approach that combines the strengths of Artificial Neural Networks (ANN) and Fuzzy Inference Systems (FIS). According to Oke et al. (2018), by merging the benefits of both methods, ANFIS provides a robust solution for tackling complex and nonlinear issues, yielding superior outcomes. Kaveh et al. (2021) highlighted the ability of ANFIS to simulate nonlinear systems with high precision and reduced modelling time, distinguishing it among computational models. ANFIS has been applied in numerous studies to model the drying characteristics of a variety of agricultural products. For example, Bousselma et al. (2021) employed ANFIS to model the microwave drying of pre-treated whole apricots. Zadhossein et al. (2021) utilized the ANFIS system to forecast the exergy and energy aspects

of drying cantaloupe slices in a microwave dryer. Ojediran et al. (2020) employed ANFIS to predict the drying kinetics of yam slices. In another study, Jahanbakhshi et al. (2020) applied the ANFIS system to model various aspects of drying pistachio kernels, including speed, effective moisture diffusivity, specific energy consumption, shrinkage, and colour, during microwave heating followed by ultrasound treatment. The analysis results offer significant insights into the ideal conditions for microwave drying with ultrasonic pretreatment. These examples highlight the flexibility and efficiency of ANFIS in modelling the drying processes for various agricultural products.

ANN and ANFIS models have been used successfully to predict the drying kinetics of other agricultural products, but there is a significant gap in the literature when it comes to using them to predict the drying kinetics of microwave-dried tomatoes. This study aims to bridge this gap by creating both ANN and ANFIS models designed specifically for forecasting the drying kinetics of microwave-dried tomatoes, offering a more precise and effective prediction method. By utilizing these advanced modelling techniques, this research seeks to improve the accuracy and efficiency of predicting the drying behavior of tomatoes under microwave drying conditions. The findings from this study can provide valuable insights for optimizing the drying process and enhancing the quality of microwave-dried tomatoes in the food industry.

2. Materials and methods

2.1. Materials

The tomatoes used in this study were UTC varieties, obtained from the Teaching and Research Farm at Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. The selection of these tomatoes was based on visual appearance, firmness, and size uniformity, ensuring they met the quality and consistency standards for commercial production.

2.2.1. Samples

Initially, the tomatoes were meticulously cleaned by washing under tap water, then rinsed with distilled water, and dried with a tissue towel, adhering to the method outlined by Hussein et al. (2016). Each pretreatment involved 12 kg of tomatoes. Three separate pretreatment methods were employed: one minute of water blanching pretreatment (WBP), five minutes of 5% w/v sodium metabisulphite pretreatment (SMP), and one minute of 5% w/v ascorbic acid pretreatment (AAP). The 1:10 (w/v) ratio of tomatoes to the dipping solution, as used by Hussein et al. (2019), was retained. Subsequent to pretreatment, the tomatoes were cut into slices of three varying thicknesses: 4 mm, 6 mm, and 8 mm, utilising a Tomato Slicer (NEMCO 56610-13/16" Roma).

Table 1. Layout of Taguchi experimental design L9 (3x3)

Test runs	Coded independent variables			Variables of experiment in their natural units		
	Pretreatment	Thickness (mm)	Microwave power (W)	Pretreatment	Thickness (mm)	Microwave power (W)
1	1.0	1.0	1.0	WBP	4	90
2	1.0	2.0	2.0	WBP	6	180
3	1.0	3.0	3.0	WBP	8	360
4	2.0	1.0	2.0	AAP	4	180
5	2.0	2.0	3.0	AAP	6	360
6	2.0	3.0	1.0	AAP	8	90
7	3.0	1.0	3.0	SMP	4	360
8	3.0	2.0	1.0	SMP	6	90
9	3.0	3.0	2.0	SMP	8	180

2.2.2. The Taguchi experimental plan and the drying process

The Taguchi experimental design was developed using Minitab 16 software, which is tailored to handle three factors at three distinct levels. This approach led to the creation of an L9 (3x3) array, resulting in nine experimental runs. These runs, detailed in Table 1, evaluated the drying kinetics, considering the interactions among pretreatment, slice thickness, and microwave power. The drying was performed with a BOSCH 25 L compact microwave oven (model number: HMT84G451), which has a maximum power of 900 W and operates at a frequency of 2450 MHz. The drying procedure followed the procedures set forth by Hussein et al. (2019).

2.2.3. Determination of drying kinetic of the dried tomato slices

The methodology outlined by Hussein et al. (2022) was employed to calculate the drying time and moisture ratio. The effective moisture diffusivities were determined based on molecular diffusion, the primary mechanism for moisture transfer within the food material. Assuming a uniform initial moisture distribution, negligible external resistance to moisture migration, and moisture release from both bottom surfaces of the tomato slices, the equation used by Workneh and Oke (2013) was utilised.

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2 D_{eff}\pi^2}{4L^2}t\right) \quad (1)$$

where;

Deff denotes the effective moisture diffusivity in dried tomato slices (m^2/s).

t represents the drying time (in seconds).

n is a positive integer.

L indicates the total thickness of the tomato slice (measured on a non-perforated dish).

It is crucial to recognise that the first term of Equation 1 gains significance with the increase in drying time. Consequently, the simplified expression is as follows:

$$MR = \frac{8}{\pi^2}\exp\left(-\frac{D_{eff}\pi^2}{4l^2}t\right) \quad (2)$$

The aforementioned equation can be further reduced to the form of a linear equation;

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{D_{eff}\pi^2}{4l^2}t\right) \quad (3)$$

The determination of the effective moisture diffusivity in dried tomato slices involved plotting the natural logarithm of the moisture ratio (ln MR) against the drying time. The resulting straight line's slope provided the necessary calculation for the effective moisture diffusivity;

$$slope = -\frac{D_{eff}\pi^2}{4l^2} \quad (4)$$

Therefore, with the thickness of the tomato slices and the slope derived from the aforementioned plot, the moisture diffusivity was calculated. The Specific Energy Consumed (SEC) for the microwave oven drying of tomato slices was estimated following the method described by Hosain (2012).

$$SEC = \frac{Pt_{on}}{M_w} \quad (5)$$

where;

SEC represents the specific energy consumption for microwave drying, measured in kWh per kg of water evaporated.

P denotes the power of the microwave in watts (W).

t_{on} refers to the total duration for which the microwave power is on, measured in hours (h).

Mw is the mass of water that has been evaporated, measured in kilograms (kg).

2.2.4. ANN and ANFIS modelling design

In order to model ANN and ANFIS, the Neural Network Toolbox 8.0 in MATLAB was used, in accordance with the method described by Hussein et al. (2023). The efficacy and performance of the model were assessed using various metrics, as outlined by Hussein et al. (2022) and Nazghelichi et al. (2011), including the following: mean square error (MSE), root mean square error (RMSE), mean absolute error (MAE), and coefficient of determination (R^2).

3. Results and discussions

3.1. Drying kinetics of the microwave dried tomato slices

The required drying time to decrease the moisture content in pretreated tomato slices from 15.67% to 0.12% varied between 0.92 and 3.75 hours. The longest drying time was recorded for the experiment using water blanching (WBP), a 4 mm slice thickness, and 90 W of microwave power. Conversely, the shortest drying time was achieved with sodium metabisulphite (SMP), a 4 mm slice thickness, and 360 W of microwave power. A higher microwave power and increased exposure time were noted to significantly reduce moisture content, thus decreasing drying time, corroborating the findings of Talih et al. (2017). These results suggest that the choice of pretreatment method and microwave power level can have a significant impact on the efficiency of the drying process for tomato slices.

The effective moisture diffusivity (D_{eff}) values for pretreated tomato samples varied from 0.28 to 2.86 x 10^{-8} m^2/s. The highest D_{eff} value was observed in the experimental run with water blanching (WBP), an 8 mm slice thickness, and 360 W microwave power, while the lowest was in the run with water blanching (WBP), a 4 mm slice thickness, and 90 W microwave power. These D_{eff} values fall within the range of 10^{-12} to 10^{-8} m^2/s reported by Doymaz (2010) for various agricultural products. They are also in line with the findings of other studies, such as Zarein et al. (2013), who reported values for apple dried at 200 W to 600 W (ranging from 3.79 x 10^{-7} to 22.7 x 10^{-7} m^2/s), Mahdhaoui et al. (2014) for olive fruit dried at 90 W to 900 W (ranging from 5.96 x 10^{-9} to 13.00 x 10^{-9} m^2/s), and Workneh and Oke (2013) for microwave-assisted hot-air dried tomato slices at 1.13 - 3.11 W/g plus hot air at 50°C (ranging from 1.68 x 10^{-9} to 4.77 x 10^{-9} m^2/s). These studies collectively demonstrate the impact of different drying methods and power levels on the effective moisture diffusivity of various agricultural products. The range of values reported highlights the importance of optimising drying conditions for specific products to achieve desired results. It also provides valuable insights for optimising drying processes in the agricultural industry.

The mass transfer process during microwave oven drying of tomatoes is complex. As microwave power penetrates the slices, it generates internal heat. This creates a vapour pressure gradient within the slice, enabling moisture to move gently to the surface and evapourate. According to Hussein et al. (2019), this leads to a moisture pumping effect that pushes water out, preventing case hardening. The study suggests that effective diffusivity rises with increased microwave power and thinner tomato slices, a finding that echoes Sadin et al. (2014) regarding infrared drying of tomatoes. Notably, the intricate interplay between microwave energy, heat, and moisture migration can enhance drying efficiency and uniformity. Nonetheless, factors such as microwave power, tomato characteristics, and slice preparation can influence the effectiveness of this method.

The Specific Energy Consumption (SEC) ranges from 0.0027 to 0.0063 kWh/kg. The minimum SEC was recorded for SMP pretreatment with 6 mm slices at 90 W microwave power, whereas the maximum SEC was noted for WBP pretreatment with 8 mm slices at 360 W microwave power. Notably, an increase in slice thickness and microwave power corresponded to a rise in SEC, indicating that factors leading to higher energy inputs also escalated the SEC. This observation is consistent with prior research by Chayjan (2012) on potato slices and Pillai et al. (2010) on plaster of Paris.

The type of pretreatment applied significantly affected the SEC, with SMP pretreatment showing a more pronounced effect compared to AAP and WBP pretreatments. This suggests that pretreatment effectively lowers the specific energy needed for drying tomato slices, thus decreasing energy consumption during the process, which is beneficial. It corroborates the initial observation that pretreatment aids in the drying of tomato slices and shortens the drying

time. Consequently, applying pretreatment before drying tomatoes could enhance energy efficiency, particularly in areas where energy costs are substantial. This result aligns with the findings of Tunde-Akintunde et al. (2014) regarding bell pepper drying. The study also highlights the importance of considering pretreatment methods in the overall energy optimisation of food drying processes.

3.2. Modelling the effect of drying conditions on the drying kinetic of microwave dried tomato slices using ANN

Exploration of various neural network topologies and neuron counts in the hidden layer was conducted to identify the optimal configuration for drying kinetics. The architecture with a 3-9-1 topology was found to be the most effective for modelling drying time and moisture diffusivity. In contrast, an ANN structure with 11 neurons in the hidden layer demonstrated superior performance for Specific Energy Consumption (SEC).

Figure 1 depicts the optimal ANN model structure for predicting drying time and moisture diffusivity, which includes three inputs (pretreatment, slice thickness, and microwave power), a hidden layer with 9 neurons employing a logarithmic sigmoid activation function, and a tangent sigmoid function at the output layer. Conversely, the best structure for SEC features a single hidden layer with 11 neurons and a tangent sigmoid activation function at both the hidden and output layers.

It is significant to mention that Lertworasirikul and Tipsuwan (2008) reported a comparable network setup featuring nine neurons in the hidden layer and a logarithmic sigmoid transfer function in the initial layer for the drying of semi-finished cassava crackers. This indicates that the selected architecture has proven successful in modelling drying processes across different applications. Moreover, the consistent use of this architecture suggests its versatility and effectiveness in capturing the complex dynamics of drying phenomena. It may be beneficial for future studies to explore its applicability in other food drying processes.

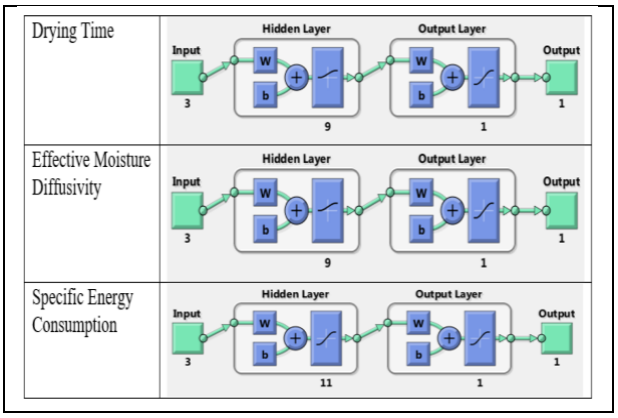
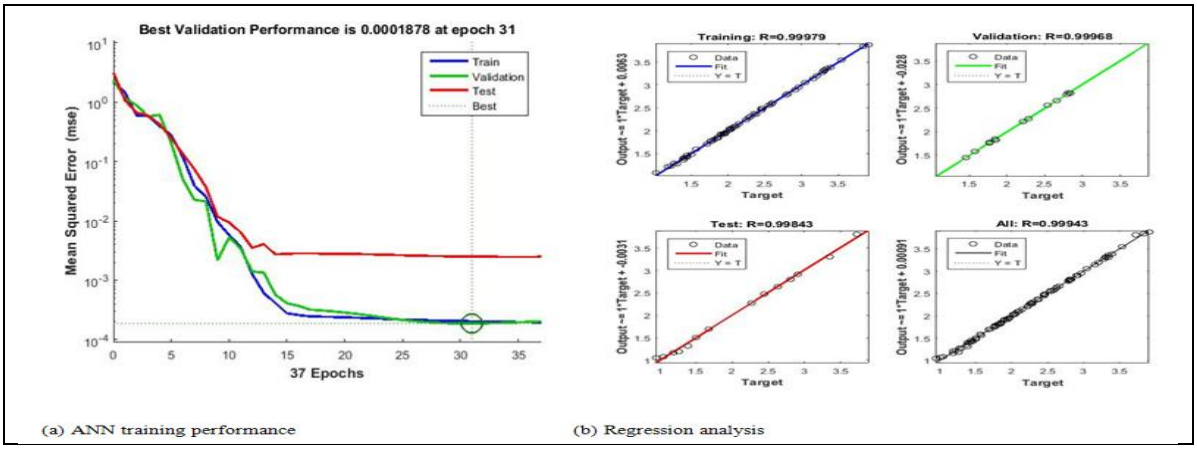


Figure 1. The optimised ANN model for drying kinetic (drying time, D_{eff} and SEC) of microwave dried tomatoes

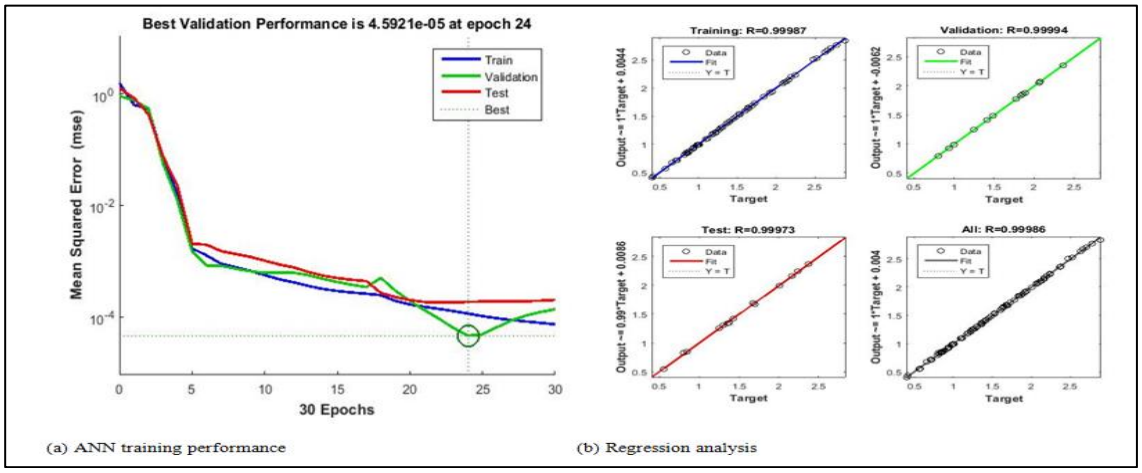
The ANN model was simulated to find the best architecture. This was based on getting a low mean squared error (MSE) and a high correlation coefficient (R). Figure 2 displays the ANN's performance during training and the regression analysis results for both the training and validation datasets concerning drying time. The training ceased after 37 epochs, as illustrated in Figure 2a, with the most appropriate ANN network identified at 31 epochs post-training. As shown in Figure 2b, the regression analysis of the ANN model's performance showed that its predictions were very good. The R values were 0.99979 for training, 0.99968 for validation, 0.99843 for testing, and 0.99943 for the overall dataset.

Furthermore, the MSE value, a measure of the model's accuracy, was exceptionally low at 31 epochs, with a value of 0.00019, affirming the effectiveness and precision of the chosen optimal architecture in predicting drying time. These results suggest that the ANN model performed exceptionally well in predicting drying time, achieving a high level of accuracy and reliability in its forecasts, both during training and when applied to unseen data.



Figures 2. The performance of ANN training and regression analysis for both training and validation datasets for drying time.

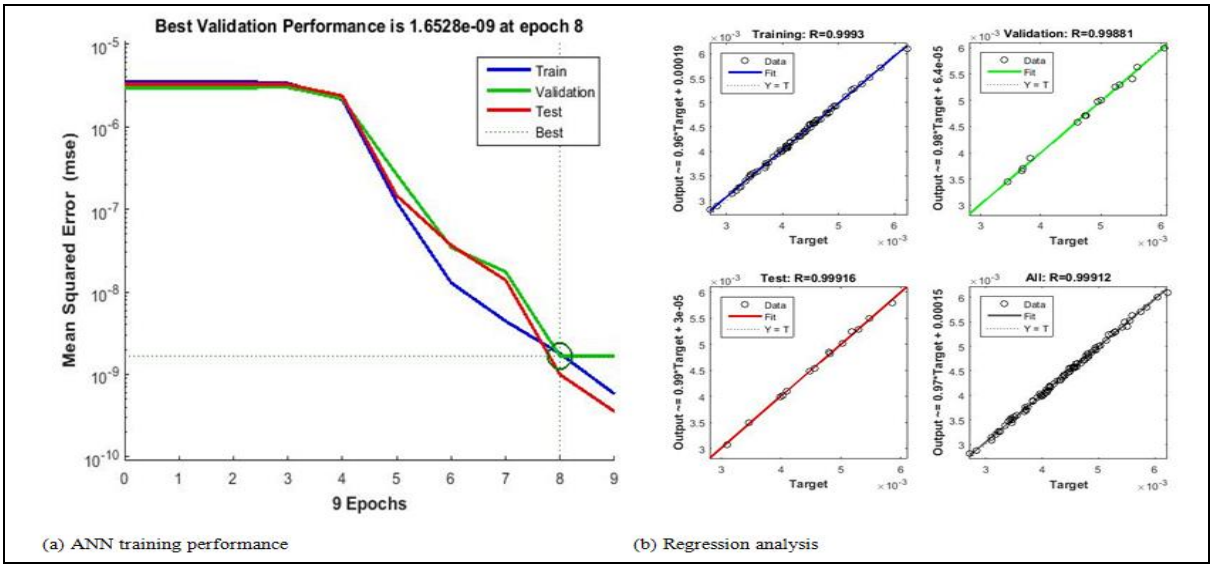
Figure 3 presents the training performance and regression analysis of the ANN concerning moisture diffusivity. Training ceased after 30 epochs, as depicted in Figure 3a, with the optimal ANN architecture emerging after 24 epochs. The regression analysis evaluated the correlation between the ANN's forecasts and the actual experimental data, showcasing the ANN model's remarkably precise predictive ability. The R was notably high, registering values of 0.99987 for training, 0.99994 for validation, 0.99973 for testing, and 0.99986 for all data, as shown in Figure 3b. Moreover, the MSE was determined to be 0.00005 at the 24th epoch for the chosen architecture. These results show that the ANN model is very good at predicting moisture diffusivity, showing that it is accurate and reliable across both training and validation data sets.



Figures 3. The performance of ANN training and regression analysis for training and validation datasets for the effective moisture diffusivity

Figure 4 illustrates the training performance and regression analysis of the ANN for SEC. The training was halted after nine epochs to avert overfitting, as shown in Figure 4a. Following this, the optimal ANN architecture was determined at the eighth epoch, prior to ceasing training. The regression analysis assessed the correlation between the ANN's predictions and the actual experimental data, confirming the model's accuracy and efficacy. The R remained impressively high, with values of 0.99930 for training, 0.99881 for validation, 0.99916 for testing, and 0.99912 for all data, as depicted in Figure 4b. Furthermore, the MSE was exceptionally low, with a value of 1.6528 x 10^{-9}, corresponding to the 8th training epoch for

the optimal ANN architecture. These findings indicate that the ANN model performed exceptionally well in predicting specific energy consumption, delivering highly accurate and reliable results for both the training and validation datasets, while also avoiding overfitting issues.

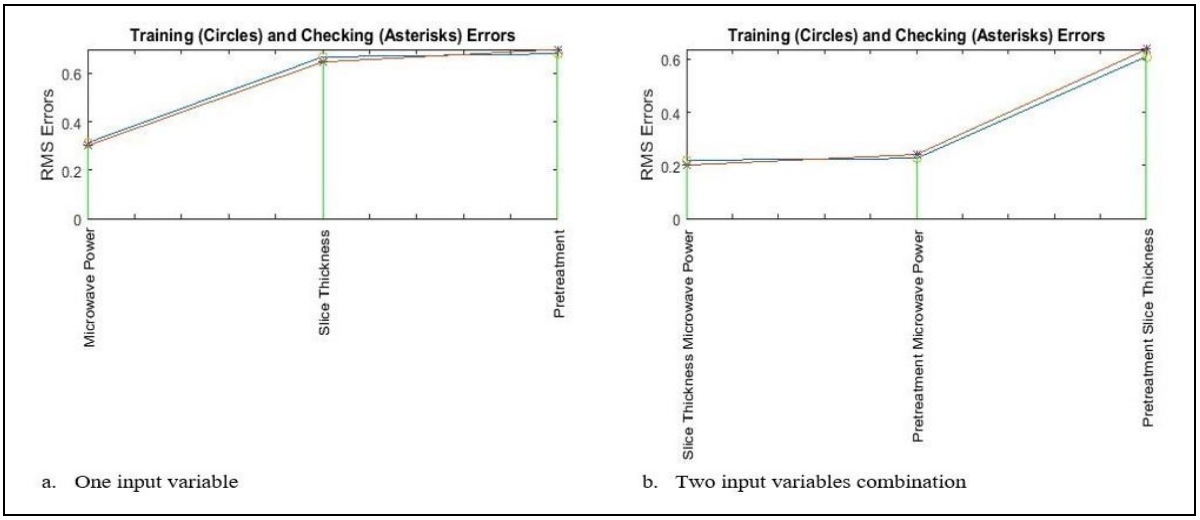


Figures 4. The performance of ANN training and regression analysis for training and validation datasets for the specific energy consumption.

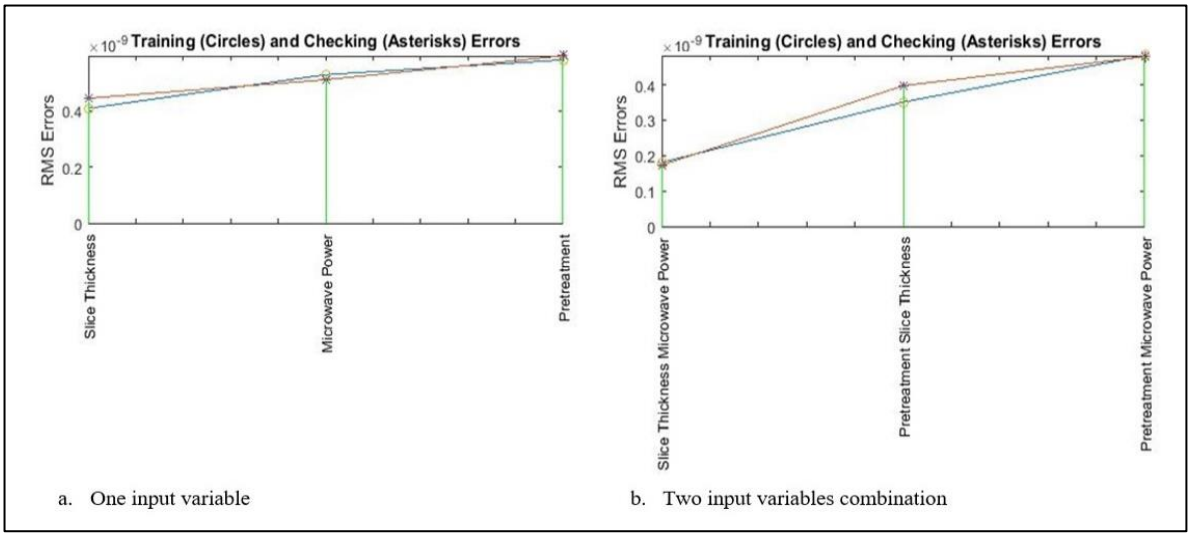
3.5. Modelling the effect of drying conditions on the drying kinetic of microwave dried tomato slices using ANFIS

An exhaustive analysis of experimental data was performed to determine the optimal combination of input variables influencing the drying kinetics of microwave-dried tomato slices using ANFIS. Analysing one input variable at a time for drying time, microwave power was found to have the lowest training error of 0.3127 and checking error of 0.3012 (Figure 5a), indicating its significant role in determining the drying time of pretreated tomato slices in microwave oven drying. Conversely, considering two input variables, the combination of slice thickness and microwave power yielded the smallest training error of 0.2194 and checking error of 0.2008 (Figure 5b), establishing their importance in affecting the drying time. These observations align with Horuz et al. (2017), who observed that microwave power penetrates the tomato slices, generating internal heat and vapour pressure that expels moisture to the surface for vaporation. Likewise, research by Kulanthaisami et al. (2010) and Hussein et al. (2019) supports that increased microwave power for a set thickness leads to reduced drying times.

With only one input variable, slice thickness had the lowest training error of 0.4082 and the highest checking error of 0.4440 for effective moisture diffusivity (Figure 6a). This shows that slice thickness has a big effect on the moisture diffusivity of microwave-dried tomato slices that have been pretreated. For two input variables, the combination of slice thickness and microwave power achieved the lowest training error of 0.1807 and a checking error of 0.1733 (Figure 6b), underscoring their importance in affecting the output performance. These observations are in line with research by Touil et al. (2014), Afolabi et al. (2014), and Onu et al. (2016), which suggests that the shorter distance moisture travels before evaporating has a significant impact on effective moisture diffusivity. Still, the big difference between the training errors and the checking errors (Figure 6) points to a possible overfitting problem during the exhaustive search. This is similar to what Aremu et al. (2014) and Oke et al. (2018) said about model development and validation.



Figures 5. The ANFIS exhaustive search illustrates the impact of one and two input variables on the drying time

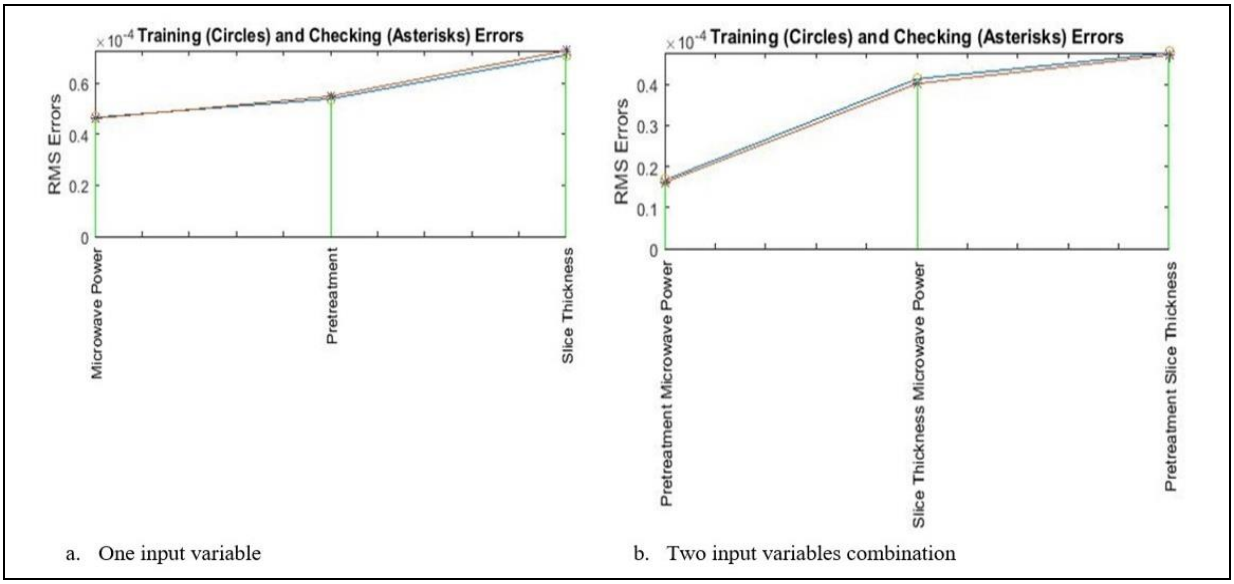


Figures 6. The ANFIS exhaustive search illustrates the impact of one and two input variables on the moisture diffusivity.

For SEC, with a single input variable, microwave power was found to have the lowest training and checking errors, both at 0.0005 (as shown in Figure 7a). This indicates that microwave power is the most significant variable in determining the SEC for pretreated tomato slices dried using a microwave oven. Conversely, with two input variables, the combination of pretreatment and microwave power yielded the lowest training and checking errors, both at 0.0002. Hence, these two variables were determined to be the most critical in influencing the output performance.

3.6. The ANN and ANFIS model's performance indices

Table 2 presents the performance metrics of the ANN and ANFIS models in predicting drying kinetics. It's evident that the ANN regression plot achieved an R^2 value greater than or equal to 0.9982, with RMSE and MAE values less than or equal to 0.02366 and 0.01496, respectively.



Figures 7. The ANFIS exhaustive search illustrates the impact of one and two input variables on the specific energy consumption.

Table 2. ANN and ANFIS drying kinetics prediction metrics

	Input Membership Function		Membership Function's Type		ANFIS's Regression Coefficient			ANFIS's Coefficient of Determination			ANN's Coefficient of Determination		
Quality Characteristics	**Input**	**Epoch**	**Input**	**Output**	**R**	**RMSE training**	**RMSE testing**	R^2	**RMSE**	**MAE**	R^2	**RMSE**	**MAE**
Drying Time	2-2-2	1000	gauss	linear	0.9999	0.00309	0.00296	1.0	0.00309	0.00296	0.9989	0.02366	0.01496
Effective Moisture Diffusivity	2-2-2	1000	tri, gbell and gauss	linear	1.0	1.45×10^{-11}	1.57×10^{-11}	1.0	1.45×10^{-11}	1.15×10^{-11}	0.9996	0.01225	0.00615
Specific Energy Consumption	2-2-2	1000	tri, gbell and gauss	linear	1.0	1.36×10^{-6}	1.30×10^{-6}	1.0	1.36×10^{-6}	9.95×10^{-7}	0.9982	0.00004	0.00003

These results demonstrate that the prediction model effectively simulated the experiments. The ANFIS model, employing two types of membership functions (gauss IMF and linear OMF), exhibited the highest predictive accuracy for drying time. Additionally, the ANFIS model with two types of MFs (tri, gbell, and gauss IMF) combined with linear OMF demonstrated superior predictive accuracy for moisture diffusivity and SEC. The correlation coefficients between ANFIS outputs and experimental data were exceptionally high (0.99999, 1.0, and 1.0) for drying time, moisture diffusivity, and SEC, respectively (Figure 8). The data points closely aligned with the ideal trend line in the regression plot indicate the model's suitability for predicting test data. Notably, ANFIS displayed very low RMSE values for both training ($\leq$ 0.00309) and testing ($\leq$ 0.00296), underscoring its ability to capture the fundamental relationships between input and output variables.

These results demonstrate the high accuracy and reliability of the ANFIS model in predicting drying time, moisture diffusivity, and SEC. The low RMSE values indicate that the model can effectively capture the complex interactions between the input and output variables, making it a valuable tool for further analysis and optimisation in this field. The simulation accuracy of the ANFIS model was evaluated to confirm its dependability. The regression plot from the simulation yielded an R^2 value of 0.9999 or higher, with RMSE and MAE values

at or below 0.00309 and 0.00296, respectively. As per Hussein et al. (2022), an R^2 value nearing one signifies a more accurate fit of the empirical model to the experimental data. Hence, given the high R^2 value along with minimal RMSE and MAE values, the ANFIS model is anticipated to simulate the drying kinetics of microwave-dried tomato slices effectively.

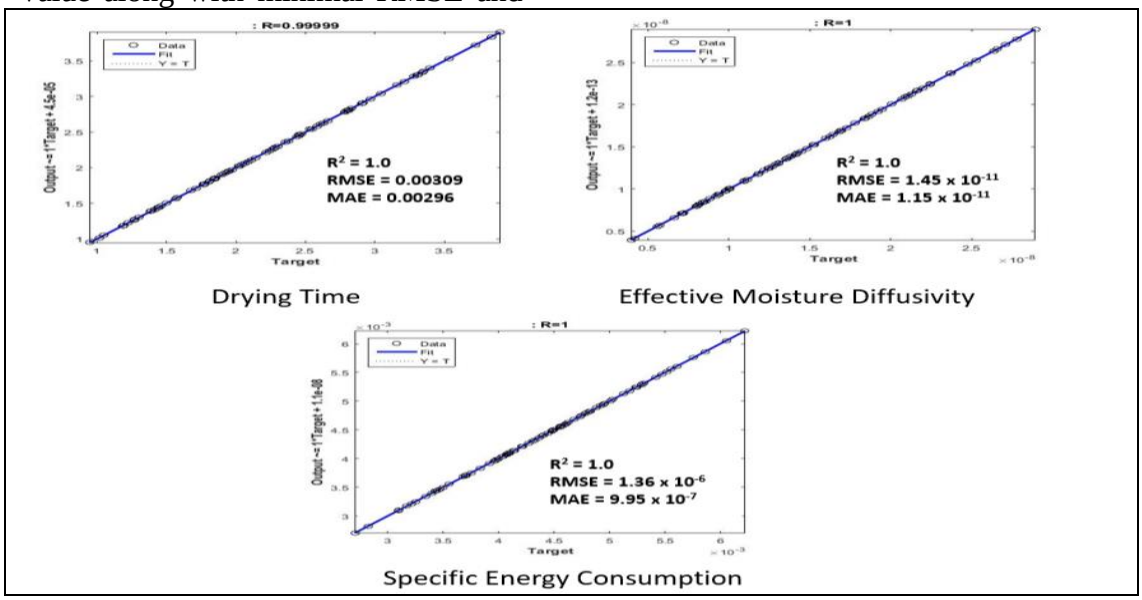


Figure 8. Regression between the experimental and predicted values for the drying kinetics

Figure 9 presents the ANFIS decision surface plot, which demonstrates the effects of pretreatment, thickness, and microwave power on drying time. The plot clearly shows that an increase in microwave power decreases the drying time, whereas a greater slice thickness leads to a longer drying time. Previous studies by Workneh and Oke (2013) and Onu et al. (2016) have indicated that higher microwave power produces more heat energy, which speeds up the movement of water molecules and enhances moisture diffusivity, thus shortening the drying time.

Furthermore, the WBP pretreatment has shown a more pronounced decrease in drying time compared to other methods. According to Kaymak-Ertekin (2002), this is due to the blanching heat's alteration of the cell membrane's physical properties, which shortens the drying time. This highlights the importance of optimising both parameters for efficient drying processes in food industry applications.

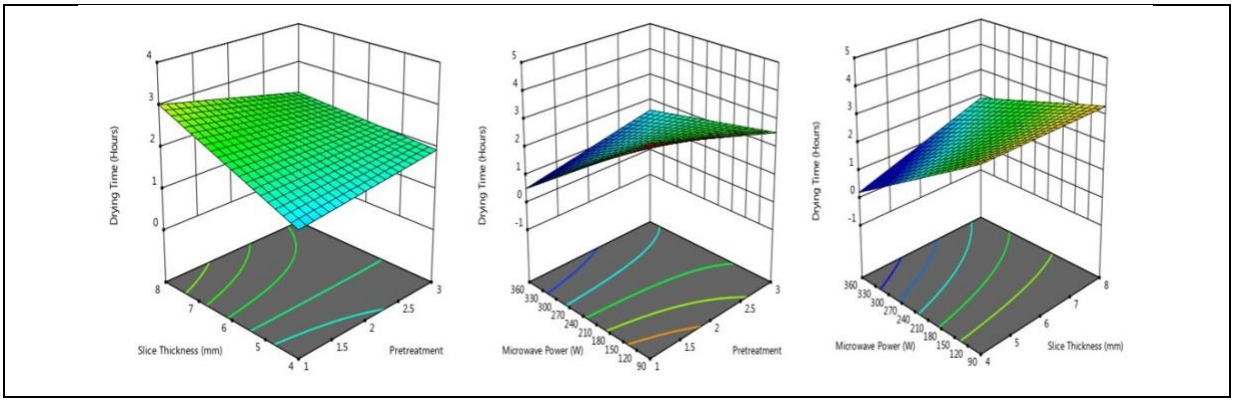


Figure 9. The ANFIS decision surface plot illustrates the impact of pretreatment, slice thickness, and microwave power on the drying time value.

The disruption of the cell membrane tissue by blanching heat allows for faster moisture evaporation during the drying process. This phenomenon is further enhanced by the higher microwave power, which intensifies the heat energy and accelerates the migration of water molecules, ultimately reducing the drying time even more significantly.

The ANFIS decision surface plot illustrated in Figure 10 demonstrates how pretreatment, thickness, and air temperature affect moisture diffusivity. The plot indicates that an increase in microwave power reduces moisture diffusivity, while a greater slice thickness also leads to lower diffusivity. Hussein et al. (2016) explain this effect by noting that water must traverse the slice's thickness from inside to the surface to evaporate. Similarly, Beigi (2016) and Torki-Harchegani et al. (2016) observed that higher microwave power enhances mass and heat transfer within the tomato slice, creating a larger vapour pressure gradient between the surface and core of the slice, thus accelerating water vapour diffusion.

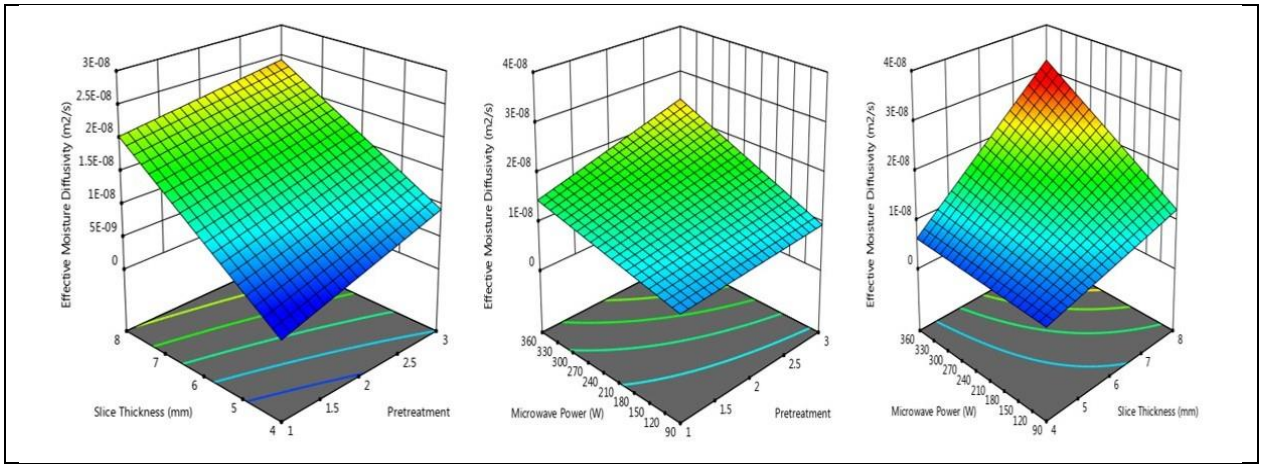


Figure 10. The ANFIS decision surface plot illustrates the impact of pretreatment, slice thickness, and microwave power on the moisture diffusivity value.

Furthermore, the WBP pretreatment exhibited higher moisture diffusivity compared to other pretreatments. According to Kaymak-Ertekin (2002), this improvement results from the blanching heat altering the physical properties of the cell membrane tissue, which shortens the drying time. The increased moisture diffusivity due to the WBP pretreatment can also be attributed to the breakdown of cell walls, which allows for easier movement of water molecules. Additionally, the shorter drying time achieved with WBP pretreatment can lead to improved product quality by preserving more of the tomato's natural colour and flavour.

Figure 11 presents the ANFIS decision surface plot, which shows the effects of pretreatment, thickness, and microwave power on the SEC. The plot indicates that an increase in microwave power correlates with a higher SEC, as does an increase in slice thickness. This is because the water has to move through the thickness of the slice from the inside to the surface to evaporate, requiring more energy. Moreover, samples pretreated with WBP showed the highest SEC, followed by those treated with AAP and SMP. This suggests that SMP pretreatment is effective in reducing the energy needed to dry tomato slices, leading to lower energy use in the drying process, which is beneficial. Similar results were observed by Sharma and Prasad (2006) in their research on microwave drying of garlic cloves and by Kumar et al. (2014) in their study of microwave-assisted hot-air drying of okra. Sharma and Prasad (2006) also found that microwaves' volumetric heating effect could decrease drying time, thus reducing SEC.

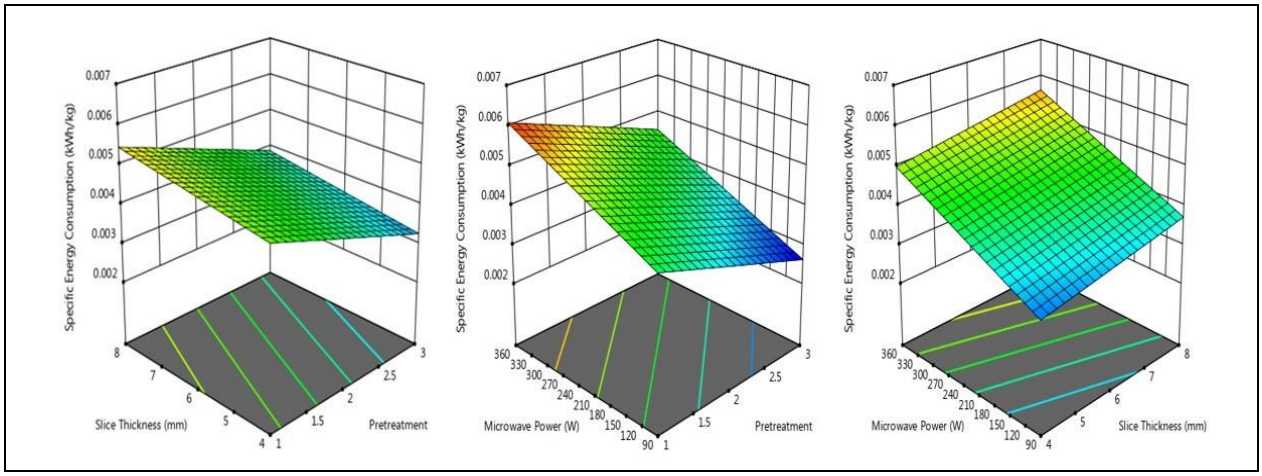


Figure 11. The ANFIS decision surface plot illustrates the impact of pretreatment, slice thickness, and microwave power on the specific energy consumption value.

These findings imply that microwave drying methods can decrease energy use and enhance drying efficiency. Furthermore, the volumetric heating effect of microwaves can shorten drying times, contributing to reduced specific energy consumption.

4. Conclusions

This study evaluated the drying characteristics of pretreated tomato slices in a microwave dryer, focussing on drying time, effective diffusivity (D_{eff}), and specific energy consumption (SEC). The most favourable results were observed under certain conditions: SMP pretreatment, 4 mm slice thickness, and 360 W microwave power resulted in a drying time of 0.92 hours, D_{eff} of 0.28 x 10^{-8} m^2/s, and SEC of 0.0027 kWh/kg. Similarly, WBP pretreatment with 4 mm slice thickness at 90 W and SMP pretreatment with 6 mm slice thickness at 90 W yielded optimal results. The study found that SEC increases with slice thickness and microwave power. Among the pretreatment methods (SMP, AAP, and WBP), SMP significantly reduced SEC. A comparison between the ANN and ANFIS models for parameter prediction showed that ANFIS was superior in modelling and optimising the drying process. The findings provide insights into the drying kinetics and energy efficiency of microwave-dried tomato slices, beneficial for both pilot and industrial-scale processing.

Additionally, these findings have the potential to mitigate postharvest tomato losses that often occur during bumper harvests. This can help reduce food waste and ensure a more sustainable food supply chain. Furthermore, the use of the ANFIS model can also contribute to cost savings and improved efficiency in tomato processing by accurately predicting drying times and optimising energy usage. Implementing this model in the industry can lead to increased productivity and reduced environmental impact, making it a valuable tool for sustainable food production.

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Acknowledgment

The authors acknowledge the financial support provided by Modibbo Adama University, Yola through TETFund PhD Study Fellowship Grant.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

SALEP MUCILAGE COATING USAGE FOR STUCK-POT RICE BASED ON POTATO AND EVALUATION THE EFFECTS OF FRYING OIL CONDITION

Kosar Mahmood-babooi[1], Mohammad Ekrami[2], Parisa Sadighara[1], Mohammadreza Rostami[1], Ebrahim Molaee-aghaee[1,3]✉

[1.] Division of Food Safety & Hygiene, Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2.] Department of Food Science, Technology and Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, Tehran, Iran

[3]Department of Food Science and Technology, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

✉emolaeeaghaee@tums.ac.ir

https://doi.org/10.34302/crpjfst/2024.16.3.11

Article history:
Received:
January 3rd, 2023
Accepted:
August 19th, 2024

Keywords:
Salep mucilage;
Frying oil;
Central-composite design;
Acrylamide;
Optimizing.

ABSTRACT

Coating hydrocolloids are appropriate barriers against carbon dioxide, oxygen and lipids, therefore the amount of absorbed oil can be decreased. Salep as a hydrocolloid source cultivated in western and northwestern Iran is known as a food and pharmaceutical substance. In the present research, Salep mucilage (SaM) was studied as a coating agent with the aim of declining oil absorption and increasing the amount of moisture of stuck-pot rice based on potato (SpP) by using a central-composite design. Salep mucilage concentration (0.75, 1 and 1.25% w/w), frying time (3, 4.5, 6 min) and frying temperature (160, 170 and 180°C) were the examined parameters of this research. The effects of frying oil type (Sunflower oil, corn oil, rice bran oil, canola oil, palm olein oil and hydrogenated vegetable oil as the frying media) and blanching (85°C, 3.5 min in hot water) were examined only at the optimum point. According to the results, optimal conditions for coating and frying processes obtained from RSM were 1.24% (w/w) SaM concentration, 3.6 min frying time at 162°C frying temperature. Salep mucilage can be used as a promising agent to coat deep-fat fried potatoes.

1. Introduction

Vegetable oils and fats as a nutritious foodstuff with a considerable consumption in the daily diet, have a key role in human health, such as in prostaglandins, blood pressure, cholesterol level and the reproductive system by providing the essential fatty acids (Askarpour et al., 2023; Eghbaljoo et al., 2023; A. Kamkar et al., 2014). The frying process refers to a dehydration treatment with mass and heat transfer. Protein denaturation, rapid water evaporation, starch gelatinization, oil absorption and surface browning are some of the changes during frying (Wang, Su, Wang, & Nie, 2019). The unbound water in the product is evaporated and oil is replaced by up to 40% affecting the quality properties of the product (Isik, Sahin, & Oztop, 2018). This is a concern–that results in adverse health effects such as heart disease and obesity. Different factors including porosity, frying temperature, food composition (protein, fat and moisture contents), the quality of the used oil and the pre-treatment process have impacts on oil absorption (Santos *et al.*, 2019). Various products like potato strips, chickpeas, cereal products, chicken nuggets and carrot slices go through different processes to decrease oil absorption when in deep-fat frying (Belkova *et al.*, 2018). Some studies showed that in moderate protein and high carbohydrate foods

such as Asian potatoes, higher levels of acrylamide have been formed under heating and frying conditions (A Kamkar et al., 2015). To achieve the goal, frying is done under special conditions at high temperatures and short time and benefits from pre-treatments like coating, blanching, immersing in an acidic solution, pulsed electric field and semi-drying in fried potatoes-based products (Ananey-Obiri *et al.*, 2018). In recent years, biodegradable coating has been used widely for different purposes such as in films and or improving food properties (Molaee Aghaee, Kamkar, Akhondzadeh Basti, Khanjari, & Kontominas, 2016). Coating hydrocolloids such as carbohydrates and plant gums are suitable barriers against carbon dioxide, oxygen and lipids, therefore they can play an acceptable role in decreasing the amount of absorbed oil (Eghbaljoo et al., 2022; Naghavi, Dehghannya, & Ghanbarzadeh, 2018a).

Salep as a new hydrocolloid source cultivated in western and northwestern Iran in two types (Branched and rounded) is known as a food and pharmaceutical substance. It contains starch (2.7%) ash (2.4%) and nitrogen compounds (5%) and can be used in different food formulations such as desserts and ice cream (Ekrami & Emam-Djomeh, 2014). Salep contains glucomannan (16-55%) so, the mucilage with viscoelastic properties is used as a gelling agent at low temperatures and also functions as an emulsifier and thickening agent, hence capable of forming edible films and batters to develop different kinds of biodegradable packaging materials (Farhoosh & Riazi, 2007; Kurt, 2019).

Decreasing the amount of linolenic acid via mixing or hydrogenating which means a change in the composition, leads to more stability in the frying process. Nowadays, genetic modification is a way to enhance the properties of products (Safaei, Aghaee, Khaniki, Afshari, & Rezaie, 2019) and alter the number of fatty acids (Uluata, McClements, & Decker, 2015). It has been proved that diminishing the levels of linolenic acid improves the stability of frying oils (Park & Kim, 2016). Oils with a high amount of oleic acid are accessible in the market. There is a great attempt in the agriculture sector to plant verities with high amounts of saturated fatty acids. Efforts to reduce linolenic acid and increase oleic and saturated acids look suitable, because these oils have some properties such as better frying stability, less polymerization, hydrolysis and oxidation. The impact of these alterations in fatty acid composition on foods fried in modified oils needs to be studied (Selani *et al.*, 2016). The vulnerability of fats and oils rich in omega (ω) fatty acids to thermal process effects, is a major concern in the food industry. Cooking oils provide a heat transfer medium which helps to improve the aroma and flavor of cooked food products. However, commercial cooking oils, rich in ω_6-fatty acids, often do not meet the thermal stability requirements and get rancid (Ghosh, Upadhyay, Mahato, & Mishra, 2019).

The chemical changes at high temperatures in fats and oils and their natural contaminants, result in polymerization, isomerization, oxidation, hydrolysis, or cyclisation reactions. The oxygen, moisture, trace elements and free radicals may promote these reactions. The amount of antioxidants in the oil may reduce, and products with lowered nutritional value and quality of the oil will be obtained (Quiles, Ramírez-Tortosa, Gomez, Huertas, & Mataix, 2002).

Considering rice as one of the major cultivated and consumed agro-products and a good source of carbohydrates and fibers, which almost provide the required calorie for a large population (Mohajer et al., 2024; Safaei et al., 2019), so, it is a relatively novel and essential approach to decrease oil absorption and increase the moisture content of stuck-pot rice. Therefore, this work aimed to use the Iranian Salep mucilage (SaM) as a new hydrocolloid source and coating agent for stuck-pot rice based on potato (SpP) and also study its effects during frying. Response surface methodology (RSM) was used to investigate the impacts of frying temperature, time and the SaM concentration on the properties of SpP. The impacts related to the type of oil during the frying process on the

stability and quality of stuck-pot rice based on potato were also studied.

2. Materials and Methods

Salep was provided during the flowering season (July) 2022 from the northwest (East Azerbaijan province) and west (Kurdistan province) of Iran. Six commercial oil (refined, bleached and deodorized) oil including corn oil, sunflower oil, rice bran oil, canola oil and hydrogenated vegetable oil palm olein oil were prepared from the local market. All chemical materials were provided by Sigma (St. Louis, Mo., USA.).

2.1. Preparation of coating solution

The SaM extraction was performed by the method demonstrated in our previous procedure (Ekrami & Emam-Djomeh, 2014). Various concentrations of SaM solution were prepared and SpP were coated by them. A mixture of powdered SaM (the size of the particles was less than 100 µm) and deionized water was provided and the achieved solution was stirred during the night to assure that the dissolution had been done completely.

2.2. Coating deep fat frying of SpP procedure

The potato variety used in this research was Monona. After storage at 13°C for a week, they were kept at 9°C for 2 weeks and the relative humidity of the place where potatoes were kept was 93-95%. The coating process lasted 30 s and drained for 15 min, respectively. Domestic rice cookers with an aluminum vessel (260×192×348 mm of capacity) were used. For every type of oil, the vessel was filled with 20 g of potato slices (4 mm thickness) at the bottom, rice pre-cooked and 40 mL oil and heated. After 15 min, the cookers were switched off and the oil was allowed to cool to room temperature at the end of the experimental process. After frying, samples wrapped on absorbing tissue paper were allowed to cool and stored in a freezer at -20°C before analysis.

2.3. Analytical methods

2.3.1. Color analysis

A colorimeter (Minolta CR 300 Series, Osaka, Japan) with 3 scales (a, b and L) was used to estimate the color of the chips. The scales for the reference sample were a*=0.2, b*= -2.6 and L*= 98.1. Various parts of each sample were taken at room temperature and the average of five measurements was calculated. The total color difference (ΔE), whiteness index (WI), browning index (BI) and yellowness index (YI) were calculated as follows (Hassannia-Kolaee, Khodaiyan, Pourahmad, & Shahabi-Ghahfarrokhi, 2016):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

Where L, a, and b color values were averaged. Also, the results were expressed as ΔE values in comparison with the control sample before frying (L=82.1, a=−3.8 and b=17.1).

2.3.2. Oil and water content

For each ground sample (5 g) after frying and cooling, the oil content was determined and reported as a percentage of dry matter weight by n-hexane solvent extraction using the Soxhlet method.

The water content was determined as the percentage of the difference between control and dried samples at 105°C for 3 h in an oven according to the following equation:

$$W_C\% = \frac{S_i - S_f}{S_i} \times 100 \quad (2)$$

Where W_C is the whole water content (%), S_i is the initial weight (g), and S_f is the final dry weight (g). All tests were conducted in triplicates.

2.3.3. Total antioxidant capacity (TAC) determination

TAC was determined by spectrophotometry and the extraction procedure was adopted by Granato *et al.*, (2018). One mL potassium acetate and 0.5 mL copper (II) chloride were mixed with 1 mL sample absorbance measurement of the resulting cuprous-neocuproine complex which was performed at

450 nm against a reagent blank prepared from pure distilled water (Granato *et al.,* 2018).

2.3.4. Lipid oxidation (LO-T) determination

The LO-T test was conducted according to the thiobarbituric acid (TBA) method with the modification described by Zainol *et al.,* (2003). Two mL of sample (with chloroform) was added to the thiobarbituric acid solution (2 mL). The mixture was then placed in a boiling water bath at 40°C for 15 min. After cooling, it was then measured at 532 nm with a UV/Visible spectrophotometer (Zainol, Abd-Hamid, Yusof, & Muse, 2003).

2.3.5. Total flavonoid content (TFC) determination

The TFC was determined according to Ekrami *et al.,* 2019 with some modifications, using quercetin as a standard of the calibration curve. About 0.5 mL (1 mg sample dissolved in 10 mL - methanol) was mixed at room temperature for 30 min with 1.5 mL of 2% (w/v) aluminum chloride and 1 mL of distilled water. The absorbance of the samples was recorded at 430 nm with a UV/Visible spectrophotometer (Ekrami, Emam-Djomeh, Ghoreishy, Najari, & Shakoury, 2019).

2.3.6. Total carotenoid content (TCC) determination

The absorbance of TCC in methanol after appropriate dilution was measured at 470 nm using a UV/Visible spectrophotometer (Fullerton, CA, USA) according to García-López *et al.,* 2016. Total carotenoids concentration (astaxanthin) in the extracts was measured by external calibration with astaxanthin standards in the same solvent (García-López, Pérez-Martín, & Sotelo, 2016).

2.3.7. Sensory evaluation

The samples were prepared and displayed homogeneously. Overall ratings of texture, odor, color and appearance were used for estimating the acceptable sensory quality of SpP. The mean value of each parameter was measured followed by a ranking test including 40 females and 20 males with a range of 22-50 years old. To evaluate the samples, a nine-point hedonic scale was used, where score 1 stands for most disliked and score 9 represented most liked. Scores higher or equal to 5 were taken as acceptable.

2.3.8. Coating and frying processes optimized by RSM

Response surface methodology (RSM) design can quantify the relationships between the measured responses (dependent variables) and the significant input factors (independent variables). A desirable location in the design space was aimed to be found. In addition, estimating the coefficients of the mathematical model which expresses the relationship between dependent and independent variables is possible by this technique. The accuracy (validation) of the predicted optimum run and its corresponding response was assessed by experimental data. As it is clear in Table 1, the impact of coating and frying parameters on the amount of oil was estimated by a Central-composite design. X1, X2 and X3 were the three independent variables showing SaM concentration (0.75, 1 and 1.25% w/w), frying time (3, 4.5, 6 min), and frying temperature (160, 170 and 180°C) respectively. The design included 20 experiments which consisted of 6 center points. The operating conditions were conducted at 5 levels coded as -2 (-α), -1, 0, +1 and +2 (+α). To investigate the optimal conditions, a second-order polynomial function was fitted to the correlated relationship between the independent variables and the response. The relationship between four factors and the response was expressed by a second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \quad (3)$$

Where Y is the response, β0 is the constant coefficient, xi (i=1–3) are non-coded variables, βi, βii, and βij are the linear, quadratic, and second-order interaction coefficients, respectively.

Table 1. Experimental conditions of the Central-Composite design studying the effect of X1, X2 and X_3: SaM concentration, frying time and frying temperature, respectively

Run	Coded			Un-coded		
	X_1	X_2	X_3	SaM concentration (%w/w)	Frying time (min)	Frying temperature (°C)
1	0	+2	0	1	7	170
2	-1	-1	-1	0.75	3	160
3	-1	+1	+1	0.75	6	180
4	0	0	0	1	4.5	170
5	0	-2	0	1	2	170
6	0	0	0	1	4.5	170
7	0	0	+2	1	4.5	187
8	+1	-1	+1	1.25	3	180
9	0	0	0	1	4.5	170
10	0	0	0	1	4.5	170
11	+1	-1	-1	1.25	3	160
12	-1	+1	-1	0.75	6	160
13	+1	+1	+1	1.25	6	180
14	+1	+1	-1	1.25	6	160
15	-1	-1	+1	0.75	3	180
16	-2	0	0	0.58	4.5	170
17	+2	0	0	1.42	4.5	170
18	0	0	0	1	4.5	170
19	0	0	-2	1	4.5	153
20	0	0	0	1	4.5	170

All tests and analytical observations were conducted in triplicate. ANOVA was used to calculate the mean of each parameter. The p-value was less than 0.05, which means that differences between treatments at a 5% level ($p < 0.05$) were considered significant.

3. Results and discussion

3.1. Optimization of coating and frying condition

3.1.1. Fitting of models

Regression analysis was performed for the experimental data. Derived models for different response variables including water content, acceptable quality and color difference are given in Table 2. The goodness-of-fit of the models was evaluated by a lack-of-fit test and R^2 analysis. R^2 and Adj R^2 values of the model for all response variables were more than 0.99 indicating that a high proportion of variability can be explained by the data. The Adeq precision measures the signal-to-noise ratio was 310.6, 325.1, 537.8 and 2126.6 for oil content, water content, acceptable quality and color difference, respectively. A ratio greater than 4 was desirable. Our ratios indicated an adequate signal and models can be used to navigate the design space. The lack of fit test is a measure of the failure of a model to represent data in the experimental domain. The lack-of-fit test (F-values) for all the models was not-significant (Fcal < Ftab). The F value for oil content water content, acceptable quality and color difference (6600.7, 6710.1, 3.3×10^4 and 3.8×10^5) were significant at the 95% level. The coefficients of variation (CV) were less than 2.24% in all responses, which shows the experiments were performed with adequate precision.

3.2. Oil content

According to Fig. 1, the amount of absorbed fat is affected by the time and temperature of frying. There is a direct connection between the amount of absorbed oil and its temperature. The control SpP contained 25% oil content while the other samples had significantly lower oil content. Results revealed that by increasing the concentration of SaM, oil uptake declined noticeably by SpP.

The amount of oil consumed during frying after coating declined. Hydrocolloids can have a noticeable impact on the frying process by controlling oil uptake (Naghavi, Dehghannya, & Ghanbarzadeh, 2018b). Moreover, decreasing the amount of oil in different foods with the aim of producing low-fat and low-calorie foods can be another advantage of using hydrocolloids (Valiahdi, Asadollahi, & Hosseini, 2019).

In other words, a decrease in the amount of oil occurred up to 47% when SaM concentration increased to maximum. Coating by other hydrocolloids revealed a similar result. For instance, the addition of almond gum declined 34% of chips oil (Bouaziz *et al.*, 2016), 41% of stripes coated with guar gum (Kim, Lim, Bae, Lee, & Lee, 2011), and Gellan gum reduced the oil uptake of fried pastry dough by 55% (Albert & Mittal, 2002). A few broad punctures which were the result of the thermal gelation or crosslinking properties of gums with low capillary pressures in gum allowed less oil to enter the pores. Akdeniz *et al.*, 2006 demonstrated that the connection between oil uptake and moisture loss was inverse. In other words, coating foods with gums resulted in less oil uptake by keeping more moisture (Akdeniz, Sahin, & Sumnu, 2006). Kim *et al.*, 2011 agreed with this result in their research and reported water evaporation resulted from a frying process that made void spaces within the food and was filled with oil, thus increasing the oil content of fried foods (Kim *et al.*, 2011).

Table 2. Regression models and ANOVA for the experimental data of SpP

Quality Parameter	ANOVA					Model equations
	Model (F value)	Lack of fit (F value)	R^2	Adq Pre	CV%	
Oil content	6600.7*	3.8**	0.99	310.6	0.52	$Y_1=+14.9-2.9X_1+1.7X_2+3.7X_3-0.1X_1X_2+0.325X_1X_3+0.4X_2X_3+1.1X_1^2+0.2X_2^2-0.8X_3^2$
Water content	6710.1*	4.1**	0.98	325.1	0.33	$Y_2=+7.3+0.8X_1-0.7X_2-1X_3+0.1AX_2-0.2X_1X_3+0.2X_2X_3+0.1X_1^2-0.1X_2^2-0.5X_3^2$
Acceptable sensory quality	$3.3\times10^{4*}$	1**	0.99	537.8	0.28	$Y_3=+8.2-0.1X_1-0.7X_2-0.9X_3-0.3X_1X_2-0.1X_1X_3+0.1X_2X_3-0.5X_1^2-1.4X_2^2-1.6X_3^2$
Color difference	$3.8\times10^{5*}$	0.4**	0.95	2126.6	0.08	$Y_4=+17.5-4.6X_1+1.3X_2+6.4X_3-0.7X_1X_2+0.1X_1X_3+0.8X_2X_3+1.5X_1^2-0.2X_2^2+1.9X_3^2$

* Significant ** Not-significant

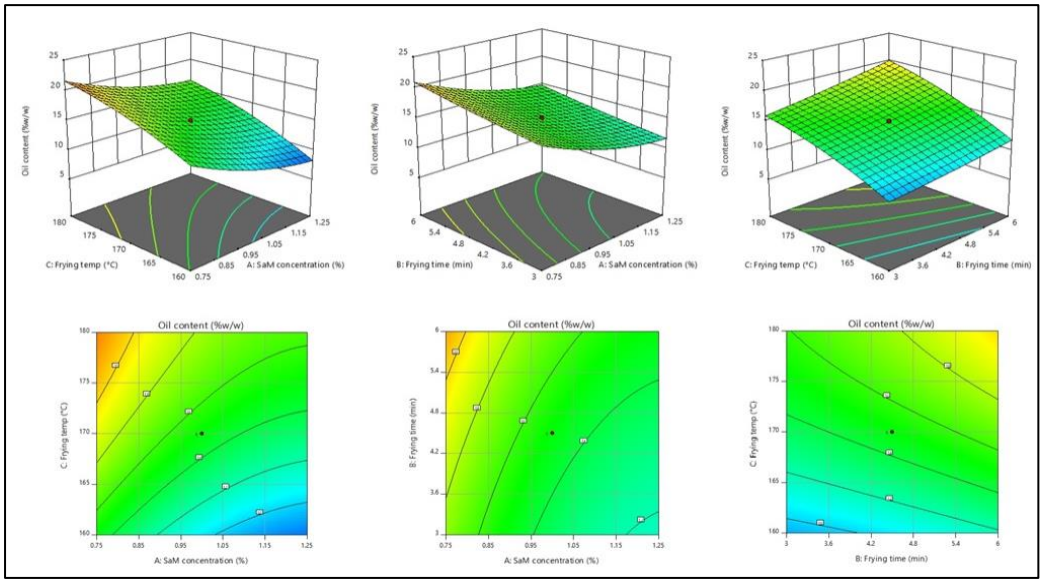

Figure 1. 3D surface/contour plots demonstrating the effect of SaM concentration, frying time and frying temperature on oil content of SpP

3.3. Water content

The frying process of SpP in which water loss occurs is classified into 3 steps: The first step consists of losing water at the cutting surface (heating potato). The second stage refers to producing water bubbles. As time passes during this step, the water content of the slices declines. The last step happens after crust production and prevents the movement of vapor bubbles which are produced by internal gas pressure (Mohammadalinejhad & Dehghannya, 2018).

According to Fig. 2 coated SpP kept a higher amount of moisture. This impact occurred because the capacity of water binding was high so, it did not allow moisture to be replaced with oil during the frying process (Ghaderi, Dehghannya, & Ghanbarzadeh, 2018).

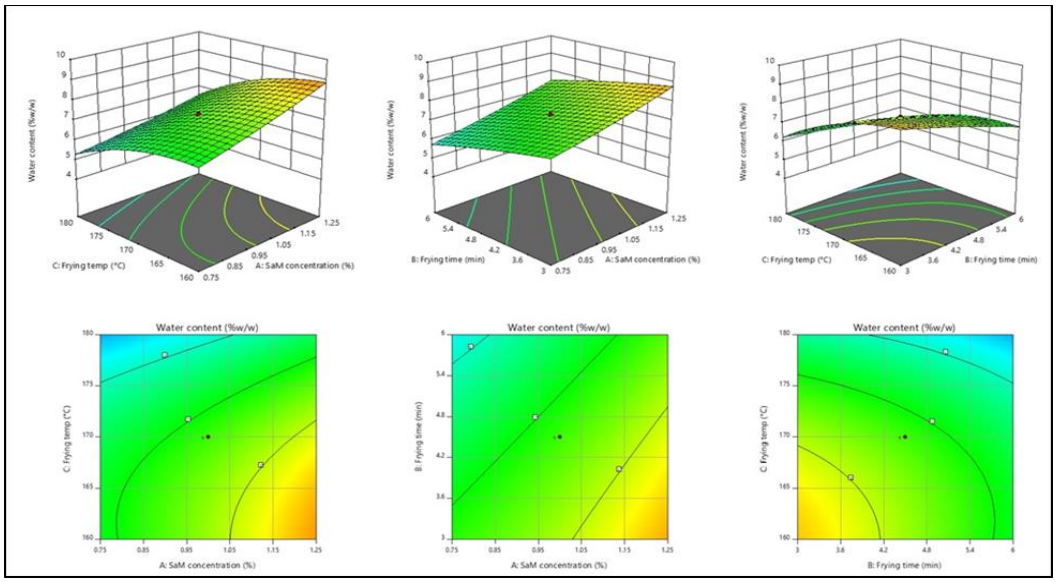

Figure 2. 3D surface/contour plots demonstrating the effect of SaM concentration, frying time and frying temperature on water content of SpP

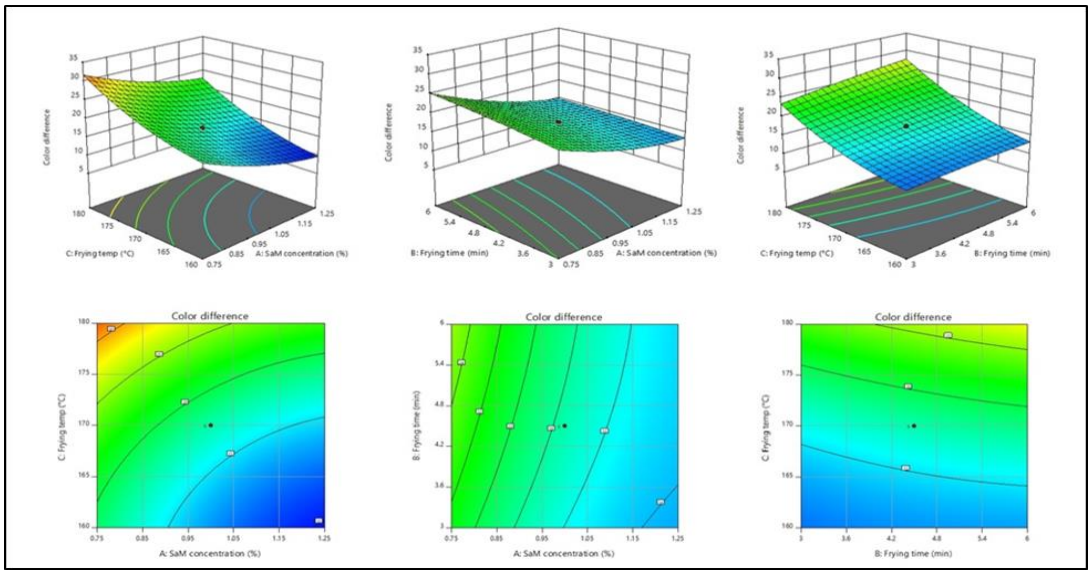

Figure 3. 3D surface/contour plots demonstrating the effect of SaM concentration, frying time and frying temperature on color difference of SpP

3.5. Sensory evaluation

Fig. 4 reveals a sensorial evaluation of coated SpP by using the ranking test mentioned above. Ordinal data were obtained from this experiment and there was proof regarding the difference between samples. A significant difference in sensory quality was observed. However, SpP coated by lower SaM was much preferable in terms of taste, appearance and crispiness (taste). The weak structure of the middle lamella matrix and the concomitant loss of intercellular adhesion, as well as the weakness of the cell walls during frying, may be the reason for the low crispiness of the control sample. Moreover, the existence of cross-linking and gel-forming agents in coated samples resulted in an increase in crispiness followed by an increase in the resistance of the film on the surface of potato (Troncoso, Pedreschi, & Zúñiga, 2009).

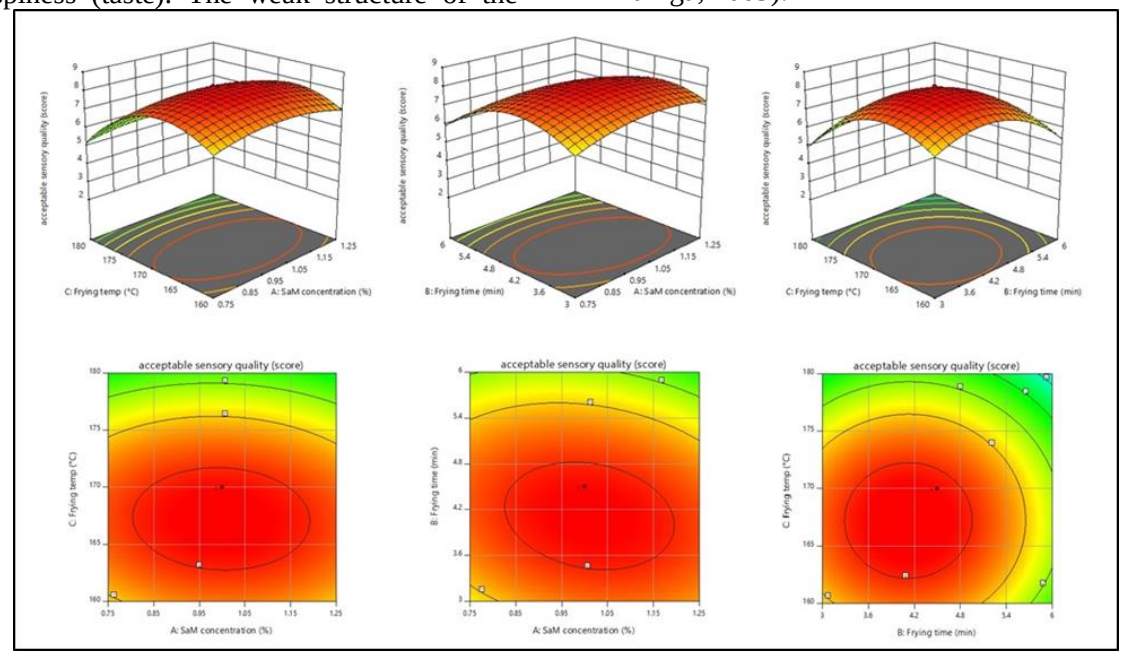

Figure 4. 3D surface/contour plots demonstrating the effect of SaM concentration, frying time and frying temperature on acceptable sensory quality (score) of SpP

3.6. Optimization

Optimized processing conditions for SpP were achieved by the numerical optimization tool of a Design expert with experimental data shown in Fig 5. The target values of the dependent variables were set in the program. The oil content and color variation were minimized, while the sensory evaluation and water content values were maximized. Therefore, the optimum conditions of the experimental data resulting in lower oil content and desirable physicochemical properties were 1.24% (w/w) SaM concentration, 3.6 min frying time at 162 °C frying temperature, resulting in oil content of 8.9%, water content of 9.2%, and a color difference of 10.5 and acceptable sensory quality score of 7.6. The optimum conditions and the corresponding predicted responses are listed in Table 3. For the validation of the optimum conditions, duplicate confirmatory experiments were performed. The measured values are closely related to the predicted data as shown in Table 3

Table 3. Constraints and criteria for optimum conditions, predicted and experimental values of responses of SpP

Factor		Limits	Weights	Predicted condition	Experimental condition	Correction (%)
Name	Code					
Salep mucilage concentration (%)	X_1	0.75-1.25	1	1.246	1.25	+0.32
Frying time (min)	X_2	3-6	1	3.622	3.7	+2.15

Frying temperature (°C)		X_3	160-180	1	162.393	165	+1.60
Response		Goal	Limits	Importance	Predicted value	Validated value	Variation (%)
Name	Code						
Oil content	Y_1	Minimize	6.3-23.9	3	8.91	9.05	+1.57
Water content	Y_2	Maximize	4.3-9.7	3	9.23	8.56	-7.82
Acceptable sensory quality	Y_3	Maximize	5-8.3	3	7.57	7.12	-5.94
Color difference	Y_4	Minimize	9.4-34.2	3	10.49	11.07	+5.52

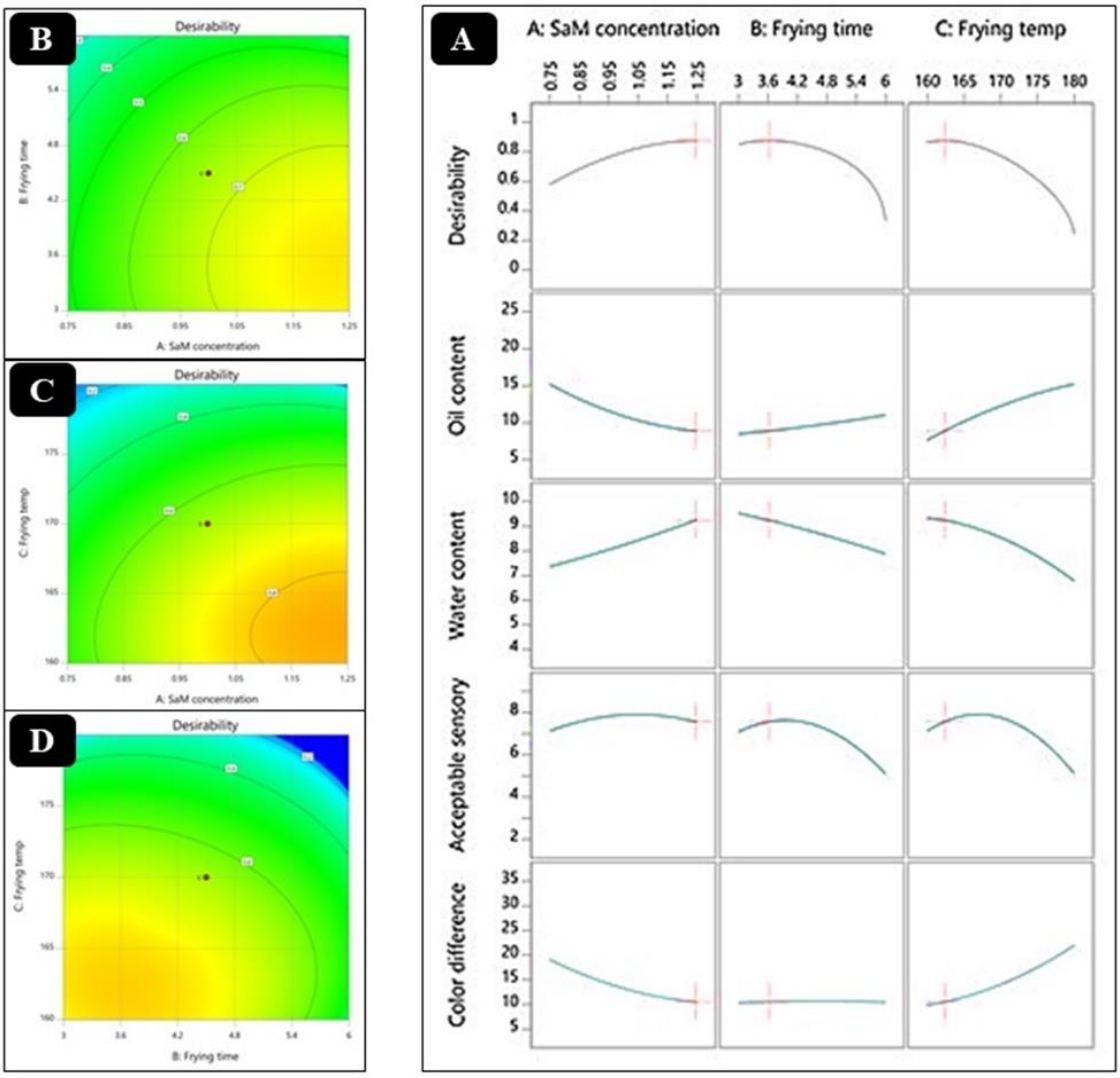

Figure 5. Optimization plot (A) and effect of SaM concentration and frying time (B), SaM concentration and frying temperature (C), frying time and frying temperature on desirability of SpP

3.7. Effect of frying oil type

TAC, LO-T, TFC and TCC measurement was the way to analyze the hydrolysis and polymerization of the frying oils. There was a direct relationship between C18:1 and FFA. According to this relationship, FFA displayed an increasing trend when the amount of C18:1 was increased. As it is shown in Table 4, the hydrogenated oil sample did not follow this rule. After using oil for 15 min, the lowest and the highest amount of TAC are referred to are hydrogenated vegetable oil (~ 35.9) and palm olein oil (~13.4) respectively.

Table 4. Total antioxidant capacity (TAC), lipid oxidation (LO-T), total flavonoid content (TFC) and total carotenoid content (TCC) of stuck-pot rice based on potato cooked with six types of oil

Type of oil	TAC (%)	LO-T (mg MDA/kg)	TFC (mg/kg)	TCC (mg/kg)
Sunflower oil	19.2±6.6	10.4±1.3	35.7±17.2	20.3±0.6
Corn oil	16.6±5.7	8.3±1.4	15.7±2.3	19.3±0.6
Rice bran oil	15.3±3.2	4.2±0.2	42.3±6.0	18.3±1.2
Canola oil	19.0±9.2	1.9±0.1	23.0±2.0	21.7±1.2
Palm olein oil	13.4±2.1	6.2±0.3	30.0±7.0	22.0±1.0
Hydrogenated vegetable oil	35.9±4.9	7.7±0.2	42.0±13.8	14.0±2.0

Except for rice bran oil (~42.3) and hydrogenated vegetable oil (~ 42), the amount of TFC was noticeably different for all types after using oil for 15 min. The range of LO-T in frying oils which were measured after using for 15 min was shown in Table 1 (1.9-10.4). The formation of lipid oxidation compounds, which shows oil deterioration, is strongly attributed to the primary and secondary oxidation that occurs during frying. When the amounts of LO-Ts reach, oil is thermally degraded and fresh oil must be used. LO-Ts during the deep fat frying process are given in Table 1. The amount of LO-Ts in sunflower oil was higher than in other samples. At the end of 15 min frying time, LO-Ts in all oils analyzed were found to be lower than 11 (mg MDA/kg) level. These results indicated that all of the oils could be used for frying potato chips for up to 15 min frying period. Khazaei *et al.* reported that the amount of LO-Ts in shrimp samples before and after the deep-fat frying process were 0.4 and 1 % respectively (mg MDA/kg) (Khazaei, Esmaiili, & Emam-Djomeh, 2016).

The TAC decreased during frying, which is the same pattern for antioxidant capacity in most deep-fat frying studies (Nsabimana, Powers, Chew, Mattinson, & Baik, 2018; Park & Kim, 2016). Antioxidants are unstable under deep fat frying conditions and may break down in the presence of high temperature, airflow, and light exposure as can be expected in deep fat frying operations (Wong *et al.*, 2019). The results of this study showed that in hydrogenated vegetable oil, the total antioxidant capacity decreases slowly.

4. Conclusions

According to the findings of the current study, Salep mucilage can be used as a promising agent to coat deep-fat fried potatoes. One of the advantages of the aforementioned mucilage was decreasing the oil absorption of SpP, which can reduce the cost of large-scale production due to the price of frying vegetable oils. On the other hand, more studies are required to investigate the impact of the combined use of this mucilage along with emerging nonconventional technologies (i.e. pulsed electric fields) to improve the quality of the products, while reducing the costs. Therefore, Salep can be introduced as a coating agent for food products.

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Acknowledgments

The authors are grateful to the Tehran University of Medical Sciences.

Authors' contributions

Mahmood-babooi, Kosar; Data curation, Methodology, Writing – original draft. Ekrami, Mohammad; Data curation, Methodology. Sadighara, Parisa; Data curation, Investigation, Methodology. Rostami, Mohammad reza; Conceptualization (Supporting), Writing – review & editing. Molaee-Aghaee, Ebrahim (Corresponding Author); Project administration, Supervision, Writing – review & editing, Methodology,

Funding

This study did not receive any funding by any institute.

Availability of data and materials

All data generated during this study are included in this published article.

Code availability

Not applicable.

Declaration ethics approval and consent to participate

All participants declare their consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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FATTY ACID PROFILE IN DIFFERENT AGE CATEGORIES OF FARMED RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM, 1792)

Kujtim Uka[1,2], Dijana Blazhekovikj - Dimovska[2]✉, Vlora Gashi - Zogjani[1], Haki Bytyqi[3]

[1]*Food and Veterinary Agency of the Republic of Kosovo*
[2]*University „St. Kliment Ohridski", Faculty of Biotechnical Sciences, Partizanska b.b., 7000 Bitola, Macedonia*
[3]*Fish farm „Trofta", Istog, Kosovo*
✉*dijana.blazekovic@uklo.edu.mk*

https://doi.org/10.34302/crpjfst/2024.16.3.12

Article history:
Received:
October 23rd, 2023
Accepted:
August 1st, 2024

Keywords:
Rainbow trout (Oncorhynchus mykiss Walbaum, 1792);
Fatty acid profile;
Fish age;
Fish farm.

ABSTRACT

The objective of this study was to compare the fatty acid profile in different age categories of farmed rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) collected from the aquaculture facility—cold-water fish farm "Trofta"—located in the Republic of Kosovo.

Considering the results of the fatty acid profile, SFA participated with 25.76 % (I age group), followed by 25.67 (II), 18.28 % (III), and 18.46 % (IV). There is an evident decrease in the SFA content with increased fish age. Of those, the most dominant are palmitic fatty acids (16.13; 16.31; 10.68; 10.69, respectively). The content of MUFA is rapidly increasing with the ages (31.28 % - I; 29.14 % - II; 46.01 % - III and 46.39 % - IV). Of those, the most dominant is an oleic fatty acid with a double increase from the 8 - 9 to 12 -14-month-old fish (20.03 %; 18.21 %; 39.41 %; 40.48 %, respectively). PUFA participates with an average of 40.00 % in total fatty acid content, from which, the most dominant at the 8- and 9-month-old fish is cervonic fatty acid (27.68 and 30.40 %, respectively), with an evident decrease at 12- and 14-months fish (10.53 and 8.52 %, respectively). The biggest increase is determined in linoleic acid, starting with 4.95 and 4.22 % at 8 and 9-month-old fish, while the content at 12 and 14-month-old fish was enormously higher (14.73 and 16.70 %, respectively).

1. Introduction

Nutritional and health benefits achieved by the consumption of fish (Burger and Gochfeld, 2009), the content of high biological value proteins, low-fat, and low cholesterol content (Conor, 2000), as well as valuable quantities of essential fatty acids (Sidhu, 2003), increased the demand of fish in the human diet.

According to Özoğul *et al.* (2007), freshwater and marine fish species are good sources of essential fatty acids. Variations in fatty acid compositions within fish species depend on many factors such as food availability, season, environmental condition, geographic location, sex, diet, and age (Gorgun and Akpinar 2007; Petterson *et al.*, 2009; Sicuro *et al.*, 2010). Due to its positive impact on health, fatty acids in fish lipids have been a subject of great interest (Wang *et al.*, 2006; Riediger *et al.*, 2009).

Salmonids are one of the most farmed fish in aquaculture due to their greater resistance to diseases compared to cyprinid fish, the excellent and balanced composition of fish meat, and the favorable content of polyunsaturated fatty acids.

Oncorhynchus mykiss (Walbaum, 1792), commonly known as rainbow trout, is a species of trout native to cold-water tributaries of the Pacific Ocean in Asia and North America. Sarma *et al.* (2013) considered that it is widely

used in aquaculture because of its rapid growth and high market value due to its flesh quality, especially the content of significant amounts of essential fatty acids.

Rainbow trout is currently a significant agricultural product preferred in the global market, fresh, frozen, or processed (mainly smoked). Usually, rainbow trout in the market is requested in portion sizes of 200 - 400 gr, but depending on the situation, purpose, and many factors, it can be requested in other sizes as well. Thanks to the favorable amount of polyunsaturated fatty acids (especially omega 3 and omega 6) compared to saturated fatty acids, it is good to know in which fish age categories this amount is the highest to prefer certain consumption sizes.

The objective of this study was to compare the fatty acid profile in different age categories of farmed rainbow trout (Oncorhynchus mykiss Walbaum, 1792) collected from the aquaculture facility—cold-water fish farm "Trofta"—located in the Republic of Kosovo.

2. Materials and methods

A total of 46 samples of rainbow trout were collected from an intensive fish farm located in Istog, Republic of Kosovo (Figure 1). Examinations were performed on rainbow trout of four different age categories, as follows: I group - 8 months old (14.80 g weight and 9.33 cm length); II group - 9 months old (34.11 g weight and 11.07 cm length); III group - 12-month-old (273.14 g weight and 23.71 cm length) and IV group - 14-month-old (444.30 g weight and 27.12 cm length) (all of these are average values).

The fish farm Trofta is supplied with water from the river Istog. In this fish farm, pelleted food from manufacturer Aller Aqua is used, with the following content (per specification):

- Aller Futura (2 mm): fish meal, wheat, rapeseed oil, soya protein concentrate, hemoglobin meal (porcine) and fish oil. Besides the main components, the feed contains the following components (per specification): crude protein (47%), crude fat (25%), fiber (1.4%), crude ash (7.1 %), total Ca (1%), total Na (0.4%), total P (1%), Vitamin A (10000 IU/kg), Vitamin D3 (1250 IU/kg), Calcium iodate (3 mg/kg), Manganese chelate (12 mg/kg), Zinc chelate (50 mg/kg), Cooper chelate (5 mg/kg), Antioxidant BHT (E321)(70 mg/kg) and Antioxidant BHA (E320)(45 mg/kg).

- Aller Amber (3 mm): soy oil, soya bean meal, wheat, pap 65% (poultry), hemoglobin, fish meal LT 70%, hydrolyzed poultry feather meal, fish oil, DDGS, mono ammonium phosphate, diamol, lysin, vit-mineral premix, methionine, choline-chloride 60%. Besides the main components, the feed contains the following components (per specification): protein (min 40%), fat (min 28%), moisture (max 8 %), fiber (max 2.4%), ash (max 5.6 %), Ca (0.9%), Na (0.2%), total P (1%), Vitamin A (6000 kJ/kg), Vitamin D3 (2000 kJ/kg), Vitamin E (200 mg/kg), Vitamin C (150 mg/kg), Lizin (min 2%) and Methionine + Cistin (min 1%);

- Aller Amber (4.5 mm): rapeseed oil, wheat, fishmeal, poultry meal, soya meal, sunflower protein concentrate, hemoglobin meal (porcine), hydrolyzed feather meal, fish oil, distiller`s grains. Besides the main components, the feed contains the following components (per specification): crude protein (40%), crude fat (28%), fiber (1.8%), ash (6.2 %), Ca (0.7%), Na (0.2%), total P (1%), Vitamin A (10000 IU/kg), Vitamin D3 (1250 IU/kg), Calcium iodate (3 mg/kg), Manganese chelate (12 mg/kg), Zinc chelate (50 mg/kg), Cooper chelate (5 mg/kg), Antioxidant BHT (E321)(70 mg/kg) and Antioxidant BHA (E320)(45 mg/kg).

- Aller Amber (6 mm): rapeseed oil, wheat, fish meal, poultry meal, soya meal, sunflower protein concentrate, hemoglobin meal (porcine), hydrolyzed feather meal, fish oil, and distiller`s grains. Besides the main components, the feed contains the following components (per specification): crude protein (40%), crude fat (28%), fiber (1.8%), ash (6.2 %), Ca (0.7%), Na (0.2%), total P (1%), Vitamin A (10000 IU/kg), Vitamin D3 (1250 IU/kg), Calcium iodate (3 mg/kg), Manganese chelate (12 mg/kg), Zinc chelate (50 mg/kg), Cooper chelate (5 mg/kg), Antioxidant BHT (E321)(70

mg/kg) and Antioxidant BHA (E320)(45 mg/kg).

In this fish farm rainbow trout are hatched, raised, and produced for the local market as well as exported to neighboring countries for retailers, restaurants, and other clients. This

is a modern farm, producing about 800 tons of trout per year. They distinguish themselves in the region by the high quality of their fish, and the fingerlings which are also exported abroad.

Gas chromatography - AOAC method 996.06 was used to determine the fatty acid composition.

Data were presented as mean ± standard deviation (SD) and subjected to analysis of variance (ANOVA).

3. Results and discussions

Considering the results of the fatty acid profile of the rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from fish farm Trofta (Istog, Kosovo), we`ve obtained the following results (Table 2, Figure 2):

Figure 1 Fish farm Trofta (Istog, Republic of Kosovo)
https://www.aller-aqua.com/media/593390/new-co-trofta-kosovo.jpg

Table 1. Fatty acid profile of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from fish farm Trofta (Istog, Kosovo) in different age categories

Lipid numbers	Name	Type	I	II	III	IV
C8:0	Caprilic acid	SFA	0.48	0.36	0.60	0.76
C14:00	Myristic acid	SFA	3.90	3.75	1.84	1.85
C15:0	Pentadeclic acid	SFA	0.28	0.29	0.16	0.14
C16:0	Palmitic acid	SFA	16.13	16.31	10.68	10.69
C18:0	Stearic acid	SFA	3.84	3.82	3.85	4.03
C20:0	Arachidic acid	SFA	1.13	1.13	1.15	0.98
TOTAL SFA (saturated fatty acid)			**25.76**	**25.67**	**18.28**	**18.46**
C16:01	Palmitoleic acid	MUFA	4.00	3.64	1.79	1.64
C18:1	Oleic acid	MUFA	20.03	18.21	39.41	40.48
C20:1	Gondoic acid	MUFA	3.91	3.89	3.15	3.02
C22:1	Erucid acid	MUFA	3.34	3.40	1.66	1.25
TOTAL MUFA (monounsaturated fatty acid)			**31.28**	**29.14**	**46.01**	**46.39**
C18:2 n-6 c	Linoleic acid	PUFA	4.95	4.22	14.73	16.70
C18:3 n-6	α- linolenic acid	PUFA	0.16	0.13	0.75	0.75
C18:3 n-3	α- linolenic acid	PUFA	1.82	1.77	4.48	4.47

C20:2 n-6	Eicosadienoic acid	PUFA	0.52	0.50	0.88	0.88
C20:3 n-6	Dihomo-γ-linoleic acid	PUFA	0.23	0.20	0.70	0.76
C20:4 n-6	Arachidonic acid (AA)	PUFA	1.03	0.98	0.88	0.80
C20:3 n-3	Eicosatrienoic acid (ETE)	PUFA	0.19	0.20	0.24	0.25
C20:5 n-3	Timnodonic acid	PUFA	6.36	6.47	2.48	1.97
C22:6 n-3	Cervonic acid	PUFA	27.68	30.40	10.53	8.52
TOTAL PUFA (polyunsaturated fatty acid)			**42.94**	**44.87**	**35.67**	**35.10**
Total SFA (saturated fatty acid)			25.76	25.67	18.28	18.46
Total UFA (unsaturated fatty acid)			74.24	74.00	81.71	81.53
Total PUFA n-6			6.37	5.54	17.06	19.01
Total PUFA n-3			36.05	38.83	17.74	15.23
n-6/n-3			0.18	0.14	1.01	1.28
n-3/n-6			5.72	7.10	1.06	0.82
UFA/SFA			2.88	2.89	4.57	4.44
PUFA/SFA			1.67	1.75	1.97	1.91
PUFA/MUFA			1.41	1.60	0.79	0.76

*I size - 8-months-old (14.80 g weight and 9.33 cm length)
II size - 9-months-old (34.11 g weight and 11.07 cm length)
III size - 12-months-old (273.14 g weight and 23.71 cm length)
IV size - 14-months-old (444.30 g weight and 27.12 cm length)

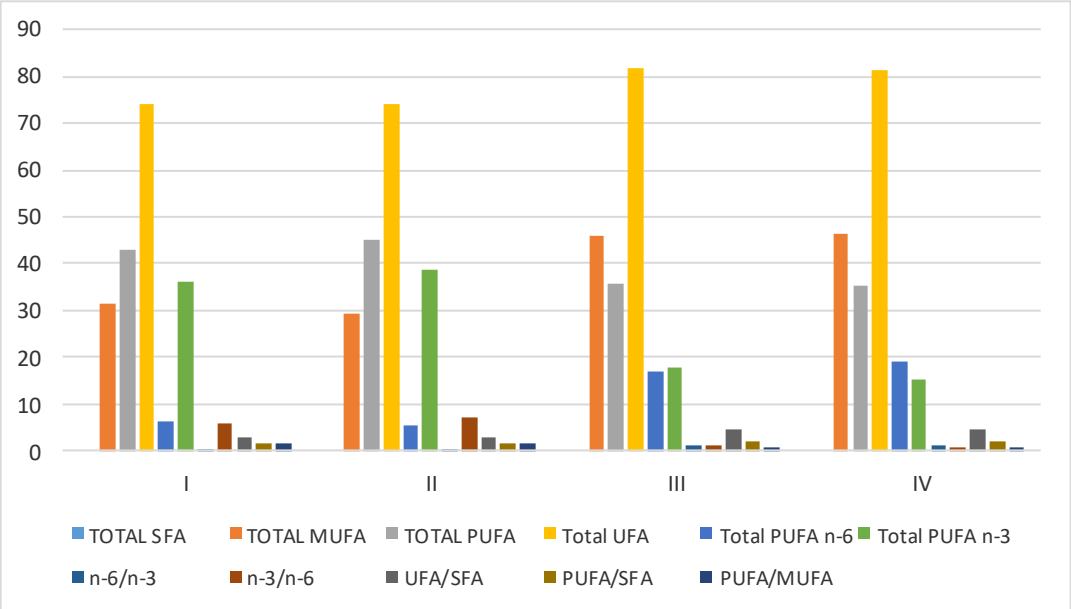

Figure 2 Comparative indicators of content and fatty acid ratio in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

***Legend:** UFA – unsaturated fatty acid, PUFA – polyunsaturated fatty acid, SFA – saturated fatty acid, n-3 – omega 3 fatty acid, n-6 – omega 6 fatty acid

Table 2 Calculation of means and standard deviations for each age group (8, 9, 12, and 14 months) across various parameters related to fatty acids

Age months	SFA Mean/ Std Dev		UFA Mean/ Std Dev		PUFA n-3 Mean/ Std Dev		PUFA n-6 Mean/ Std Dev		n-6/n-3 Mean/ Std Dev		n-3/n-6 Mean/ Std Dev		UFA/SFA Mean/ Std Dev		PUFA/SFA Mean/ Std Dev		PUFA/MUFA Mean/ Std Dev	
8	25.41	0.43	74.59	0.43	37.80	5.48	6.36	0.52	0.17	0.02	5.97	0.56	2.89	0.10	1.72	0.08	1.41	0.31
9	25.66	0.50	74.34	0.50	37.35	4.27	5.74	0.63	0.16	0.03	6.55	0.81	2.97	0.13	1.79	0.18	1.52	0.25
12	17.77	2.12	82.23	2.12	15.45	3.78	16.35	1.66	1.16	0.28	0.87	0.21	4.69	0.53	2.05	0.29	0.73	0.09
14	17.34	2.27	82.66	2.27	15.41	3.75	17.36	1.80	1.13	0.26	0.88	0.19	4.69	0.43	2.04	0.25	0.76	0.06

Table 3 ANOVA tests for age-dependent variations in Dietary Fatty Acid categories and significance

Fatty Acid Category	p-value	Significance
SFA (Saturated Fatty Acid)	0.00000291	Significant Difference
UFA (Unsaturated Fatty Acid)	0.00000532	Significant Difference
PUFA n-3 (Polyunsaturated Fatty Acid Omega-3)	0.00117	Significant Difference
PUFA n-6 (Polyunsaturated Fatty Acid Omega-6)	0.21882	No Significant Difference
n-6/n-3 Ratio	0.38664	No Significant Difference

Considering the results of the fatty acid profile of the rainbow trout from the fish farm Trofta, in total fatty acid content, saturated fatty acid (SFA) participates with 25.76 % (I age group), followed by 25.67 (II), 18.28 % (III) and 18.46 % (IV). There is an evident decrease in the SFA content with the increase in fish age. Of those, the most dominant are palmitic fatty acids (16.13; 16.31; 10.68; 10.69, respectively).

The content of monounsaturated fatty acids (MUFA) is rapidly increasing with the ages (31.28 % - I; 29.14 % - II; 46.01 % - III and 46.39 % - IV). Of those, the most dominant is an oleic fatty acid with a double increase from the 8 - 9 to 12 -14-month-old fish (20.03 %; 18.21 %; 39.41 %; 40.48 %, respectively).

Polyunsaturated fatty acids (PUFA) participate with an average of 40.00 % in total fatty acid content, from which, the most dominant at the 8 and 9 months old fish is cervonic fatty acid (27.68 and 30.40 %, respectively), with an evident decrease at 12 and 14 months old fish (10.53 and 8.52 %, respectively). The biggest increase is determined in linoleic acid, starting with 4.95 and 4.22 % at 8 and 9-month-old fish, while the content at 12 and 14-month-old fish was enormously higher (14.73 and 16.70 %, respectively).

Sabetian *et al.* (2012) determined that the total SFA, MUFA, and PUFA content in rainbow trout (511.75±6.92 g weight and 35.2±0.18 cm length) from fish farms in Iran were 26.3%, 33.8% and 24.62% of total fatty acids, respectively. The ratio of n-3/n-6 fatty acids was 2.06.

N-6 (omega 6) fatty acids participate with 6.37 (I); 5.54 (II); 17.06 (III) and 19.01 (IV) %, with the higher content in older fish, while n-3 (omega 3) participate with 36.05 (I); 38.83 (II); 17.74 (III) and 15.23 (IV) %, with the lower content in older fish, and good values at younger fish.

Many authors suggested that the n-3/n-6 ratio is a good parameter to determine the nutritional value of oil present in fish meat. In our study, the n-3/n-6 ratio was very high in 8-9-month-old fish (5.72 and 7.10, respectively), with lower values in older fish (1.06 and 0.82, respectively). According to Simopoulos (2002), the n-3/n-6 ratio in freshwater fish ranges from 1 to almost 4. The n-3/n-6 ratio was appreciably higher in the study of Malenica Stavera *et al.* (2012) ranging from 8.05 - 14.77, probably due to the high n-3/n-6 ratio in the feed and environmental condition. This finding is in correlation with our results.

Simopoulos (2004) considered that excessive amounts of n-6 PUFA and a very high n-6/n-3 ratio promote cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 PUFA apply suppressive effects.

In our study, the n-6/n-3 ratio is quite similar in all age categories (0.18; 0.14; 1.01, and 1.28, respectively), with a slight increase with age, as well as, PUFA/SFA ratio.

Simopoulos (2010) concluded that a ratio of 1:1 to 2:1 n-6/n-3 fatty acids should be the ideal ratio for beneficial to health. The Department of Health of the United Kingdom (1994) recommends n-6/n-3 value below 4. Consuming higher dietary quantities of n-3 PUFAs is an approach to normalizing high n-6/n-3 ratios (McDaniel *et al.*, 2013). In the study of Guler *et al.* (2017), the n-3/n-6 ratio was found to be 1.56 in cultured rainbow trout. All of these findings are in correlation with our results.

According to H.M.S.O. (1994), the ideal n-6/n-3 ratio of fatty acids is up to 4. Moreira *et al.* (2001) considered that ratios greater than this value are harmful to health and may

promote cardiovascular diseases. In our research, the n-6/n-3 ratio ranged from 0.14 - 1.28, from which it can be concluded that it is favorable.

According to H.M.S.O. (1994), a minimum value of PUFA/SFA ratio is recommended as 0.45. Our findings of 1.67 - 1.97 indicate that fish meat with good nutritional quality because the obtained values are higher than the minimum (0.45) which is recommended for a healthy diet in humans.

According to AFSSA (2003), the UFA/SFA ratio of fatty acids in fish fat is very important and its value should be over 3. In our study, UFA/SFA ratio is 2.88 and 2.89 in 8 -9 months old fish, with increasing in older fish (4.57 - III and 4.44 - IV), so, favorable values are obtained by consuming 12 - 14 months old fish.

The age categories of trout displayed significant variations in SFAs content, with a significance level of $p<0.05$. In Group I (8 months), SFAs constituted 25.76% of the fatty acids. Group II (9 months) exhibited a notably higher proportion of SFAs, amounting to 25.67%. Conversely, in Group III (12 months), the SFAs proportion was 18.28%, and in Group IV (14 months), it was 18.46%. These results underline substantial alterations in SFA levels with age. Additionally, significant discrepancies in the proportions of UFAs were detected, also at $p<0.05$. In Group I (8 months), UFAs comprised 74.24% of the fatty acids. For Group II (9 months), the proportion was quite similar at 74.00%. However, in Group III (12 months), UFAs constituted 81.71% of the fatty acids. In Group IV (14 months), the proportion of UFAs reached 81.53%. Moreover, significant differences ($p<0.05$) were identified in the n-3 fatty acids content. Group III (12 months) demonstrated the highest share of n-3 fatty acids, accounting for 17.74% of the total fatty acids. In Group I (8 months), the proportion of n-3 fatty acids was 36.05%, and in Group II (9 months), it stood at 38.83%. On the other hand, in Group IV (14 months), the proportion of n-3 fatty acids amounted to 15.23%. Further differences were observed in the proportion of n-6 fatty acids. In Group III (12 months), the proportion of n-6 fatty acids was 17.06%, which was significantly higher ($p<0.05$) compared to Group II (9 months) with 5.54% and Group I (8 months) with 6.37%.

The fatty acid ratios in the trout samples indicate age-related shifts, such as a decrease in the n-6/n-3 ratio from 0.18 at 8 months to 0.14 at 9 months, and a substantial increase in the n-3/n-6 ratio from 5.72 at 8 months to 7.10 at 9 months, with notable differences observed in the UFA/SFA and PUFA/SFA ratios across age groups. The fatty acid ratios in the trout samples demonstrate age-related variations, highlighting changes in the balance of essential fatty acids as the fish mature.

Saturated Fatty Acids (SFA): Our statistical analysis indicates a significant relationship between age and the levels of saturated fatty acids in the diet. As individuals age, there is a noticeable change in the consumption of SFA. This aligns with previous research, such as the study by Mozaffarian and Wu (2010), which highlights the importance of monitoring SFA intake, especially among older adults, due to its association with cardiovascular health.

Unsaturated Fatty Acids (UFA): The statistical analysis also demonstrates a significant association between age and the levels of unsaturated fatty acids. The intake of UFA appears to vary with age. It's worth noting that UFA, including monounsaturated and polyunsaturated fats, is generally considered healthier and is linked to various health benefits (Micha *et al.,* 2017). Our findings suggest that dietary habits change as individuals grow older, potentially indicating a shift towards healthier fat choices.

Polyunsaturated Fatty Acids Omega-3 (PUFA n-3): The data reveals that the levels of PUFA n-3, often associated with heart and brain health, are significantly influenced by age. This finding is in line with research that emphasizes the importance of omega-3 fatty acids in the aging population (Dyall, 2015). It underscores the need for older individuals to maintain an adequate intake of these essential fatty acids.

Polyunsaturated Fatty Acids Omega-6 (PUFA n-6): Interestingly, our analysis did not find a substantial age-related effect on PUFA n-

6 levels. This suggests that the intake of omega-6 fatty acids remains relatively constant across different age groups. Omega-6 fatty acids, although essential, should be consumed in balance with omega-3 to avoid an imbalance that could promote inflammation (Simopoulos, 2002). The stability in PUFA n-6 levels across age groups may be attributed to consistent dietary habits.

Balance between Omega-6 and Omega-3 (n-6/n-3 Ratio): The balance between omega-6 and omega-3, as indicated by the n-6/n-3 ratio, does not exhibit significant age-related differences. This observation is noteworthy as an optimal balance is essential for overall health (Simopoulos, 2008). It's vital to maintain this equilibrium throughout life to support various physiological processes.

The fatty acid profile of fish is known to be influenced by a combination of metabolic, physiological, and environmental factors. Among these, age is a crucial determinant that plays a significant role in shaping the composition of fatty acids within the fish. Here, we will explore the metabolic and physiological reasons behind this age-related influence on fatty acid profiles:

• Metabolic Adjustments: Fish exhibit distinct metabolic patterns throughout their life stages. Younger fish are characterized by elevated metabolic rates to fuel rapid growth and development. This heightened metabolic activity influences the synthesis and utilization of fatty acids. Younger fish may favor the incorporation of specific fatty acids to support their energy and growth requirements. As fish mature, metabolic demands change, leading to a different allocation of fatty acids.

• Dietary Shifts and Prey Selection: Fish are opportunistic feeders and tend to modify their dietary preferences as they age. Younger fish often feed on different prey items, which vary in fatty acid content. The diversity in their diets during different life stages can lead to variations in fatty acid profiles. As fish grow older, their dietary choices may evolve, affecting the fatty acids they ingest.

•Physiological Development: Age-related physiological changes can significantly influence fatty acid profiles. Life stage transitions, such as larval to juvenile or juvenile to adult, are marked by shifts in organ development, particularly the liver and gonads. These changes can impact the synthesis and storage of specific fatty acids, especially those essential for reproduction and egg development in mature females. These physiological developments contribute to age-dependent variations in fatty acid composition.

•Environmental Dynamics: Fish encounter varying environmental conditions throughout their life. Changes in factors like water temperature, oxygen levels, and prey availability can alter their metabolic and physiological responses, including how they process and store fatty acids. Different seasons may present different prey options, affecting the intake of particular fatty acids.

•Stress Responses: Aging fish experience stressors such as predation threats, competition for resources, and environmental challenges. Stress responses, including the release of cortisol, can lead to alterations in fatty acid metabolism. These responses can, in turn, affect the overall balance of fatty acids within the fish.

4. Conclusions

Our statistical analysis reveals that age plays a substantial role in shaping dietary habits, especially concerning the intake of saturated and unsaturated fatty acids, as well as PUFA n-3. However, the balance between omega-6 and omega-3 fatty acids remains relatively stable across age groups. These findings underscore the importance of age-tailored dietary recommendations and interventions to ensure that individuals, particularly older adults, maintain healthy fat consumption patterns to support overall well-being.

In summary, the influence of fish age on their fatty acid profiles is a multifaceted interplay of metabolic adaptations, dietary shifts, physiological developments, environmental changes, and stress responses. Researchers keen on understanding fish nutrition and health need to consider these age-

related factors when studying fatty acid composition. Recognizing the intricate relationship between age and fatty acid profiles is critical for fisheries management, aquaculture practices, and the development of age-specific nutritional interventions to promote fish health and ensure the production of fish products with desired fatty acid profiles. It underscores the importance of age as a crucial variable in fish nutrition research.

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Acknowledgment

Special thanks to the Fish farm "Trofta" (Istog, Kosovo) for providing the necessary material of rainbow trout for analyzes.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

MICRONUTRIENT, PHYSICOCHEMICAL AND ACCEPTABILITY RESPONSES OF "MOI-MOI" AS A FUNCTION OF COWPEA (*VIGNA UNGUICULATE L. WALP*) PARTIAL SUBSTITUTION WITH YELLOW MAIZE

I. N. Okwunodulu[1]✉, C. C. Osuagwu[2], A. U. Onwuzuruike[3], S. C. Ubbor[4]

[1,2,3,4]*Department of Food Science and Technology, College of Applied Food Sciences and Tourism, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.*
✉*ncntokwu@yahoo.com*

https://doi.org/10.34302/crpjfst/2024.16.3.13

Article history:
Received:
September 9th, 2023
Accepted:
August 10th, 2024

Keywords:
Quality implications;
Yellow maize;
"Moi-moi";
Cowpea;
Partial substitution.

ABSTRACT

Neither legume nor cereal alone can meet all the nutrient needs of the body to prevent hidden hunger including bone health and development except when both complement and supplement each other. This study aimed at investigating the nutritional and acceptability changes of "moi-moi" from cowpea partially substituted with yellow maize in the ratios of 95: 5, 90: 10 and 80: 20%. The "moi-moi" produced with 100% cowpea served as control. Proximate, mineral, vitamin and physicochemical composition were investigated on both substituted and control with standard methods while sensory characteristics were evaluated subjectively with 20 untrained panellists. With increasing substitution levels of yellow maize, there were significant ($p<0.05$) decrease (lower than the control) in moisture (47.27-32.37%), crude protein (20.09-18.94%), and fat (10.02-8.66%). While ash content (1.45-1.73%), crude fibre (1.38-1.46%) and carbohydrate (18.86-36.85%) increased more than the control. Vitamin A (1.39-1.82 µg/100g) increased significantly ($p<0.05$) more than the control while vitamin C (0.06-0.04 mg/100g had no significant decrease. Mineral contents increased significantly ($p<0.05$) from 17.30-19.06 mg/100g, 12.44-13.43 mg/100g and 7.48-9.42 mg/100g for calcium, magnesium and phosphorous respectively more than their respective controls. The pH (6.12-6.00) and colour intensity (0.12-0.15) decreased significantly ($p<0.05$) lower than their respective controls. Acceptability decreased with yellow maize substitution level increase. The "moi-moi" produced with 100% cowpea had the best organoleptic properties (7.80) followed by 5% yellow maize substitution (6.85). Nutrient composition, calcium to phosphorous ratio, negative correlation of vitamin C with minerals and acceptability changed with increasing yellow maize substitution for all the substituted samples.

1. Introduction

"Moi-moi" is a popular protein-rich cowpea pudding usually served as a convenient snack or as a component of breakfast in Nigerian homes. It is consumed with pap (*ogi*), gari soaked in water, *akamu* (maize gruel) and cereal foods like rice or alone. "Moi-moi" preparation is the same all over West Africa. Cowpea is steeped in tap water for some hours to ease seed coat removal, hand dehulled and the hulls remove by water floatation. Dehulled cowpea cotyledons are then ground into smooth pastry slurry. Water and ingredients such as onion, pepper, oil, salt, water and other ingredients are added according to preference and mixed thoroughly with spoon. The pasty slurry is then scooped into aluminium plates with lids or wrapped with leaves or foil and steamed for 30-50 min to produce a steamed bean pudding called "moi-moi". Aside from wet cowpea cotyledons and maize seeds, 'moi-moi'

could be prepared with their flours (Akusu and Kiin-Kabari, 2012) or with improved maize and other bean (Ejima and Ejima, 2015) using same processing technique of paste preparation and mixing.

Cowpea is an economic and readily available source of plant protein in West Africa (Henshaw, 2008; Mamiro *et al*., 2011). Unlike other legumes such as soybean and groundnuts, which are oil-protein seeds, cowpea is a starch-protein legume with a wider spectrum of utilisation than other legumes in West Africa (Henshaw, 2000). Cowpea seeds contain 36% to 68% carbohydrate, 2.7% to 5.8% crude fibre, 2.5% to 4.2% total ash and 0.9% to 2.4% fat. More so, protein, iron, calcium, phosphorus, potassium, magnesium, sodium and vitamin B complex are some of the important micronutrients found in cowpea (Okereke and Hans-Anukam, 2015). The calcium and iron content of cowpea are higher than that of meat, fish and egg while their thiamine, riboflavin and niacin content are comparable with that found in lean meat and fish (Achuba, 2006). Cowpea is especially rich in lysine, but is deficient in sulphur-containing amino acids (Ilesanmi and Gungula, 2016). Owing to the taste, ease of incorporation in other recipes and nutritional value, cowpea is widely used in Nigeria for everyday delicacies like "moi-moi", "akara", "danwake" amongst others (Adegbola *et al*., 2013; Akajiaku *et al*., 2014).The presence of anti-nutritional factors is one of the main drawbacks that limits the utilization of the nutritional and food quality of cowpea. The common anti-nutrients found in cowpea are trypsin inhibitors, tannin, phytate and lectins (Vadivel and Jonardhanen, 2003) but are removed during processing.

Maize (*Zea mays*) is a cereal crop widely cultivated in Nigeria and in the tropics. It is nutritionally superiority to other cereals in many ways except in protein value. The grains are rich in vitamins A, C and E, carbohydrate and essential minerals (Brownson, 2016). Maize is higher in fat and iron than wheat and rice (Mejia, 2003), but lower in protein as half of its protein is made up of zein which is low in two essential amino acids, lysine and tryptophan (Mejia, 2003; Akusu and Kiin-Kabari, 2012). About 50 species of maize exist and consist of different colours, size and shape. The white and yellow varieties are preferred by most people depending on the region. White maize seeds contain reasonable concentration of thiamine while yellow maize contains carotene, a precursor of vitamin A (Adu, 2010). Maize is also rich in dietary fibre (IITA, 2009). It is prepared and consumed in many ways which vary from region to region or from one ethnic group to the other. For instance, maize grains are used in the production of "Akamu", "Agidi", "Kokoro", "Epitit", "Ipekere" in Nigeria or as popcorn which is eaten all over West Africa.

Cowpea and maize, despite their great nutritional and economic importance, are highly susceptible to many diseases and pests during storage (IITA, 2009) and therefore need to be preserved through processing. One of the problems facing the poor socio-economic groups of developing countries such as Nigeria is protein-energy malnutrition due to consumption of starchy foods with little or no nutrient value. Substituting cowpea with that of maize is a legume-cereal technology that leads to complementation of their nutrient contents. For instance, vitamin A deficiency is a common disease among children of school age and pregnant/lactating mothers in developing countries like Nigeria (WHO/FAO, 2006). Yellow maize contains vitamin A precursor which along with good quality protein from cowpea will solve both protein malnutrition and vitamin A deficiency problem. This study therefore aimed at evaluating the nutritional changes of "moi-moi" from cowpea partially substituted with yellow maize.

2. Materials and methods

2.1. Materials

2.1.1. Source of raw material

Cowpea (Figure 1), yellow maize (Figure 2) and ingredients used for the "moi-moi" preparation were procured from Ubani main market in Umuahia North Local Government Area, Abia State, Nigeria.

Figure 1. Cowpea

Figure 2. Yellow maize

2.1.2. Preparation of "moi-moi"

Cowpea and maize were first sorted to remove extraneous materials and steeped in stainless steel buckets separately with tap water covering the seeds. This is to hydrate their endosperms and make it easier for the separation of cowpea seed coat from the endosperm to obtain the cotyledons. Steeping will also hydrate and soften maize endosperm for easier milling. The cowpea cotyledons and maize were blended according to formulation ratio (Table 1), milled into fine and smooth slurry with variable speed kitchen blender (QLink, Japan). Similarly, 100% cowpea was milled same as control. The "moi-moi" samples were prepared separately (Figure 3) by mixing properly the slurry of the control and the substituted blends with same ingredients (Table 2) in a stainless steel bowl with stainless steel spoon. The mixed slurry obtained was dispensed (200 ml) into each 3 inch square formed aluminium foil, closed and steamed in aluminium pot for 30 min with a gas cooker. Then the 'moi-moi' wraps (Figures 4, 5, 6 and 7) were removed and allowed to cool to room temperature (25^{o}C) before all the analyses were carried out.

Table 1. Formulation ratios (%) for "moi-moi' preparation from cowpea partial substituted with yellow maize.

Sample codes	Cowpea	Yellow maize
CP100	100	0
CPYM1	95	5
CPYM2	90	10
CP YM3	80	20

CP100=100% cowpea, CPYM1=95:5% cowpea-yellow maize blend, CPYM2=90:10% cowpea-yellow maize blend and CPYM3=80:20 cowpea: yellow maize blend.

Table 2. Recipe per 400 g paste used for "moi-moi" preparation

Ingredients	Quantity
Warm water	1104 ml
Vegetable oil	200 ml
Ground red pepper	50 g 10 g
Onion	40 g
Salt	11 g
Crayfish	10 g
Mixed spice	1 g
Maggi cube	1 g

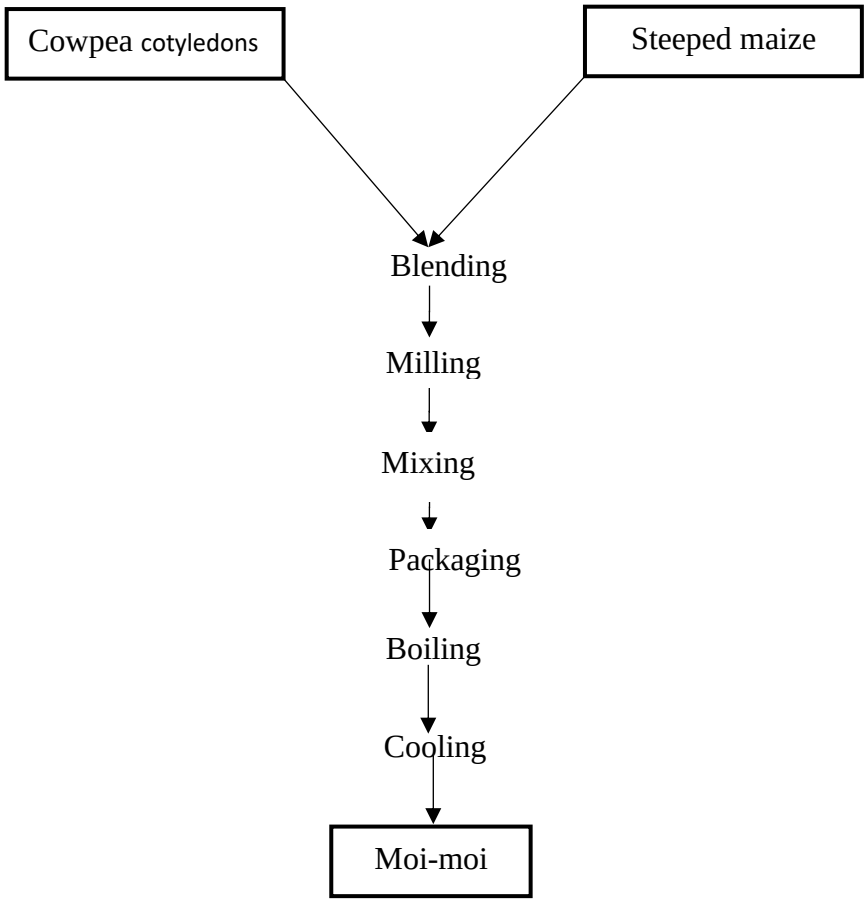

Figure 3. Preparation of *"*moi-moi*"* with cowpea cotyledons and maize seed blends.

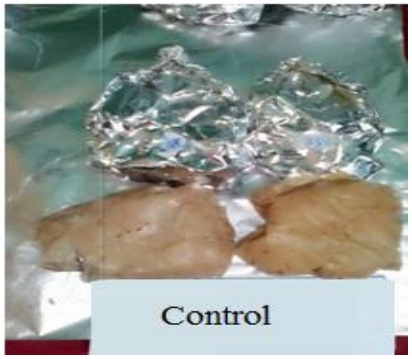

Figure 4. 100% cowpea.

Figure 5. 95% cowpea

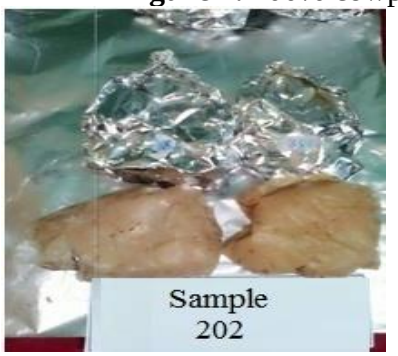

Figure 6. 90% cowpea: 10% yellow maize

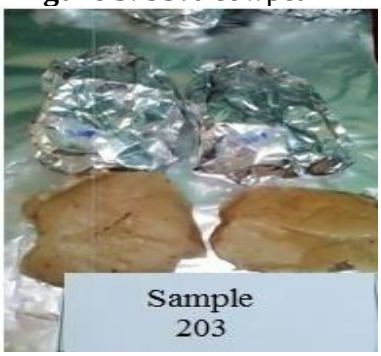

Figure 7. 80% cowpea: 20% yellow maize

2.2. Methods

2.2.1. Proximate analysis

2.2.1.1. Moisture content

The moisture content was determined according to AOAC (2010) method and calculated thus:

$$\% \text{ moisture} = \frac{weight\ of\ sample\ before\ drying - weight\ of\ sample\ after\ drying}{original\ weight\ of\ the\ sample} \frac{X\ 100}{1} \quad (1)$$

2.2.1.2. Crude protein

This was carried out using micro-kjeldahl digestion method described by AOAC (2010) and crude protein was calculated as:

$$\% \text{ Crude protein} = \frac{(0.01x6.25x280xT)}{Weightofsample} \text{ x} \frac{100}{1} \quad (2)$$

Where T= titre value.

2.2.1.3. Ash content

The method as described by AOAC (2010) was used to determine the ash content which percent content was calculated as.

$$\% \text{ Ash} = \frac{Weight\ of\ crucible + Ash - Weigh\ of\ crucible}{Original\ weight\ of\ sample} \text{ x} \frac{100}{1} \quad (3)$$

2.2.1.4. Fat content

Fat content was determined by the continuous solvent extraction in a soxhlet reflux apparatus (AOAC (2010) and the fat content was calculated as shown.

$$\% \text{ fat} = \frac{W_2 - W_1}{Weight\ of\ sample} \text{ x } \frac{100}{1} \quad (4)$$

Where
W_1= Weight of empty extraction flask
W_2 = Weight of flask + oil extracted.

2.2.1.5. Crude fiber

Crude fiber was determined by the method described by James [27] and calculated thus:

$$\% \text{ Crude fibre} = \frac{Loss\ in\ Weight\ on\ incineration}{Weightof\ original\ sample} \text{ x} \frac{100}{1} \quad (5)$$

2.2.1.6. Carbohydrate

Carbohydrate was calculated by difference (James, 1995) as indicated:-

% Carbohydrate =100% - (% moisture + % ash +%fat+% crude fibre + %crude protein). (6)

2.2.2. Vitamin determination

2.2.2.1. Vitamin A

Vitamin A was determined with Spectrophotometric method of Onwuka (2018) and calculated thus:

$$\text{Vitamin A (mg/ 100g)} = \frac{100}{W} \text{x } \frac{au}{as} \text{x C} \quad (7)$$

Where: au = absorbance of the test sample.
as = absorbance of the standard solution.
c = concentration of the test sample.
w = weight of sample (g).

2.2.3. Vitamin C

Vitamin C was determined according to the method of Okwu and Josiah (2006) and calculated as shown:

$$\text{Vitamin C (mg/100g)} = 0.88 \text{ x} \frac{100}{10} \text{ x } \frac{vf}{20} \ x \ \frac{T}{1} \quad (8)$$

Where:
V_f = Volume of extract
T = Sample titre – blank titre

2.2.4. Mineral Determination

2.2.4.1. Calcium and magnesium

Calcium and magnesium were determined with the Complexiometric titration method of Onwuka (2018). Total calcium and magnesium content were calculated separately using the formula:

$$\text{Calcium (mg/mg)} = \frac{100}{W} \text{ x T – B (N X Ca/ mg) x } \frac{vf}{va} \quad (9)$$

Where W=Weight of sample
T = titre value of sample
B= Titre value of blank
Ca = Calcium equivalence
Mg =Magnesium equivalence
Va = Volume of extract titrated
Vf = Total volume of extract
N=Normality of titrant (0.02N EDTA).

2.2.3.2. Phosphorus

The molybdate protocol of Onwuka (2018) was used and phosphorous content was calculated as shown:

$$\% \text{ Phosphorus} = \frac{\text{graph reading x solution volume}}{100} \quad (10)$$

Calcium: Phosphorous ratios were calculated using the method of Okwunodulu et al. (2022).

2.2.5. Physicochemical analysis

The pH was determined by the method described by Akpakpunam and Sefa-Dedeh (1995) while the colour intensity of the samples was determined using ELICO spectrophotometer model SL. 171 (Nyderabad) India as described by Sharma (2003).

2.2.6. Sensory evaluation

The method described by Iwe (2010) was used with 20 semi-trained panellists randomly selected from the staff and students of Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike. Nigeria. The panellists scored the samples according to 9-point Hedonic scale where 9 is like extremely, 5 is neither like nor dislike and 1 is dislike extremely.

2.2.7. Statistical analysis

One-way analysis of variance of a completely randomized design using the Statistical Package for Social Science version 22 was carried out on the data obtained. Treatment means were separated using Duncan multiple range test at 95% confidence level ($p<0.05$). Also, vitamin C was used to correlate the minerals using Pearson Correlation method.

3. Results and discussion

3.1. Effects of partial substitution of cowpea with yellow maize on the proximate composition

3.1.1. Mosture content (MC)

Proximate composition results as presented in Table 3 showed that MC (wet basis) of the cowpea-yellow maize "moi-moi" decreased significantly ($p<0.05$) with increasing yellow maize substitution of cowpea from 47.27% in sample CPYM1 (95% cowpea:5% yellow maize) to 32.27% in sample CPYM3 (80% cowpea:20% yellow maize).

Table 3. Proximate composition of "moi-moi" as affected by partial substitution of cowpea (%).

Samples	Moisture	CP	Crude fibre	Fat	Ash	Carbohydrate
CP100	$50.20^{a}\pm0.28$	$20.70^{a}\pm0.01$	$1.37^{c}\pm0.02$	$10.67^{a}\pm0.02$	$1.38^{d}\pm0.02$	$15.70^{d}\pm0.32$
CPYM1	$47.27^{b}\pm0.01$	$20.09^{ab}\pm0.01$	$1.38^{c}\pm0.01$	$10.02^{b}\pm0.02$	$1.45^{c}\pm0.01$	$18.86^{c}\pm0.04$
CPYM2	$41.64^{c}\pm0.01$	$20.03^{ab}\pm0.04$	$1.42^{ab}\pm0.02$	$9.58^{c}\pm0.02$	$1.49^{b}\pm0.01$	$25.79^{b}\pm0.04$
CPYM3	$32.37^{d}\pm0.19$	$18.94^{b}\pm0.03$	$1.46^{a}\pm0.01$	$8.66^{d}\pm0.01$	$1.73^{a}\pm0.01$	$36.85^{a}\pm0.21$

Values are means of triplicate determinations ± standard deviation. Values in the same column with different superscript are significantly different ($p<0.05$). CP100-100% cowpea, CPYM1-95: 5% cowpea-yellow maize, CPYM2-90:10 cowpea-yellow maize and CPYM3-80:20%-cowpea-yellow maize. CP- crude protein.

The decrease which was less than 50.20% from the control sample (CP100 =100% cowpea) could be attributed to increasing carbohydrate content with increase yellow maize substitution (Brownson, 2016). Carbohydrate increases total solids which in turn decrease MC (Okwunodulu and Nwabueze, 2019). The decrease in MC of the "moi-moi" samples may signify increase in stability, better texture chewability and acceptability due to higher dry matter (Adepoju and Etukumoh, 2014; Onoja *et al.*, 2014). The MC of the control sample (50.20%) was lower than 55.40% reported for cowpea "moi-moi" (Ejima and Ejima, 2015) which may be attributed to the variety of maize, ingredients and the water ratio used. However, MC of cowpea-yellow maize "moi-moi" obtained in this study was within the

range (36.10% - 44.10%) reported by Akusu and Kiin-Kabari (2012) for "moi-moi" prepared with cowpea and maize flour blends. This may be due to ingredient type, quantity as well as water: flour ratio used in the formulations.

3.1.2. Crude protein (CP)

Decreases in crude protein content due to increasing yellow maize substitution were lower than 20.70% from the control which points to protein reduction. The decrease (20.09-18.94%) could be aligned to the higher protein content of cowpea than yellow maize which was diluted with substitution. Higher crude protein (20.70%) obtained in the control sample confirmed the report that legumes such as cowpea contain substantial quantity of crude protein (Egouniety *et al*., 2002). Maize is a carbohydrate-rich crop without substantial amount of crude protein (Shah *et al.,* 2015). The decreasing protein trend (20.09-18.94%) in this study analogized with but slightly higher than 20.25-15.40% obtained by Akusu and Kiin-Kabari (2012) for "moi-moi" produced with cowpea and maize flour. Perhaps, drying of cowpea and maize had no significant effect on their protein content. Protein in foods helps to build and maintain healthy muscle mass, supports tendon, ligaments and growth. Protein also helps to prevent spikes in blood glucose which are especially important for preventing type 2 diabetes, balancing energy level and keeping appetite and mood in check (Voet *et al*., 2008).

3.1.3. Crude fibre (CF)

The CF offers a variety of health benefits and is essential in reducing the risk of chronic disease such as diabetes, obesity, cardiovascular disease and diverticulosis. It lowers the concentration of low-density lipoprotein cholesterol in the blood, possibly by binding with bile's acids (Ishiwu and Tope, 2015). Crude fibre content of the cowpea-yellow maize "moi-moi" samples obtained in this study increased (1.38-1.46%) significantly ($p<0.05$) more than the control with increasing level of yellow maize substitution. The increase may have stemmed from high fibre content of yellow maize (IITA, 2009). These results implied that the fibre content of "moi-moi" and its associated health benefits could be increased by complementing it with yellow maize. The samples could be an ideal food for the obese and maintenance of weight. The results obtained in this study was higher than 0.75% to 0.83% reported by Nwosu (2011) for "moi-moi" produced from cowpea and asparagus bean seeds which could be attributed to variation in raw materials used and the processing methods used. Also, the increasing fibre trend in this study (1.38-1.46%) aligned with but slightly lower than 1.38% to 1.85% reported by Akusu and Kiin-Kabari (2012) for "moi-moi" produced with cowpea and maize flour blends. Variations in cowpea and maize varieties as well as ingredients used in the formulations may have contributed as well.

3.1.4. Fat

The fat content of the blended samples decreased (10.02%-8.66) with increasing levels of yellow maize substitution levels. These significant ($p<0.05$) decreases were lower than the control sample (10.67%) and may imply higher fat absorption by the carbohydrate and calcium content of the yellow maize (Igyor *et al*., 2011). The fat decrease could signified reduced fat-soluble vitamins and calorific value with yellow maize substitution (Coppin and Pike, 2001). Acceptability and swallowing may as well be reduced as they are dependent on fat content levels. Similar decreasing trend (14.75 to 9.98%) was reported by Akusu and Kiin-Kabari (2012) for "moi-moi" produced with cowpea and maize flour blends. Their fat values than those obtained in this study may be due to drying of cowpea and maize.

3.1.5. Ash

Ash content represents total minerals content in foods and therefore serves as an index for nutritional evaluation of mineral (Lienel, 2002). Significant ($p<0.05$) ash content increase (1.45-1.73%) due to increasing yellow maize substitution justified higher mineral of yellow maize than cowpea and mineral improvement in all the cowpea-yellow maize samples more than 1.38% from the control. Maize is a useful source of minerals (Gopalan *et al*., 2007; Shah *et al*.,

2015). Similar increasing trend (1.70-1.96%) was reported by Akusu and Kiin-Kabari (2012) for "moi-moi" produced from cowpea and maize flour blend which is higher than those reported in this study probably due to variety and drying of cowpea and maize.

3.1.6. Carbohydrate

This is one of the energy substrate which content in the cowpea-yellow maize "moi-moi" samples increased (18.86%-36.85%) with increase in yellow maize inclusion levels higher than the control sample (15.70%). The increase could be due to high carbohydrate content of yellow maize (Brownson, 2016). This may likely increase the energy levels of the samples thereby making it a good weaning food. These results are in agreement with but higher than 21.39%-34.72% reported by Akusu and Kiin-Kabari (2012) from "moi-moi" made with cowpea-soybean blend, 30.79% -36.63% with cowpea-soybean reported by Ogundele *et al*. (2015) and 32.05%-35.53% reported by Nwosu (2011) from cowpea "moi-moi*"* complemented with asparagus bean. The variation could be due to bean variety, ingredients and the preparation technique used.

3.2. Effects of cowpea partial substitution with yellow maize on the vitamin composition of "moi-moi"

3.2.1. Vitamin A

Table 4 depicts the vitamin results that revealed significant ($p<0.05$) vitamin A increase (1.39-1.82 µg/100mg) of cowpea-yellow maize "moi-moi" samples more than the control (1.23 µg/100mg) with yellow maize substitution. The increase is vitamin A could be due to high content of beta carotene in yellow maize (Omafuvbe *et al*., 2003) which is a good source of pro-vitamin A (Tawanda *et al*., 2011). Vitamin A is a fat-soluble vitamin and a powerful antioxidant that plays a critical role in maintaining healthy vision, neurological function, healthy skin and support immune function. It is involved in reducing inflammation by fighting free radical damage. Vitamin A values obtained in this study were higher than 1.23 µg/100g obtained from the control sample, but below 2.67 µg/100g obtained from 100% cowpea *"*moi-moi" reported by Ejima and Ejima (2015). The ingredients used as well as varietal and processing technique employed could be sources of variation. This study showed that cowpea-yellow maize "moi-moi" samples could be beneficial for sight improvement especially for vulnerable low-income earners.

Table 4. Effects of the partial substitution on the vitamin composition of "moi-moi" produced with cowpea-yellow maize blend

Sample	Vitamin A (µg/100g)	Vitamin C (mg/100g)
CP100	1.23^{d}±0.04	0.07^{a}±0.04
CPYM1	1.39^{c}±0.01	0.04^{b}±0.01
CPYM2	1.48^{b}±0.01	0.05^{ab}±0.01
CPYM3	1.82^{a}±0.03	0.06^{ab}±0.01

Values are means of triplicate determinations ± standard deviation. Values in the same column with different superscript are significantly different ($p<0.05$). CP100-100% cowpea, CPYM1-95:5% cowpea-yellow maize, CPYM2- 90:10% cowpea-yellow maize and CPYM3 -80; 20% cowpea-yellow maize.

3.2.2. Vitamin C

Just like vitamin A, vitamin C increased (0.04-0.06 mg/100g) with increase in yellow maize substitution but lower than the control (0.07 mg/100g) with sample CYPM3 (80:20% cowpea-yellow maize) having the highest value (0.06 µg/100g). The increment is not significant ($p>0.05$) at levels of 10-20%. Vitamin C is

associated with numerous health benefits like bone and joint development, maintenance of healthy immune system, purifies the blood and is an antioxidant (Cohen *et al.,* 2000). Vitamin C levels in this study were considerably low which could be attributed to the effect of heat during steaming as vitamin C is heat and light labile and possibly steeping since it is water soluble.

3.3. Mineral composition of "moi-moi" as affected by partial substitution of cowpea with yellow maize

3.3.1. Calcium

Mineral results as shown in Table 5 revealed that calcium content of cowpea-yellow maize "moi-moi" samples increased (17.20-19.06 mg/100g) significantly ($p<0.05$) with increase in yellow maize substitution. These increases were significantly ($p<0.05$) higher than the control (15.33 mg/100g) and could be attributed to higher calcium content of yellow maize than cowpea (Shah *et al.,* 2015). Therefore, yellow maize substitution is calcium enrichment compare to 100% cowpea 'moi-moi'. The values obtained in this study were within 12.10-19.10 mg/100g reported by Ejima and Ejima (2015) for improved maize and cowpea "moi moi" samples. Calcium is important in the body as it helps to build and maintain teeth and plays a key role in our cells. The samples are therefore good for infant, elderly and recovering patients as calcium-rich diet will helps to maintain bone strength and prevent rickets infants, prevent osteoporosis in adults and is needed for patient recovery.

Table 5. Partial substitution effects on the mineral composition of the "moi-moi" samples (mg/100g).

Samples	Calcium	Magnesium	Phosphorous	Ca: P
CP100	$15.33^{d}\pm0.04$	$9.33^{b}\pm0.04$	$6.92^{c}\pm1.35$	$2.22^{a}\pm0.31$
CPYM1	$17.20^{c}\pm0.14$	$12.44^{ab}\pm0.03$	$7.48^{bc}\pm0.04$	$2.30^{a}\pm0.30$
CPYM2	$17.88^{b}\pm0.01$	$12.56^{ab}\pm0.02$	$7.73^{b}\pm0.04$	$2.31^{a}\pm0.02$
CPYM3	$19.06^{a}\pm0.03$	$13.43^{ab}\pm0.01$	$9.42^{a}\pm0.02$	$2.02^{a}\pm0.04$

Values are means of triplicate determinations $\pm$ standard deviation. Values in the same column with different superscript are significantly different ($p<0.05$). CP-100% cowpea, CPYM1-95:5% cowpea-yellow maize, CPYM2-90:10% cowpea-yellow maize and CPYM3-80:20% cowpea yellow maize.

3.3.2. Magnesium

The magnesium content of yellow maize substituted "moi-moi" samples also increased (12.44-13.43 mg/100g) though without significant ($p<0.05$) variations with increasing yellow maize substitution more than 9.33 mg/100g from control. This may possibly mean that substitution levels had no significant ($p<0.05$) magnesium improvement. Magnesium values in this study were higher than 8.00 - 9.20 mg/100g reported by Ejima and Ejima (2015) from "moi-moi" produced with improved maize, and beans. These increases confirmed higher magnesium content of maize (Gopalan *et al*., 2007) which also reflected same on the moi-moi samples alongside the associated health benefits. Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation (Soetan *et al*., 2010). It is required for energy production, oxidative, phosphorylation, glycolysis, contributes to the structural development of bones, synthesis of DNA, RNA and the antioxidant glutathione. Higher magnesium content of the substituted samples than the control predisposes it to a better diet to infants, elderly and recovery patients. It works in synergy with adequate ratios of calcium and phosphorous for bone health and development (Okwunodulu *et al*., 2022).

3.3.3. Phosphorous

Phosphorous values like other minerals increased (7.48 - 9.42) mg/100g) with significant ($p<0.05$) variation as the yellow maize substitution increased more than the control (6.92 mg/100g). Higher phosphorous content of yellow maize than cowpea may be responsible. Yellow maize substitution improved the phosphorous content of "moi-moi" which is a valuable nutrient for bone formation. Phosphorus synergizes with calcium to form calcium-phosphate that builds strong bones and teeth. Therefore the substituted samples will provide adequate phosphorous more than the control that will synergise with calcium to give a better calcium: phosphorous ratio.

3.3.4. Ca: P ratios

Calcium to phosphorous content in the body which is maintained by sufficient diet intake is important for infants' bone growth and development. Any imbalance results in rickets and high risk of bone fracture in children as well as osteomalacia and osteoporosis in adults. This is because the ratio determines calcium absorption, its retention and regulation in the body. Also, high phosphorous intake aids hypocalcaemia and bone fracture in infants. Maintaining appropriate ratio in the body through dieting is therefore mandatory for optimal body function and nutritional adequacy. Though there were no significant ($p>0.05$) variations among the entire Ca: P ratios in this study, but slight increase were recorded as yellow maize inclusion increased. The ratios increased (2.30-2.31) from 5-10% and decreased to 2.02 at 20% yellow maize inclusion levels. This simple suggest that yellow maize substitution beyond 10% in "moi-moi" preparation will start affecting bone health and development. Though the decrease was lower than the control, but they were within the recommended values (1.5: 1, 1:1, 1:2 and 2:1) by the nutritionists for bone health and development (Okwunodulu *et al*., 2022). Therefore, the samples will be good for infants, elderly and recovery patients to prevent rickets and osteoporosis.

3.3.5.Correlation of vitamin C with calcium, magnesium and phosphorous

Table 6 depicts the Pearson correlation of vitamin C with calcium, magnesium and phosphorous content of the ''moi-moi'' samples. All the results showed negative correlation irrespective of the blending ratios of cowpea and yellow maize. Calcium had slight stronger significant ($p>0.05$) correlation with vitamin C followed by magnesium and phosphorous. This could be associated to the decreasing vitamin C content of the 'moi-moi' samples due to increase in yellow maize inclusion thereby resulting to the decrease in vitamin C enhancement of mineral absorption. The maximum absorption order of the minerals is shown as phosphorous > magnesium > calcium. These concretised with the assertion that vitamin C enhances mineral absorption. Each mineral including vitamin C correlated maximally at 1.0 with each counterpart thereby showing that minerals are function of the overall mineral content of the samples. These notwithstanding, calcium correlated with magnesium at $p<0.01$ (99% confident level) and phosphorous at $p<0.05$ (95% confident level). These aligned with the literature report that magnesium enhances calcium-phosphorous synergy for strong bones and teeth formation (Okwunodulu *et al*., 2022). Therefore, yellow maize substitution should not be above 20% for maximum mineral absorption and their health benefits. The samples may likely contribute in preventing osteoporosis in adults and rickets in infants.

3.4. Influence of cowpea substitution on the physicochemical properties of 'moi-moi'

3.4.1. pH

Physicochemical results as presented in Table 7 showed that the pH of the yellow maize substituted "moi-moi" samples decreased (6.07-6.00) with increasing yellow maize inclusion. The decrease is significantly ($p<0.05$) lower than the control (6.12) and signified acidity content of yellow maize following steeping. With the pH decrease, the samples were moderately acidic and within the acceptable

threshold with an advantage of lesser susceptibility to microbial proliferation that may cause gastrointestinal problems.

Table 6. Physicochemical properties of cowpea substituted "moi-moi" samples

Samples	pH	Appearance
CP100	6.12^{a}±0.00	0.18^{a}±0.00
CPYM1	6.07^{ab}±0.01	0.12^{c}±0.01
CPYM2	6.03^{bc}±0.01	0.13^{c}±0.01
CPYM3	6.00^{bc}±0.03	0.15^{b}±0.00

Values are means of triplicate determinations ± standard deviation. Values in the same column with different superscript are significantly different ($p<0.05$). CP100-100% cowpea, CPYM1-95:5%cowpea-yellow maize, CPYM2-90:10% cowpea-yellow maize and CPYM3-80:20% cowpea-yellow maize.

Table 7. Sensory scores of "moi-moi" produced with cowpea-yellow maize blends

Samples	Taste	Texture	Appearance	Flavour	G A
CP100	7.75^{a}±0.79	7.05^{a}±0.51	6.85^{a}±1.35	7.10^{a}±1.52	7.80^{a}±1.15
CPYM1	7.10^{ab}±0.79	6.25^{cd}±1.12	5.80^{a}±1.64	5.70^{b}±1.87	6.85^{b}±1.39
CP90YM2	6.45^{b}±1.88	6.30^{c}±1.17	6.60^{a}±1.14	5.65^{bc}±1.39	6.65^{bc}±1.39
CPYM3	5.05^{c}±2.11	6.60^{b}±1.14	6.70^{a}±1.49	5.25^{c}±1.71	6.10^{c}±1.74

Values are means of triplicate determinations ± standard deviation. Values in the same column with different superscript are significantly different ($p<0.05$). CP100-100% cowpea, CPYM1-95:5% cowpea-yellow maize, CPYM2-90:10% cowpea-yellow maize and CPYM3- 80:20% cowpea-yellow maize. GA- general acceptability.

3.4.2. Appearance

The appearance of the substituted samples increased (0.12-0.15) with yellow maize inclusion but lower than control (0.18). The improvement was only significantly ($p<0.05$) at 20% inclusion level. This could be due to steeping and yellow pigments in the yellow maize. Besides, caramelizaton during steaming of simple sugars released during yellow maize steeping may have contributed too (Nwosu, 2011). Steeping may have activated the dormant enzymes in yellow maize which in turn hydrolysed the starch to release the simple sugars. The sugars also may have reacted with the amine groups of the proteins and resulted in Maillard browning. Appearance attracts the consumers to initiate acceptability. Therefore, yellow maize substitution influenced the appearance of the substituted samples compared to the control by improving the aesthetic appeal and may likely improve the acceptability (Figures 5, 6 and 7).

3.5. Effect of substitution of cowpea on sensory characteristics

3.5.1. Taste

The sensory scores presented in Table 7 showed that taste scores of yellow maize substituted "moi-moi" samples decreased (7.10-5.05) significantly ($p<0.05$) with an increase in yellow maize substitution lower than the control (7.75). Decreasing taste scores may suggest acceptability reduction or rejection if yellow maize substitution increased beyond 20% used in this study depending on choice. These decreases may have contributed to the preference of the control to the substituted samples by the panellists alongside familiarity with the control sample.

3.5.2. Texture

The scores increased (6.25-6.60) with significant ($p<0.05$) variation as yellow maize substitution increased may be due to higher carbohydrate content of yellow maize (Table 3). Though the increased texture scores were significantly ($p<0.05$) lower than the control sample (7.05), but substitution levels beyond 20% may be higher provided acceptability is not compromised. Therefore, yellow maize inclusion improved cowpea-maize "moi-moi" texture which is one of the acceptability criteria.

3.5.3. Appearance

The same appearance improvement (5.80-6.70) trend was also obtained here as yellow maize inclusion increased, except there was no significant ($p>0.05$) variation in their improvement. May be the substitution levels had no significant ($p<0.05$) variation in appearance to the consumers. Though these increases were lower than the control (6.85), substitution beyond 20% may be more appealing to the consumers depending on choice. Therefore yellow maize inclusion improved the aesthetic appeal of the cowpea "moi-moi" depending on choice

3.5.4. Flavour

The scores decreased (5.70-5.25) significantly ($p<0.05$) with increasing yellow maize content which is in consonant with taste score as flavour is a combination of taste and smell. The decrease was lower than the control (7.10) and could be attributed to the inherent flavour lowering components of cereals compared to legumes (Ouoba *et al*., 2005). The substitution may have diluted the desirable beany flavour of the control sample which the panellist were used to. However, these decreases point to acceptability decrease and may lead to complete rejection at above 20% inclusion levels.

3.5.5. General acceptability (GA)

The GA decreased (6.85-6.10) significantly ($p<0.05$) with increase in yellow maize inclusion with control sample being the most preferred (7.80) followed by sample CPYM2 (95: 5% cowpea-yellow maize). This may follow that substitution level of lower than 5% may improve better or equal to the control. The 20% substitution level was the least preferred (6.10) may be due to higher substitution level. Similar decreasing acceptability trend (8.6-5.6) was reported by Akusu and Kiin-Kabari (2012) from "moi-moi" made from cowpea-maize flour blend. The acceptability scores of their control and substituted samples were higher than those obtained in this study probably due to maize and bean varieties, type and quantity of ingredients used, preparation method adopted and cowpea-maize proportion used.

4.Conclusion

The results of this study justified legume-cereal nutrient complementation and supplementation. With increase in yellow maize substitution levels of up to 20%, there were improvement in ash, crude fibre, and carbohydrate. Vitamins A and C, calcium, manganese, phosphorous and appearance also increased. Crude protein, moisture, fat and pH contents decreased. Some nutrients increased below the control. Higher nutrient may be obtained at higher inclusion levels, but that may compromise acceptability as maximum acceptability for the blends was obtained at 5% substitution level. The "moi-moi" produced from 100% cowpea was best preferred followed by that with 5% yellow maize substituted sample while most nutrients peaked at 5-10 yellow maize inclusion but may be increased up to 20% for maximum mineral absorption. Therefore, yellow maize substitution of cowpea for 'moi-moi' preparation becomes a matter of choice between increasing the substitution level to increase the nutrient and acceptability levels.

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Acknowledgments

We are grateful to Food Science and Technology Department of the University for supply of the reagents used and laboratory space.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

DETECTION METHODS OF FOODBORNE PATHOGENS

Omolara Adenaike[1]✉, Evelyn N. Fatokun[2], Grace O. Abapka[3] and Oluseye A. Ogunbayo[4]

[1]Dept. of Biological Sciences, Oduduwa University, Ipetumodu, Nigeria.
[2] Dept. of Biological Science and Biotechnology, Caleb University, Imota. Nigeria.
[3]Dept. of Microbiology, Federal University of Health Sciences, Otukpo, Nigeria.
[4]Edinburgh Surgery Online, University of Edinburgh, UK.
✉adeoyelara2003@gmail.com

https://doi.org/10.34302/crpjfst/2024.16.3.14

Article history:
Received:
October 23th, 2023
Accepted:
August 15th, 2024

Keywords:
Culture-based method;
Detection;
Foodborne pathogens;
Immunological method;
Molecular methods;
Next generation sequencing.

ABSTRACT

Food borne pathogens are present in a variety of foods giving rise to foodborne illnesses that have become a major threat to human health globally. Detection of these pathogens is critical to ensure safe food supply and prevention of foodborne illnesses. There are varied ways of detection and the methods are categorized under two broad techniques which are culture dependent and culture independent. This review highlights different types of detection methods classified under culture-based and culture-independent methods namely, immunological assays, nucleic acid-based methods, biosensor-based, microarray based as well as the next generation sequencing; their strengths, limitations and challenges. Next-generation sequencing (NGS) technology has advanced our understanding of food microbiome by allowing the discovery and characterization of unculturable microbes with prediction of their function over other diagnostic assays. The need for improved, low cost, rapid and reliable detection techniques cannot be overemphasized and are highly recommended.

1.Introduction

Food safety can be defined as the assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. Food is often contaminated by bacteria, viruses, fungi and parasites, which induces several enteric diseases (Saravanan et al., 2021). The ability to rapidly detect viable pathogens in food is important for public health and food safety reasons (Foddai and Grant, 2020). Research in the food safety and security field continuously focuses on the search for improved methods of foodborne pathogen detection that are sensitive, accurate, rapid, and cost-effective (Vanegas et al., 2017). Detection methods of bacterial foodborne pathogens can broadly be divided into culture-based and culture-independent methods (Fig.1) (Park et al., 2023). This paper aimed to review different types of detection methods classified under the broad categories of culture-based and culture-independent methods.

2.Culture-based methods

Culture- based conventional methods also known as traditional techniques have been the oldest methods in detecting microorganisms, even the pathogenic strains (Priyanka et al., 2016). They are considered to be the "gold-standard" and are well known for their cost-effectiveness, sensitivity, ability to confirm cell viability, and ease of standardization (Senturk et al., 2018). Culture is a term used to describe the biological amplification of viable and cultivatable bacteria with manufactured growth media (Amani et al., 2015). It is the microbial growth on or in a nutritional solid or liquid medium; the increased number of the organisms simplifies detection as well as enhances the identification by the

morphological and biochemical studies of the microbial cells (Vazquez-Pertejo, 2023).

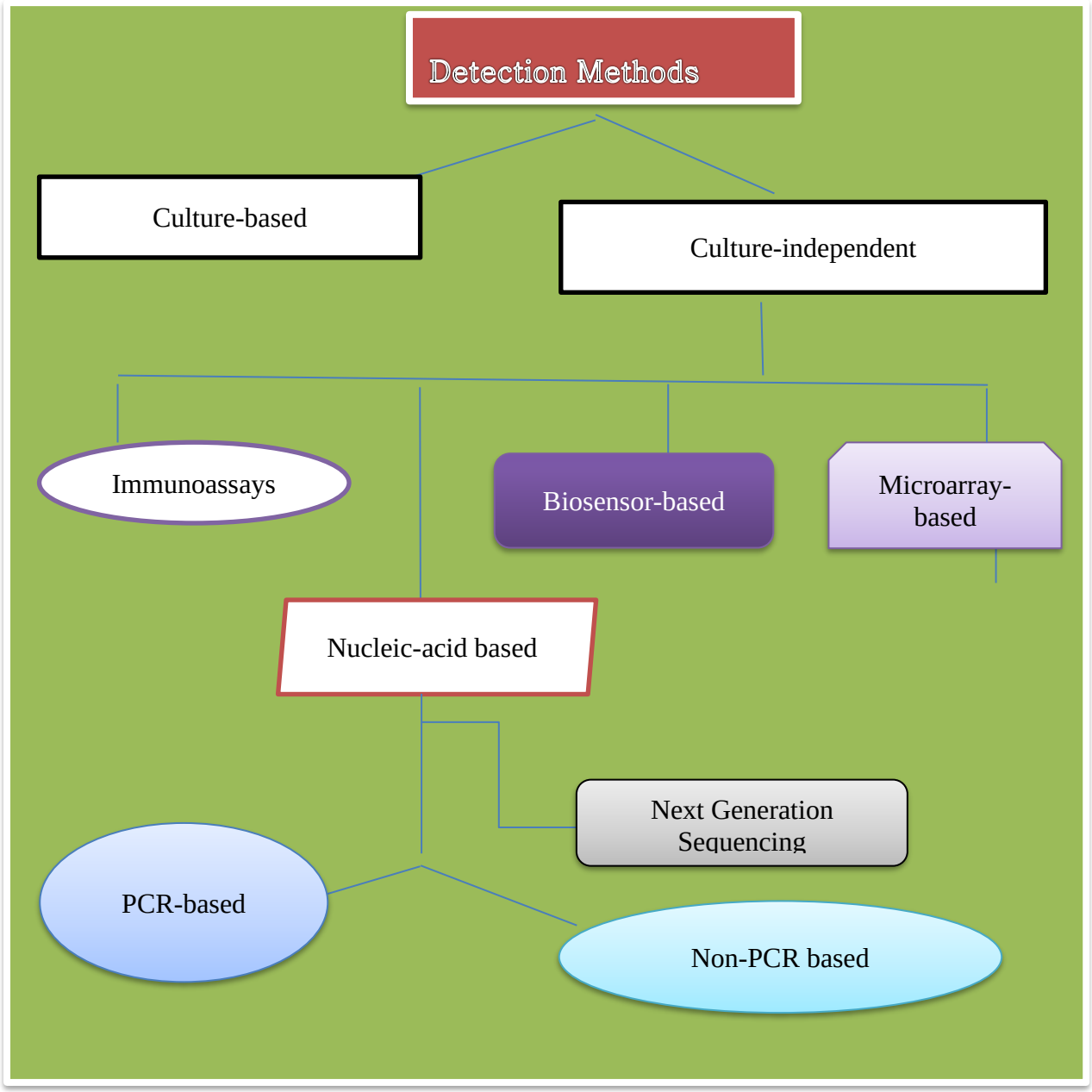

Figure 1. Detection methods of Foodborne Pathogens

Culture-based methods mainly rely on growing the pathogens on agar plates which are exhausting processes and take more than three days to a week, from preliminary observations to confirmation of the pathogens (Reddy et al., 2022). Routine detection of bacterial pathogens in most Nigerian laboratories is based on enrichment culture, microscopic observation and biochemical assays (Nasrabadi et al., 2017). Culture based methods have the advantage of delivering a bacterial isolate that can be subjected to further analyses such as antimicrobial susceptibility testing, test for virulence determinants (e.g. toxins) and also typing to support investigation in an epidemiologic study (Kaprou et al., 2021). Despite the merits stated and the benefits of being widely used and reproducible,

these methods are time-consuming, relatively expensive, prone to contamination due to extended time-lag, and the processes are cumbersome. Furthermore, the difficulty of quantitative analysis; that is the culture method is not appropriate for a number of important bacterial pathogens because they are difficult or impossible to grow under laboratory conditions (Nasrabadi et al., 2017). This has given rise to the phenomenon described as 'the great plate count anomaly'. A term coined by Staley and Konopka in 1985 to describe the difference between the numbers of cells from natural environments that form viable colonies on agar medium and the numbers obtained by microscopy (Harwani, 2013). This is largely explained by the development of cells into a dormant state regarded as viable but not culturable (VBNC) state. Cells in the VBNC state exhibit very low, but detectable, metabolic activity compared to actively growing cells. Such dormant cells maintain the integrity of their cell membranes and continue to express genes at low levels. When they are transferred to solid nutrient media, they fail to form colonies, as opposed to active cells (Ayrapetyan and Oliver, 2016). The presence of VBNC cells in food is widely documented. This was resulted due to stress and harsh environmental variations during the stages of food processing from farm to fork. Given that pathogenicity is maintained by some species during the VBNC state and the inability of culture method to detect the cells that are in a dormant state, poses a substantial food safety and public health risk (Ramamurthy et al., 2014). The use of such standard culture methods for the detection of pathogenic organisms is to be given urgent attention for improvement. It is vital that we be aware of their existence and understands how these dormant bacteria affect our experiments, and when their impact warrants the use of alternate methodologies (Ayrapetyan and Oliver, 2016). Other drawback of the culture-based method is the inability to identify the pathogen to species level.

2.1.Use of chromogenic media

Over the last few decades, a range of chromogenic media has been developed that are designed to target pathogens with high specificity. They serve as alternatives for rapid microbiological identification, as they make it possible to presumptively differentiate bacterial species and/or groups according to colony colour, reducing the need for biochemical tests (Garcia et al., 2021). These media exploit enzyme substrates that release coloured dyes upon hydrolysis, thus resulting in pathogens forming coloured colonies that can easily be differentiated from commensal flora. Ideally, commensal bacteria should either be inhibited completely by selective agents or form colourless colonies thus allowing pathogens to 'stand out' against background flora. This allows clear differentiation of microbes producing the target enzyme from those that do not. This is especially important when attempting to detect specific pathogens within polymicrobial cultures (Manal et al., 2015). The combination of agar media operating on different biochemical principles and characterized by different sensitivities and selectivities could allow for a more rapid and accurate detection of a broad spectrum of group members in food samples. Chromogenic reactions of enzymatic cleavages of substrates and the release of chromogens leading to higher specificities and improvement over the use of the conventional microbiological growth media (Fuchs et al., 2022). These culture media have therefore been found to outperform other conventional microbiological rapid methods in terms of specificity, sensitivity, and accuracy, in addition to identifying contamination in the samples more efficiently (Garcia et al., 2021). Chromogenic media are therefore regarded at least as convenient and sensitive as well as being more cost effective (Perry, 2017).

2.2. Microscopic techniques

Simple light microscopic observation and staining are the basis on which the oldest detection methods are formed after culture on

selectivity growth media, but often do not provide a clear answer (Mobed et al., 2019). It has the advantage of being inexpensive, rapid, and simple to perform. Microscopy however, is labour intensive and requires highly skilled scientists for optimal diagnostic performance (Anderson et al., 2014). Microscopic techniques over the years have been used for bacterial observation as a complement to scientific research and conventional diagnostic tests. Nowadays, they are applied in the areas of food and water quality amongst others, where biological detection and quantification are significantly important (Nasiłowska et al., 2021). The conventional light microscope suffers from limited throughput, relatively high cost, bulky size, lack of portability, and requirement for focus adjustment. These drawbacks partially limit the use (Zhang et al., 2015). Therefore, it is best used as an adjunct to traditional culture or molecular methods (Mobed et al., 2019). Several advancements in microscopy have evolved over the years and illustrated to achieve inspiring outcomes (Liu et al., 2021). Electron microscopy surpasses the use of light microscopy due to high resolution power and hence transmission electron microscopy becomes important to find number of bacterial cells and their biomass (Mishra et al., 2016). The scanning electron microscope (SEM) is quite useful to reveal morphological features of isolated organisms as well as for diagnosis, but difficulty with specimen preparation methods have in the past limited the use of SEM for routine Microbiology (Golding et al., 2016). One of the innovative techniques to improve microscopy is stimulated emission depletion (STED) microscopy (Ghithan et al., 2021). STED microscopy is a typical laser-scanning super-resolution imaging technology for studying live biological samples on a nanometer scale. The ability of the high laser power to induce severe phototoxicity and photobleaching, limits the application for live cell imaging and this has led to the development of low powered STED (Zhang et al., 2021). STED microscopy has many practical benefits including the production of details of objects smaller than 50nm in a direct optical implementation (Alonso, 2013).

The several drawbacks of the culture-based detection methods have led to the development of novel "rapid" detection methods. These methods decreased detection time dramatically.as they do not require cultural enrichment (Wang and Salazar, 2016). They are advanced techniques that have been developed and optimized as alternatives to or for use in combination with these traditional techniques (Alegbeleye et al., 2018). The methods have been found to be user-friendly, easy, precise, portable, cheap, rapid, and provide simultaneous results in the detection of pathogens. Broadly, these methods can be categorise into four major groups: immunological, molecular, biosensor- and microarray-based techniques amongst others (Senturk et al., 2018). It should be noted that no method has 100% superiority over another but rather the newly developed methods are improvements over the older methods. Each method has its benefits and limitations as stated in Table 1.

Table 1. Some Foodborne pathogen detection methods

S/N	Pathogen detection methods	Principle	Advantages	Limitations
1.	Culture-based methods	Microbial growth in a medium under controlled laboratory conditions involving steps like pre-enrichment, selective enrichment or/ and	Relatively inexpensive, easy quantification of cells. High sensitivity with appropriate media. Allows study of colonial morphology,	Labour intensive, time consuming, less accurate, overlooks microbes that are viable but not culturable (VBNC).

		selective plating, followed by biochemical identification and serological confirmation of the results.	phenotypic characterization of organisms e.g. antibiotic susceptibility testing.	
2.	Immunological methods	Based on highly specific binding between an antigen and an antibody.	High specificity, sensitivity and efficiency. Reagents are safe, eco-friendly; cost-effective and do not require complicated procedures.	Assay preparation is laborious and time consuming. Risk of false-positives, some methods are expensive (e.g. conventional ELISA)
3.	Nucleic acid-based methods	Amplification of genetic material of the organism or directly target specific sequences of the organism's genome.	Very much accurate, having high specificity and sensitivity, and very fast. Provides more information at relatively faster speed.	Highly skilled personnel needed. High cost of the complex operations and machines. Some types have risk of false positives.
4.	Biosensor-based methods	Generation of signals and transduces in response to a specified quantity of a physical phenomenon.	Quite sensitive, easy to design, specific, and accurate.	Susceptible to interference or food matrix effects caused by components present in food. Poses challenges with detection limit, detection time, and specificity.
5.	Microarray-based methods	The ability of DNA to bind to itself and to RNA, i.e. complementary sequences will bind to each other	Provides new insights into gene function, disease pathophysiology, disease classification and drug development. Allows for full sequencing of the whole transcriptome	High cost, low-specificity, lack of control over the pool of analyzed transcripts. Relatively low accuracy, precision and specificity. The purity and degradation rate of genetic material, amplification process may impact the estimates of gene expression.
6.	Next Generation Sequencing	Relies on capillary electrophoresis. It involves fragmenting DNA/RNA into multiple pieces, adding adapters, sequencing the libraries,	Highly efficient, fast and accurate identification of microbial taxa, including uncultivable	Huge datasets produced. Computational resources required for analysis of sequencing data.

		and reassembling them to form a genomic sequence	organisms and low frequency variants. Provides superior characterization of food-chain microbiomes.	

3. Immunological methods

The recent development of immunological-based methods in several studies has made it easier and faster to detect pathogens in foods (Hormsombut et al., 2022). Immunological assay is the gold standard for highly sensitive detection of biochemical targets, providing a good platform for food contaminants detection. Immunological detection relies on the reaction between an antigen and an antibody (Ahmed et al., 2020). In immunoassay, the antibody or antigen conjugated labels are the most important units because they play roles to convert the information of target analytes to easily detected signal (Pan et al., 2021). They have the advantage that only viable cells lyse and therefore are potentially quantitative assays or detection of antigens as opposed to the characterization of the antigens. They are usually one day rapid test techniques and also provide information of previous infections and therefore are crucial in vaccine development (Yu et al., 2015; Muralidharan et al., 2020). They also have the ability to detect both contaminating organisms and their toxins that may not be expressed in the organism's genome (Amani et al., 2015). However, immunological methods are limited by a number of shortcomings. These include the lengthy development time required to prepare the immunoreagents for new analytes; the unsuitability for multiresidue analysis as immunoassays are usually only capable of detecting about two to five related compounds. Also, very few immunoassays have official status as well as having very limited amount of information delivered in the assays (Ahmed et al., 2020). Two broad categories of culture-independent immunological methods for food pathogen detection are ELISA and Lateral flow immunoassay (Wang et al., 2016).

3.1. Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is one of the immunoassay methods commonly used for the detection of surface markers on pathogens and pathogen-produced toxins based on the specificity of antibody–antigen interactions (Nehra et al., 2022). It is commonly used to measure antibodies, antigens, proteins, and glycoproteins in biological samples (Gouzalez et al., 2018). ELISA technique combined with the effective catalytic properties of enzymes; provide highly specific and sensitive detection of some special analytes. ELISA-based detection has been employed in a variety of formats, mostly immunofluorescence and lateral flow immunochromatography as well as in the development of immunosensors (Paramithiotis, 2023). ELISA has been used as a diagnostic tool in biotechnology, as well as a quality control check in various foodborne pathogen detection tests that relies on the specificity of the antibody–antigen interaction. Other immunological assays include enzyme-linked fluorescent assay (ELFA), and enzyme immunoassay (EIA). Double antibody sandwich technique is the standard immunoassay performed by ELISA. In this case, an antigen (pathogen or toxin) on the food pathogen binds to a specific antibody coated onto a solid support such as a microtiter plate. It is then washed and the pathogen is detected by the addition of an enzyme linked second specific antibody (Nehra et al., 2022; Iha et al., 2019). Enzymes usually used include alkaline phosphatase and horseradish peroxidase.

Detection of the conjugated enzyme linked antigen-antibody 'sandwich' can be performed using different systems including colorimetric substrates, chemiluminescence or fluorescence, and impedance (Iha et al., 2019; Alamer et al., 2018). The use of different substrates in ELISA has a major advantage as the substrates will bind to the respective conjugates specifically and will develop colouration which can be read in an ELISA reader in terms of wavelength. The colour change is visible to the naked eye. However, one of the disadvantages is that the binding of the chemical and conjugate is very specific, and contamination in the intermediate stages can lead to false positive result (Priyanka et al., 2016). The conventional ELISA method however is time-consuming and a costly plate reader to read the result. It is typically conducted in 96 well plates suitable for high throughput assays, thereby, allowing for many samples to be measured in a single experiment. It can take several hours to complete due to long incubation and blocking times needed. Furthermore, large volumes of expensive reagents, and the need for highly complicated and specialized instruments, limits their application in some point of care applications and in low-resource settings and developing countries (Iha et al., 2019). To circumvent these limitations, paper-based immuno-chromatographic strips have emerged for the rapid, reliable, easy-to-use, and on-site detection of pathogens (Gouzalez et al., 2018; Zhao et al., 2020). A novel paper-based enzyme-linked immunosorbent assay (p-ELISA) with shorter operation duration, lower cost, relatively higher sensitivity and wider application has been developed (Pang et al., 2018). This method provides result in less than an hour and requires 5μL of sample to complete the detection. The advantages of p-ELISA over the conventional ELISA includes the capacity to directly measure biomarker concentrations with significant increased sensitivity, use of inexpensive materials within a short duration made this platform handy for detection of pathogens especially in areas such as developing countries, lacking advanced analytical equipment (Tsao et al., 2018). The latest development, in 2012, was an ultrasensitive enzyme-based ELISA that manipulates nanoparticles as chromogenic reporters. This technique can generate a colour signal visible by naked-eye, with blue colour for positive results and red colour for negative results. Its advantage is in its ease of use, portability and speed of analysis. However, this method is qualitative and can determine only the presence or absence of an analyte and not its concentration (Alhajj et al., 2023). The technique has the challenge of being compromised by the food matrix and its accompanying microbiota. As its removal demands sample preparation and selective enrichment steps which are time consuming (Paramithiotis, 2023). Inadequate sensitivity often necessitates an enrichment step to increase bacteria count in the food sample, thus increasing the time required to deliver the result (Zhang, 2013).

3.2 Lateral flow immunoassay

A lateral flow immunoassay (LFIA) is a simple, paper-based diagnostic device operating on the chromatographic-like migration of a labelled analyte through multiple membrane endings in the visible result of an immobilized captured reagent (Tsai et al., 2018). Lateral flow immunoassays (LFIAs) are one of the urgent and prevalently applied quick recognition methods that have been settled for, recognizing diverse types of analytes (Sohrabi et al., 2022). It is a widely accepted technique owing to its on-site results, low-cost analysis, and ease of use with minimum user inputs, even though sensitivity is not quite equivalent to that of standard laboratory equipment (Jung et al., 2019).

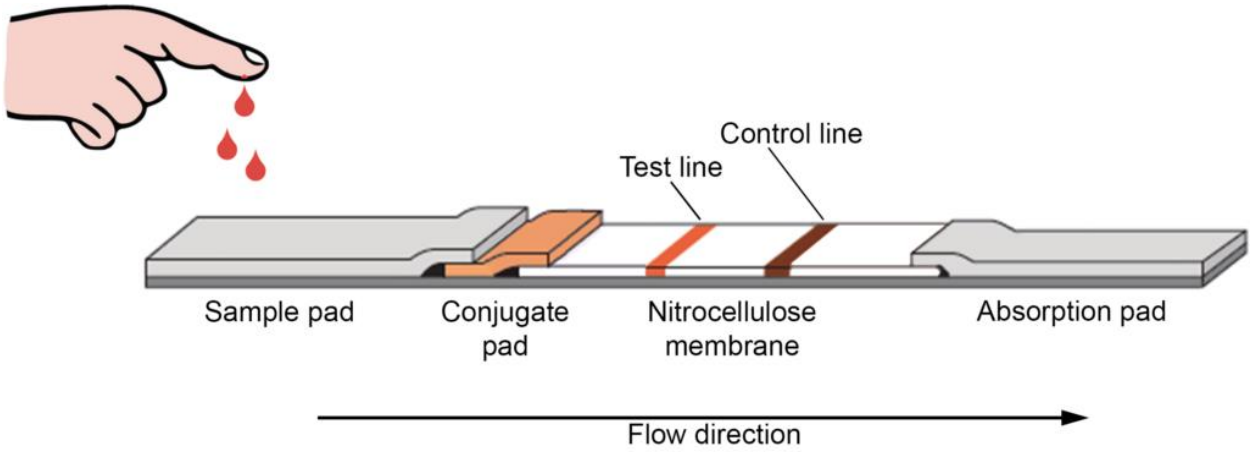

Figure 2. Schematic of a typical lateral flow assay device (Lateral flow assays, 2023)

As alternative to ELISA, LFIAs, which are designed in form of dipsticks and immuno-chromatographic strips, have emerged for the simple yet reliable detection of pathogens (Nehra et al., 2022). They have relatively long shelf-life and do not require refrigeration for storage and therefore suitable for use in resource-limited developing countries, small ambulatory care settings, remote regions and battlefields as well as refugee or internally disposed persons' camps (Contreras, 2019; Koczula and Gallotta, 2016). These tests are mostly used for qualitative assays by a visual interpretation of results. For the interpretation of the results, the colour intensity of the test zone is therefore very significant (Manta et al., 2020). Lateral flow immunoassay device is made up of four sections which are arranged orderly on a plastic backing, with sample pad starting at the bottom, followed by conjugate pad, nitrocellulose membrane which is further divided into test and control lines and then absorbent pad (Fig.2) (Nardo et al., 2021; Sajid et al., 2015).

The sample pad is the support where the sample is placed to perform the test. It absorbs the sample and allows the sample to migrate laterally to the conjugate pad. The dry conjugate is kept stable in the conjugate pad overtime (Contreras, 2019). On flowing to the conjugate pad, the analyte (sample) bind to the label biomolecules embedded in the conjugate pad and then flow together (analyte-conjugate complex) to the membrane where testing and control area are bound with immobilized protein for capturing the analytes. This part is significant as it determines the sensitivity of the assay (Kasetsirikul et al., 2020). The capillary flow-time is the duration required for the liquid to flow to fill the strip of the membrane (Koczula and Gallotta, 2016). The analyte-conjugate complex laterally flows through the third element, the nitrocellulose membrane, where specific biological compounds (typically antibodies, protein, or nucleic acids) are immobilized at pre-defined lines. The analyte, analyte-conjugate complex, and conjugates should react specifically to the compounds dispensed on the membrane. Lastly, the fourth element, the absorbent pad, should absorb any remaining sample of interest and conjugate complex (Lei et al., 2022). The captured antibodies immobilized on the test and control line on the nitrocellulose membrane form the basis of detection (Borse and Srivastava, 2019). Lateral flow immunoassays however, are not without limitations. They are of low sensitivity, they give qualitative and not quantitative results, and variability of the paper-based assay is also a concern as well as the fact that they are designed for single use and not for multiple sample analysis (Hristov et al., 2019).

The use of LFIAs in food safety procedures can help greatly in the management of foodborne risks by increasing the number of analyses, making them accessible, fast, and inexpensive. It can be recommended for used during food

safety monitoring alongside production chain, from raw materials to ready-to-eat products. It is the ideal device for testing in the Hazard Analysis and Critical Control Points (HACCP) procedures (Nardo et al., 2021).

4.Nucleic acid-based methods

Nucleic acid-based methods are by far the most sensitive and effective for the detection of a low number of target pathogens whose performance is greatly improved by combining with the sample preparation methods. They were previously considered unsuitable for routine testing of food products for pathogens because the techniques were only comprehended in research laboratories with skilled technicians. However, in the recent years, nucleic acid-based methods have gradually been mastered for use to replace or complement culture-based methods and immunochemical assays in routine laboratory analysis for food control (Lim and Kim, 2017; Souii et al., 2016). Polymerase chain reaction (PCR) is the foundational method of most DNA- and RNA-based rapid detection methods (Pleitner et al., 2014). Therefore nucleic acid-based detection methods can be broadly categorise into PCR-based and non-PCR based methods of pathogen detection (Jamdagni et al., 2016). Nucleic acid-based detection generally offers high sensitivity, but can be time-consuming, costly, and require trained staff (Cassedy et al., 2021). Also the separate detection of viable and dead bacteria is a major issue in nucleic acid-based diagnostics (Rudi et al., 2002).

4.1. Simple or conventional PCR

PCR is the oldest method of nucleic acid-based analysis; it has a dramatically reduced detection time compared with culture-based methods and provides increased sensitivity, thereby improving the possibility of detecting bacterial pathogens (Kim and Oh, 2021). PCR is a laboratory technique for DNA replication that allows a "target" DNA sequence to be selectively amplified (Wang, 2021). It is currently a widely used and incredibly potent technology that allows for rapid exponential amplification of a specific target sequence, reducing the need for culture enrichment (Akkina et al., 2022). It is one of the most commonly used molecular based methods for detection of foodborne pathogens (Wang, 2021*)*. The requirements for a PCR process include thermo cyclers, DNA template, two primers, Taq polymerase, nucleotides, buffers etc (Rajalakshmi, 2021). PCR is based on three simple steps required for any DNA synthesis reaction: first is the denaturation of the template into single strands; second is the annealing of primers to each original strand for new strand synthesis; and the third is the extension of the new DNA strands from the primers. These reactions may be carried out with any DNA polymerase and result in the synthesis of defined portions of the original DNA sequence (Delidow et al., 1993). PCR provides various benefits over culture-based and other traditional procedures; in terms of sensitivity, specificity, accuracy, speed and the ability to detect minute amounts of target nucleic acid in samples (Akkina et al., 2022). However, it cannot effectively distinguish the bacteria with different physiological states (Chen et al., 2022). Also, methods derived from PCR require sophisticated instruments and lifting temperature, and they are not suitable for the point-of-care testing (POCT) (Liu et al., 2019).

4.2. Real-time PCR/quantitative PCR

Real-time PCR or quantitative PCR (qPCR); it should be noted that the usage of RT-
PCR is inappropriate as this abbreviation is dedicated to reverse transcription PCR (Kralik and Ricchi, 2017). Real-time PCR is a variation of the PCR assay to allow monitoring of the PCR progress in actual time. Real-time PCR is currently one of the most powerful molecular approaches and is widely used in biological sciences and medicine because it is quantitative, accurate, sensitive, and rapid (Artika et al., 2022). However, there are limitations with assays based on qPCR. They

include its inherent incapability of distinguishing between live and dead cells. Its usage is limited to the typing of bacterial strains, detection, and possibly quantification in foods. To overcome this problem, a pre-enrichment of sample in culture media could be placed prior to the qPCR procedure. This step may include enrichment step or the use of specific selective media for the respective bacterium. This procedure is primarily intended to allow resuscitation or recovery and subsequent multiplication of the bacteria for the downstream qPCR detection (Kralik and Ricchi, 2017). This brings back the ill-experience of conventional culture-based technique. Also, qPCR is machine-dependent which is often expensive and requires regular maintenance (Foo et al., 2020).

4.3. Multiplex PCR

The basic principle of mPCR is similar to conventional PCR (Law et al., 2015). Multiplex PCR has the advantage of detection of many specific DNA markers in the same reaction and under specific experimental conditions (Al-Hindi et al., 2022). This has the benefit of saving time, workload, and efficiency is improved (Nguyen et al., 2016). Primer design is very important for the development of mPCR, as the primer sets are multiple and should have similar annealing temperatures in order for the mPCR assay to be successful and not produce primer dimers (Law et al., 2015). Despite the numerous advantages, mPCR has several pitfalls that have restricted its further development and broad application in food safety researches. These include the self-inhibition among different sets of primers; low amplification efficiency; and the no identical efficiency on different templates (Xu and Shang, 2016). Standardization of mPCR assays that enables the simultaneous detection of multiple pathogens has been carried out by several Researchers (Babu et al., 2013; Boukharouba et al., 2022).

4.4. Loop-mediated isothermal amplification (LAMP)

The Loop-Mediated Isothermal Amplification (LAMP) is a unique nucleic acid amplification technique for diagnosis of various pathogens which is simple, easy, rapid and cost effective when compared to PCR due to its high specificity, sensitivity, and rapidity (Akram,. 2017). LAMP results can also be easily read with the naked eye through colour-based reporters that can be added to the reaction mixture (Al-Hindi et al., 2022). Over several isothermal-based techniques, the loop-mediated isothermal amplification technique (LAMP) has many applications in the field of point-of-care (POC) testing as well as valuable means for food testing (Garg et al., 2021). LAMP assays can be carried out in regular laboratories, with minimal incubation sources like water bath or heating block and therefore suitable for application in resource-limited regions of the world (Xu et al., 2013; Al-Hindi et al., 2022). LAMP is highly specific and increases the amount of amplified DNA even up to a billion copies over less than an hour, compared to a million copies yielded by the PCR with the use of several primers (from four to six), which can distinguish up to eight specific locations on the DNA template, compared to only two in typical PCR (Soroka et al., 2021). LAMP is considered a promising alternative method to PCR technique (Bashar et al., 2022).

4.5. Nucleic acid sequence-based amplification (NASBA)

Nucleic acid sequence based amplification (NASBA), also known as self-sustained sequence replication (3SR) or transcription mediated amplification (Fakruddin et al., 2013). It is an mRNA based technology. NASBA is a continuous, isothermal, enzymatic RNA-based nucleic acid amplification technique developed in 1991 by J. Compton. NABSA mimics the retroviral RNA replication (Gilbridle, 2013; Anjorin et al., 2019; Oliveira et al., 2021). The technique is isothermal (41 °C), and the RNA is amplified

to a billion-fold within a duration of about 2 h (Zhai et al., 2019). The reaction typically consists of three enzymes, including T7 RNA polymerase, RNase H, and avian myeloblastosis virus (AMV) reverse transcriptase (RT), all of which act together to amplify sequences from an original single-stranded RNA template. The reaction also includes buffering agents and two specific primers and takes place at approximately 41°C (Zhao et al., 2014). Nucleic acid sequence-based amplification methods rely on the detection of certain gene sequences (signature sequences) in the target organism's genotype. Sequences can be chosen to detect a certain group, genes, species, or strain of the microbe (Lopez-Campos et al., 2012). NASBA operates by detecting specific DNA or RNA sequences of the target pathogenic organism (Manta et al., 2020). NASBA mechanism is in two phases: first is the non-cycling phase, where the target RNA is converted to dsDNA by reverse transcription; and second is the cycling phase, where the dsDNA molecules are actively transcribed into RNA products, leading to a yield of 10–100 copies of RNA from each template molecule (Oliveira et al., 2021). NASBA has the advantages of simple operation, strong specificity, high sensitivity and being less prone to genomic DNA contamination and therefore more suitable for applications where the testing of microbial viability is important (Jaksik et al., 2015). It eliminates the need for a thermal cycler and may therefore facilitate potential clinical testing in resource-poor settings (Zhai et al., 2014). Continuous research is on-going in different parts of the world to make these methods available technically and economically as alternative for polymerase chain reaction (Fakruddin et al., 2013).

Although NASBA is more commonly used for detection of RNA viruses, it has also being used to detect pathogenic bacteria in food and environmental samples, such as, *Campylobacter* spp., *Listeria monocytogenes*, *Vibrio cholera*, *Escherichia coli*, *Salmonella enterica* and even *Cryptosporidum parvum* in water (Everett et al., 2010; Zhai et al., 2014; Adetunji et al., 2023; Srinivasa et al., 2023). NASBA does have some disadvantages. First, to make it isothermal reaction thermolabile enzymes must be used. This means that the reaction must be effected at a lower temperature than the PCR process using thermocycling, sometimes resulting in an increase in nonspecific primer interactions. NASBA has some pittfalls nevertheless, dead or inactivated cells do not always have compromised cell membranes, so false positives may result. It is relatively expensive and requires highly specialized skills. It also requires highly specialized skills. Besides, the technology is mostly in the research stage, and a true constant temperature for the amplification is not realized due to the requirement of preheating treatment prior to the testing (Jaksik et al., 2015; Gao et al., 2022).

5.Biosensor- based methods

Biosensors are sensing devices that can be used to analyze and diagnose substances by transforming a biological response into a signal (Yasmin et al., 2016). A biosensor is a combination of two elements: the biological sensing element and a transduction unit (sensor) to produce an electrochemical, optical, mass, or other type of signal in proportion to the quantitative information on the analyte of interest in a given sample (Fig. 3) (Chepyala, 2020). The sensor or a transducer is a device that can convert energy from one form to another (Naresh and Lee, 2021). The bio-element (receptor) receives the physical or chemical stimulus and transmutes this information in the form of electrical energy while transducer performs the function of transducing this energy into valuable analytical signal which can further be analyzed and presented in an electronic form (Ali et al., 2017).

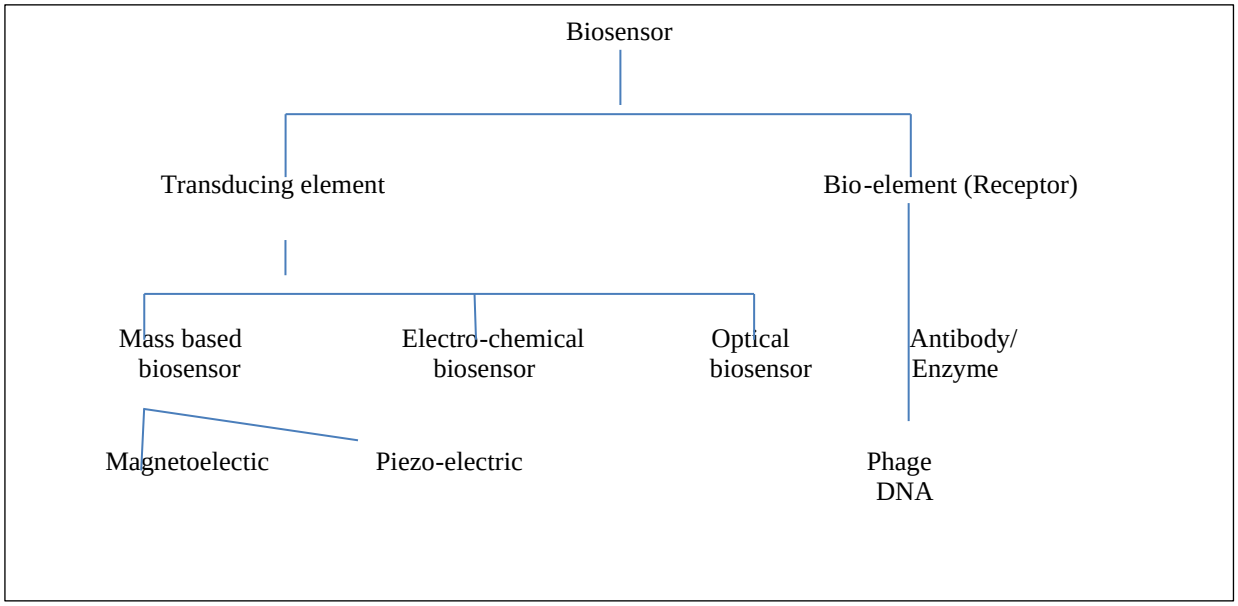

Figure 3. Working principle of biosensors. (Annonymous, 2023)

Biosensors function by coupling a biological sensing element with a detector system using a transducer (Punasiya et al.,. 2019). Biosensors are of significance use in a variety of disciplines and are employed in biochemical, electrochemical, agricultural, and biomedical fields. They are integrated in various point-of-care applications, such as disease monitoring, drug discovery, and detection of pollutants, disease-causing micro-organisms and markers that are indicators of a disease in bodily fluids (blood, urine, saliva, sweat), as well as in the food, healthcare, environmental monitoring, water quality, forensics, drug development, and other biological domains (Bhalla et al., 2016; Kulkarni et al., 2022). In Food processing, biosensors are used for the detection of pathogens such as *Escherichia coli* in vegetables, which is a bio-indicator of faecal contamination in foods (Mehrotra, 2016). A general working principle of biosensors is shown in Fig. 3.

The important components of a biosensor can be divided into three: first is a bioreceptor (e.g., enzymes, antibody, microorganism, or cells); the second is a transducer of the physicochemical signal, and lastly is a signal processor to interpret the information that has been converted. (Fatoyinbo et al., 2012). Biosensors can be classified according to transducers employed. The transducers are of different types such as electrochemical, optical, calorimetric, piezoelectric, microbial biosensor or enzyme biosensor (Dar et al., 2018).

5.1. Bacteriophage-based detection methods

Bacteriophages (phages) are viruses that can infect bacteria alone; they are predominant in nature, with more researchers paying attention toward bacteriophages as a promising tool to treat bacterial pathogens (Wei et al., 2019). With the advances made in genetic engineering and synthetic biology, phage-based methods for bacterial detection have become more iterative and elegant. Leveraging the unique characteristics of phage provides a wide variety of potential applications in the food and health industries (Wang et al., 2021). The high specificity and natural affinity of bacteriophages for their host cells make-phage based methods an attractive proposition (Foddai and Grant, 2020). Bacteriophages are reported as only been able to replicate inside living cells, hence phage-based methods can be used to demonstrate cell viability (Richter et al., 2018). Phages therefore could also assist in the discrimination between dead and living or viable but non-culturable bacteria (Paramithiotis, 2023). Bacteriophages

(i.e., viruses with bacterial hosts) pose advantages such as great specificity, robustness, toughness and cheap preparation, making them popular biorecognition elements in biosensors and other assays for bacteria detection. There are several possible designs of bacteriophage-based biosensors. The development of phage-based biosensors as a tool for the direct detection of live pathogens in food is an important and attractive approach (Richter et al., 2018).

6.Microarray-based techniques

Microarray is the advanced form of the southern blotting technique (Al-Hindi et al., 2022). Although originally developed for whole genome gene expression analysis, microarrays have become applicable in the detection of foodborne pathogens and in the investigation of the evolutionary relatedness between different bacterial species. This is due to the advantageous combination of powerful nucleic acid amplification strategies with an immense screening capability of the technique, to produce a high level of sensitivity, specificity, and throughput capacity (Sharma et al., 2022). Presently, different platforms are being used for microbial diagnostic microarrays. Microarrays not only allow characterization of microbes by information provided for specific identification of isolates, but also enhance the understanding of microbial pathogenesis based on the presence of virulence genes and an indication of the evolutionary trend of new pathogenic strains (Parolin et al**.** 2017). There are different types of microarrays, such as DNA microarrays, cellular microarrays, protein microarrays, antibody microarrays, etc (Hormsombut et al., 2022). They can be differentiated according to characteristics such as the nature of the probe, the solid surface support used, and the specific method used for probe identification and/or target detection (Sarengaowa et al., 2020).

6.1. Oligonucleotide DNA microarray

A DNA microarray is a nucleic-acid sequence based microarray technique composed of a collection of microscopic dots in which DNA is arranged and attached to a solid surface or membrane (Sarengaowa et al., 2020). Information on the differential expression of genes between food samples can be ascertained by DNA microarray. This technique uses small DNA probes capable of hybridizing complementary DNA (cDNA) of an mRNA, extracted from samples. The cDNA from food samples are tagged with fluorescent tags to facilitate the study of the differential expression of genes. Microarray can estimate the copy number of genes which in turn would aid in the study of the relative gene expression (Nehra et al., 2022).

The traditional gene chip arrays comprise of some probes that mark out the coding sequence of the virulence gene of pathogens of interest. Arraying many specific probes with molecular diagnostic markers and setting a significantly high threshold for the positive identification of pathogens that may be present, would considerably circumvent the false-positive results due to cross-contamination between foodborne pathogens. However, targeting only one area of the genome is no longer considered reliable for the identification of foodborne pathogens (Parolin et al**.** 2017). Rapid developments in the field of DNA arrays have led to a number of methods for their preparations (Sharma et al., 2022).

The strategy of tiling arrays on the gene chip can target the adjacent genome regions of the foodborne pathogen of interest, and detect the base sequences of the target gene (Parolin et al**.** 2017). However no such chip is commercially available in microarray detection technology therefore it is necessary to design and synthesize the tiling arrays for specific purposes. Microarrays have been criticized to be expensive for routine use and it is also said to be a method which fails to identify relevant information that can be transferred directly into clinical application (Miller and Tang, 2009; Everett et al., 2010).

6.2. In situ-synthesized array

In situ-synthesized arrays are extremely high-density microarrays where oligonucleotide probes are synthesized directly on the surface of

the microarray. Because in situ-synthesized probes are typically short (20–25 bp), multiple probes per target are included to improve sensitivity, specificity, and statistical accuracy (Jaksik et al., 2015; Lopez-Campos et al., 2012). For the rapid detection and identification of 10 food and waterborne bacterial pathogens including *Escherichia coli*, *Shigella* spp. *Vibrio cholera, Salmonella* spp., *Brucella* sp., and *Legionella pneumophila*, specific long oligo-microarray probes were designed (Dasari and Alex, 2014). The DNA microarray chip was able to identify all 10 bacterial agents tested simultaneously; however a professional bioinformatician would be needed for the design of the appropriate multifunctional microarray probes in order to increase the accuracy of the outcomes; developed an in situ-synthesized gene chip with 141 specific probes for the detection of *L. monocytogenes, S. aureus, E. coli* O157:H7, *Salmonella typhimurium*, and *V. parahemolyticus* on fresh-cut fruits and vegetables. They recorded a detection limit of approximately 3 log cfu/g without culturing and with a detection time of 24 h for the five target pathogens. They concluded that their detection technology can rapidly detect and monitor the foodborne pathogens on fresh-cut fruits and vegetables throughout the logistical distribution chain, from processing to sale (Parolin et al**.** 2017; Dasari and Alex, 2014). Despite some interesting features, most microarrays have integrated detection systems that require to be further developed and improvements in sensitivity and stability (Hormsombut et al., 2022). Furthermore, microarray analysis of food samples requires the application of specific conditions, and a major point of consideration is that the target microbes have to be detected in a background microflora with varying composition and abundance, depending on the type of food sample. Apart from been expensive for laboratory routine use, microarrays have also been criticized for their relatively low accuracy, precision and specificity. Also, the lack of control over the pool of analyzed transcripts is a concern since most of the commonly used microarray platforms utilize only one set of probes designed by the manufacturer (Everett et al., 2010; Ranjbar et al., 2017).

7.Next generation sequencing

Next-generation sequencing (NGS) provides new ways of detecting microorganisms beyond the microbial culture-based methods (Adenaike et al. 2023). It can allow detection in scenarios where traditional methods have generated negative or inconclusive results (Parker et al., 2023). This method with powerful bioinformatic approaches are revolutionizing Food Microbiology and serves as a powerful tool for rapidly and cost-effectively identifying and characterizing microbial species present in mixed food samples. The technology is developing at a rapid pace, with continuous improvement in quality and cost reduction and is having a major influence on Food Microbiology (Kostić and Sessitsch, 2012; News Story, 2021). Application of NGS in food safety issues does not only predict the existence of microorganisms in food samples but also to elucidate the molecular basis of their response to both intrinsic and extrinsic factors associated with the food.

This offers tremendous opportunities to predict and control the growth and survival of desirable as well as undesirable microorganisms in food (Jagadeesan et al. 2019). It also enables both culturable and non-culturable taxa to be characterized (Solieri et al., 2013).

One of the most impressive advantages of NGS over several other diagnostic assays is that it requires little or no prior knowledge of the pathogen. Therefore for pathogen discovery and detection, NGS is very valuable and reliable. NGS in Food Microbiology is applied in two major approaches: whole genome sequencing (WGS) and metagenomics. Whole-genome sequencing (WGS) is a comprehensive method for analyzing entire genomes. It identifies the entire gene content and when coupled to transcriptomics or proteomics, allows the identification of functional capacity and biochemical activity of microbial populations.

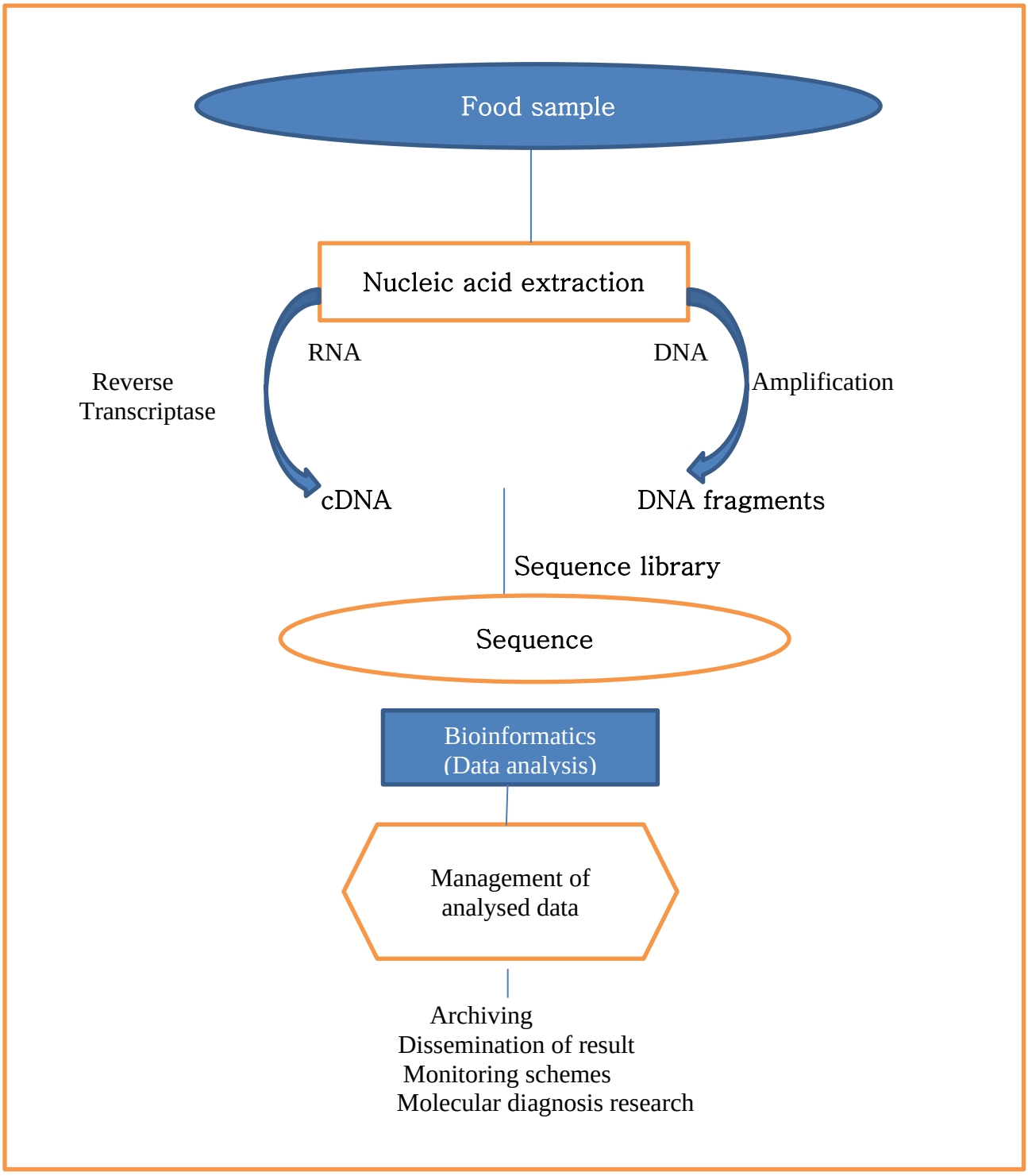

Figure 4. Stages of Next Generation Sequencing in Food sample analysis (Zhou et al., 2022)

Whole genome sequencing (WGS) of isolates consists of quality control, read trimming and assembly, bacterial characterization, strain typing, antimicrobial resistance characterization, variant calling, phylogenetic analysis and visualization tasks. WGS results can be obtained within few days rather than weeks, also provides accurate type and sub-type identification (Truchado and Randazzo, 2022; Frey and Bishop-Lilly, 2015; Jagadeesan et al. 2019; Qiagen Digital Insights, 2021). Metagenomics ensures the genetic analysis of the genomes contained in a food sample. It provides access to the functional gene composition of microbial communities and thus gives a much broader description (Zhou et al., 2022).

Next generation sequencing provides a more direct approach that does not rely on PCR and hence avoiding many of the PCR-associated potential biases (Thomas et al., 2012).

8.Conclusion

Foodborne illnesses pose important challenge to public health and cause significant economic problems in many countries of the world (Kozińska et al., 2019). Pathogens exist along the food chain and impact the quality and safety of foods in several negative ways. Identifying and understanding the behaviour of these microbes enable the implementation of preventative or corrective measures in public health and food industry settings; thus, emphasis on methods of pathogen detection as a means of prevention and control of food-borne diseases cannot be overemphasized (Adenaike et al., 2022). Classical culture-based methods can be applied to a broad range of pathogens but have long turnaround times and are not sufficient to detect and prevent all outbreaks of food-borne illnesses (Priyanka et al., 2016). Advances in technology like next-generation sequencing (NGS) have led to an explosion in the discovery and characterization of microbes, because NGS methods do not rely on traditional culture techniques and can thus detect the unculturable microbes. In foods, it is of great importance to distinguish between viable, active and inactive cells. (Yap et al., 2022; Wensel et al., 2022). Therefore, Next-generation sequencing-based methods have rapidly evolved and more or less to replace existing detection methods and platforms (Mayo et al., 2014). The continuous research for rapid, sensitive and low cost detection of pathogens in food is recommended (Ji et al., 2022).

9.References

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ACKNOWLEDGEMENTS

The Authors acknowledged their institutions.

FUNDING

No funding was provided for this work by any organization.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Author OA-Conceptualization. Formal analysis and writing original draft. ENF-Writing original draft, proofread final draft. GOA-Writing original draft. OAO-Proofread first draft, made extensive contributions for improvement.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

CHEMICAL CHARACTERIZATION OF BISCUITS (COOKIES) AS FUNCTIONAL FOOD PRODUCT SUPPLEMENTED WITH QUINOA FLOUR

Mohamed El-Hadary [1], Samy Abu-Elmaaty [1], Magdy Abdel-Hady [2], Ibrahim Eid Elesawi [3] and Amr El-Sayed [1]✉

[1]*Food Science Department, Faculty of Agriculture, Zagazig University, 44511, Zagazig, Egypt.*
[2]*Food Technology Research Institute, Agricultural Research Centre, Egypt.*
[3]*Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig, 44511, Egypt*
✉*nutrition66@gmail.com*

https://doi.org/10.34302/crpjfst/2024.16.3.15

Article history:
Received:
May 23th, 2024
Accepted:
September 17th, 2024

Keywords:
Quinoa seed;
Wheat flour;
Biscuits;
Rheological characteristics;
Functional food.

ABSTRACT

In recent years, quinoa has gained renewed relevance as an alternative crop to cereals due to its excellent nutritional value. The aim of this work was to utilize quinoa seed flour as a substitute supplementation for wheat biscuits. The physicochemical properties of quinoa seed flour were studied. Wheat flour was substituted with 25, 50 and 75% of quinoa seed flour. The obtained results declared that the rheological characteristics of the mixtures dough were altered by increasing the ratio of quinoa seed flour. Substitution with 25 and 50% quinoa seed flour had the best results which were relatively close to that of the control sample. The physicochemical properties of biscuits enriched by quinoa seed flour that characterized by increasing density, texture, water-holding capacity and oil-holding capacity. The chemical composition of enriched biscuits, for moisture, ash, dietary fiber, protein and minerals content were increased while fat and available carbohydrate content lowered. Sensory evaluation showed that substitution with 25% and 50% quinoa seed flour had the best sensory characteristics, and increased its content of protein, fat, minerals and vitamins.

1.Introduction

Concerns about food are developing, mostly about supplying the people with safe and sustainable food, as well as important nutritional characteristics that contribute to relieving hunger and malnutrition problems. Malnutrition, primarily protein energy, is expected to impact 10% of the global population (Torres-Tiji et al., 2020). As a result, the use of functional foods presents itself as an intriguing option in population nutrition and helps to the reduction of the risk of certain diseases (Nystrand & Olsen, 2020). In this context, and in response to the growing consumer demand for health-related products, technologies in vegetable matrices such as quinoa have been widely used to enhance nutritional effects as well as the extraction of compounds that can be incorporated into the formulation of novel foods (Foucault et al., 2014; Obaroakpo et al., 2020; Vera et al., 2020).

Quinoa *(Chenopodium quinoa Willd.)* belongs to Chenopodiaceae family, which also includes spinach and beet is an endemic to South America. However, it was domesticated by people living in the Andes, it is highly resistant to weather, climate, and soil conditions. Quinoa has traditionally been used by a variety of native populations in South America Citation (Jancurová et al., 2009)**.** It draws attention with its high nutritional value, but more importantly, the seeds are the most economical and scientifically significant parts. It has a balance of proteins rich in sulphur amino acids and

lysine as well as lipids, and has been consumed by people as a holy plant due to its rich protein content and incredible balance of essential amino acids (Repo-Carrasco-Valencia et al., 2010; Vega-Gálvez et al., 2011)**.**

Quinoa has gained popularity as a functional food due to its high levels of proteins, lipids, fibers, vitamins, minerals, amino acids, and phytochemicals such as saponins, phytosterols, phytoecdysteroids, phenolic compounds, and bioactive peptides (Chen et al., 2020; Pereira et al., 2019). The FAO proclaimed 2013 the "International Year of Quinoa" because of its nutritious content and it has been described as one of the cultures meant to offer food security in the twenty-first century (Nowak et al., 2016). According to the FoodEx 2 food categorization and description system, the European Food Safety Authority (EFSA) has also put quinoa in the list of functional foods/products with health claims (A000R).

Similar to rice, its seeds are used to make soups, morning cereal by puffing them, or baked goods including cookies, bread, pasta, crisps, tortillas, and pancakes by flouring them (Bhargava et al., 2006)**.** In addition, quinoa seeds can be fermented to make beer or a kind of traditional alcoholic drink used for a religious ceremony called chicha in South America (Miranda et al., 2011). It is additionally utilized as a nutrient-rich source for feeding farm animals like cattle, pigs, and chickens (Bhargava et al., 2006)**.** This study's objectives were to assess the physio-chemical characteristics of quinoa seeds, use them to fortify biscuits at three different levels (at 25%, 50%, and 75%), and assess the impact of the fortification on the characteristics of the finished products.

2.1. Materials

- Quinoa seeds (grown in Egypt in the 2022 season) obtained from agriculture research center from season 2021–2022crop and kept at 5°C until used in technological studies
- Wheat flour 72% extraction, margarine, vanilla, sugar, skim milk powder, ammonium bicarbonate and baking powder were obtained from local market, Zagazig, Sharkia Governorate, Egypt.
- All chemicals used in this study were analytical grade and purchased from El-Gomhoria Co., Zagazig, Sharkia Governorate, Egypt.

2.2. Technological process and preparation of biscuit samples

2.2.1. Preparation of the Quinoa flour

Quinoa flour was prepared according to Al Shehry (Al Shehry, 2016) with some modifications. To remove saponins, the seeds were washed repeatedly with tap water until there was no longer any froth in the washing water. The seeds were then dried in an air ventilation oven (SHEL LAB 1370 FX, Germany) at 50°C until dried. Quinoa seeds were ground to fine powder in an electric grinder (Quadrumat Junior flour mill, Model Type No: 279002, ©Brabender ® OHG, Duisburg 1979, Germany) and sifted through a 60 mesh, stored at 4 ±1°C until used.

2.2.2. Preparation of the treated biscuits samples

The recipes listed in Table (1) were followed in the preparation of the biscuit treatments. 25%, 50%, and 75% of quinoa flour were used to replace some of the 72% extraction rate of wheat flour. Ammonium bicarbonate and water were added and blended for 2 minutes after sugar, fat, and vanilla were creamed in a mixing bowl for 15 minutes. The mixture of skim milk powder, baking powder, and wheat flour was then supplemented with quinoa flour in varying quantities. The mixture was then thoroughly combined to create uniform dough. The latter was taken out of the mixing bowl and used by a cutting machine to be laminated, sheeted, and formed. Baking took place for 10 minutes at 250°C. After baking biscuits were cooled at room temperature and then warped tightly by aluminum foil and kept for sensory evaluation as reported by Mesías et al. (Mesías et al., 2019).

2.3. Assessments methods for biscuit samples

2.3.1. Proximate chemical composition

According to the procedures outlined by AOAC (A. O.A.C, 2005), the chemical composition of various biscuit samples, including their moisture, ash, crude protein, fiber, mineral, and crude lipid contents (%), was determined at the Central Laboratory of the Faculty of Agriculture at Zagazig University in Zagazig, Sharkia Governorate, Egypt.

2.3.2. Determination of vitamin

B1 (thiamine), B2 (riboflavin), B3 (niacin), B6 (pyridoxine) and total Folate determined according to the method according to Li (Li, 2018) .

2.3.3. Saponin analysis:

Quantification of saponins by spectrophotometric analysis by the method described by (Lozano, et al. (Lozano et al., 2012). Quantification was performed with a standard Saponin curve (50–350 lg/mL) and the results were expressed as % dry sample.

2.3.4. Physical properties of biscuit

- Hardness Determination

The hardness (N) of different biscuit samples was determined at Food Technology Research Institute, Giza, Egypt, according to Mesías et al. (Mesías et al., 2016) using a Texture Analyzer (Texture Technologies Corporation, USA) outfitted with a 50 kg load cell, a probe (Warner Bratzcer, HDP/BSK knife model) with a compression speed of 1 mm. s-1, and a distance prolongation of 10 mm to measure the hardness (N) of various biscuit samples.

Table 1. The recipe used for biscuits making

Ingredient	Amount (g)			
		Treatments		
	Control	25%	50%	75%
Wheat flour 72% Quinoa flour	100 0	75 25	50 50	25 75
Margarine	24	24	24	24
Sugar	30	30	30	30
Vanilla	0.1	0.1	0.1	0.1
Ammonium bicarbonate	1.5	1.5	1.5	1.5
Skim milk powder	1.0	1.0	1.0	1.0
Baking powder	0.8	0.8	0.8	0.8
Water	12.0	12.0	12.0	12.0

- **Density measurement**

The density of different biscuit samples was determined using Archimedes (buoyancy) method according to Amiri *et al.* (Amiri et al., 2017). Was determined according to the following: Density = mass/volume (g/cm^{-3})

- **Color measurement**

The color of biscuit crust was measured according to Zenoozian *et al.* (Zenoozian et al., 2007) using a Minolta colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) based on three color coordinates; L* (Lightness), a* (redness/ greenness), b* (yellowness/blueness). The measurement for each sample was replicated and the average value was recorded for each color parameter.

- **Water Holding Capacity**

According to Chau and Huang (Chau & Huang, 2003), the water holding capacity (WHC) of one gram of wheat flour (72%), which was substituted with quinoa seed flour, was tested after standing the mixture for one hour at room temperature. The hydrated sample underwent a 10-minute centrifugation at 1500 rpm. The extra supernatant was properly decanted after centrifugation. WHC was calculated as ml of retained water divided by 1g of the sample's dry weight (ml/g).

- **Oil Holding Capacity**

Oil Holding Capacity (OHC) was measured according to Garau *et al.* (Garau et al., 2007), with slight modification. 20 ml of maize oil and one gram of wheat flour (72% quinoa seed flour) were combined, and the mixture was left to stand for one hour at room temperature. After that, the sample was centrifuged for ten minutes at 1500 rpm. Decanting the extra oil was done with caution. WHC was calculated as ml of retained oil divided by 1 g of dry sample weight (ml. g).

2.3.5. Rheological Behavior

Farinograph and extensograph of dough rheological properties were measured according to AACC (AACC, 2000).

- **Dough properties by Farinograph**

Water absorption (%), dough development (min), stability time (min) and dough weakening were determined by Brabender Farinograph (model 810114, Brabender, Duisburg, Germany) at Food Technology Research Institute Giza, Egypt.

- **Dough properties by extensograph**

Dough extensibility E (mm), resistance to extension R (BU), Energy (cm^2) and proportional number R/E were determined by Brabender Extensograph (model 860702, Brabender, Duisburg, Germany), Food Technology Research Institute Giza, Egypt.

2.3.6. Sensory Evaluation

The sensory evaluation was done in accordance with Hooda and Jood's (Hooda & Jood, 2005) instructions. To assess the sensory qualities of the biscuits in terms of their look, color, flavor, and general acceptability, ten members of the food science department staff at the Faculty of Agriculture, Zagazig University, were given coded samples of the biscuits. The rating was based on a hedonic scale from 1 to 9, where (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 =like very much, 9 = like extremely).

2.3.7. Statistical Analysis

The obtained data were statistically analyzed by a statistical for social science package "SPSS" version 20 for Microsoft windows, SPSS Inc. according to Dominick and Derrick (Dominick & Derrick, 2001).

3.Results and discussions

3.1. Proximate Chemical composition of quinoa and wheat flour:

The proximate chemical composition of quinoa and wheat flour under are shown in Table (2). The obtained results declared that, wheat flour had higher values of moisture content and Carbohydrate, while the highest values of ash, protein, fat and fiber contents were recorded by quinoa flour (3.78, 15.45, 5.87 and 3.88%) respectively in compared to wheat flour which recorded the lowest value of ash, protein, fat and fiber content (0.59, 11.53, 2.15 and 0.74%) respectively. The obtained results nearly agreed with those of Sharoba *et al.* (Sharoba et al., 2009) and Atef et al. (Atef et al., 2014).

Concerning the mineral content of Calcium, iron and zinc , the obtained results referred to the highest value of calcium, iron and zinc was found in quinoa flour (165.71, 9.30 and 4.17 mg/100g) respectively in compared to 34.72, 4.27 and 1.58 mg/100g of calcium, iron and zinc, respectively in wheat flour. On the other hand, wheat flour had a higher content of phosphor (329.32mg/100g) compared to quinoa flour (220.73 mg/100g), The obtained results were in general agree with those of Beniwal *et al.* (Beniwal et al., 2019) and Konishi *et al.* (Konishi et al., 2004).

Table 2. Proximate Chemical composition of raw materials:

Parameter	Quinoa	Wheat
Ash (%)	3.78±0.21[a]	0.59±0.04[b]
Moisture (%)	9.67±0.43[b]	12.27±0.29[a]
Protein (%)	15.45±0.43[a]	11.53±0.48[b]
Fat (%)	5.87±0.04[a]	2.15±0.18[b]
Fiber (%)	3.88±0.32[a]	0.74±0.05[b]
Carbohydrate (%)	61.34±0.93[b]	72.72±0.03[a]
Iron mg/100g	9.30±0.57[a]	4.27±0.33[b]
Calcium mg/100g	165.71±2.89[a]	34.72±0.79[b]
Zinc mg/100g	4.17±0.31[a]	1.58±0.21[b]
Phosphor mg/100g	220.73±0.51[b]	329.32±0.55[a]

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$).

3.2. Effect of soaking on quinoa of saponin content:

Generally, saponin caused the bitter test in cooking quinoa, The results of saponin indicate that the raw quinoa seeds had saponin of 3.46% figure 1, these results agreed with **Nickel *et al.*** (Nickel et al., 2016) who found that the saponin content spectrophotometric analysis was 3.33% in natural quinoa grains, soaking quinoa seed for after the substitution; they increased gradually by increasing the level of quinoa addition. WHC increased by 6.3%, 17.4% and 30.7% while OHC increased by 4.1%, 7.5% and 12.2% with 25, 50 and 75% in wheat flour substituted by quinoa flour, respectively compared with wheat flour 72% (control) sample may be due to the high content of fiber in quinoa (3.88%) compared whit those of wheat flour (0.74%). Oil and water holding capacity is the flour's ability to retain oil and water under a centrifugal gravity force, in addition, this property affects the flavor and mouth feels of the product, these results are in near agreement with those reported by **Pellegrini *et al.*** (Pellegrini et al., 2018)**.** 48h revealed an 85.26% decrease in saponin content, leading to enhanced sensory properties.

3.3.Water and oil holding capacity:

Table (3) shows the water and oil holding capacity (WHC and OHC) of wheat flour (72 %) and blends of wheat-quinoa flour. The obtained results showed that both WHC and OHC values increased as a general tendency

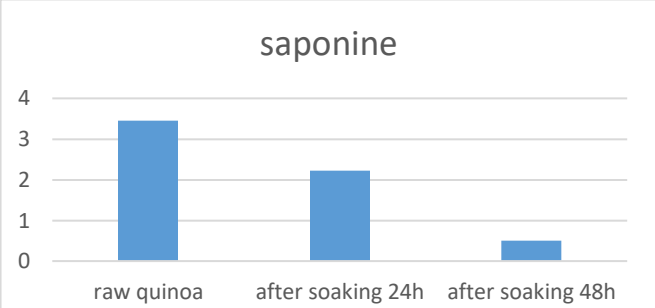


Figure 1. Effect of soaking on quinoa of saponin contint.

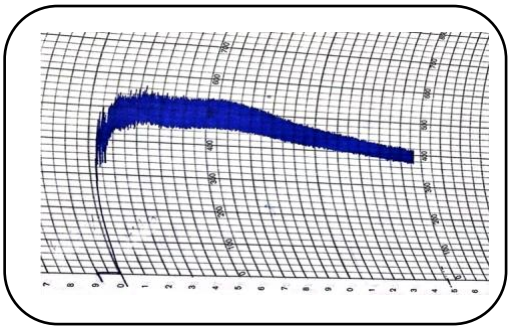
Table 3. Water and oil holding capacity of substituted wheat flour by different levels of Quinoa seed flour:

Property/sample	wheat flour 72%	Quinoa seed flour		
		25%	50%	75%
WHC (mg/100g)	180.9±1.9[d]	192.2±.95[c]	218.1±2.1[b]	260.5±2.1[a]
OHC (mg/100g)	172.2±1.86[d]	179.4±1.18[c]	186.7±1.5[b]	193.8±0.8[a]
WHC**:** Water holding capacity OHC: oil holding capacity				

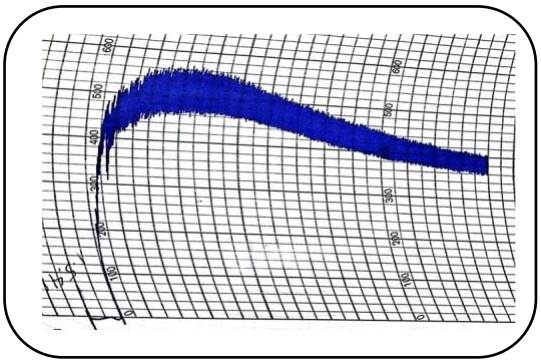
3.4.Farinograph results

Farinograph measurements are summarized in Figure 2. Results showed that the water absorption of dough increased gradually from 57.4% of control to 63.1%, 64.1% and 66.2% by adding 25, 50 and 75% of quinoa flour, respectively.

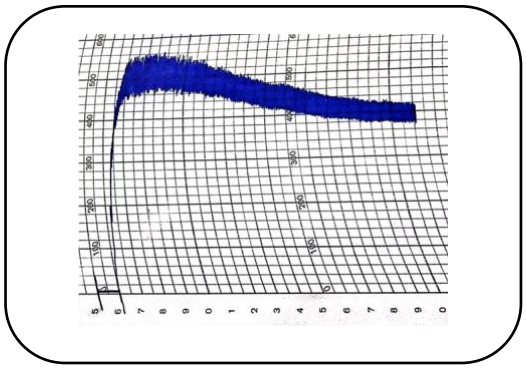
The obtained results showed that increasing the addition of quinoa flour by more than 25% led to a weakling effect on dough characteristics. Farinograph indices like arrival time, development time and dough stability were lowered in 50 and 75% quinoa flour mixtures, so the degree of weakening effect (a degree of softening, the vertical space in BU between the end of Farinograph after 12 min. of its peak and the 500 BU line) increased from 100 to reach 260 BU, at 75% quinoa seed flour These results are in agreement with **Moawad *et al.*** (Moawad et al., 2018)**.**



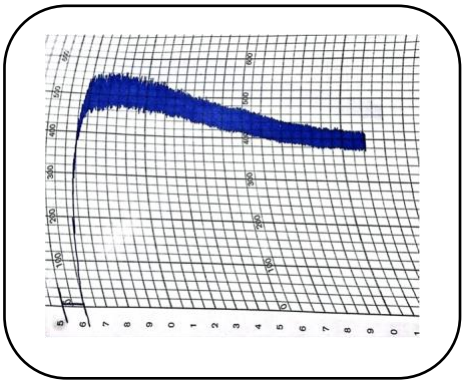
100%Wheat flour (72%)



75%Wheat flour+25% quinoa flour



50%Wheat flour+50% quinoa flour



25%Wheat flour+75% quinoa flour

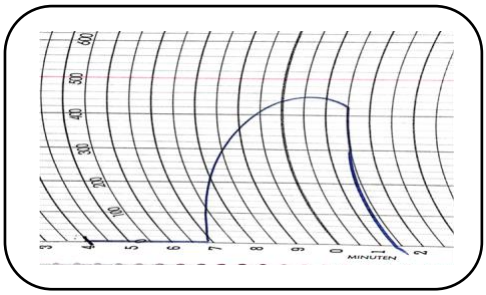
Figure 2. Effect of wheat flour substituting by different levels of Quinoa seed flour on Farinograph characteristics.

3.5.Extensograph results

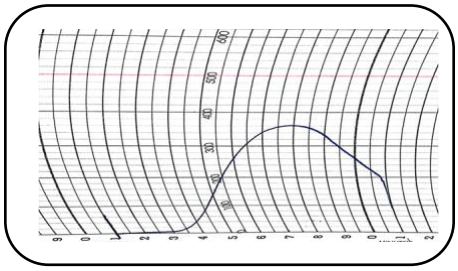
Results of Extensograph parameters of wheat flour blended with 25, 50 and 75% quinoa seed flour were given in Figure 3. Results showed that elasticity and resistance to extension (R), of dough containing up to 25%, 50% and 75% quinoa flour decreased from 420 BU for control to 390, 360 and 240 BU, respectively. Increasing the addition of quinoa flour lowered the elasticity to about half of control. On the other hand, the addition of quinoa flour caused a gradual decrement in dough extensibility (E) with increasing the adding level of quinoa.

The proportional number (R/E) is an overall index that indicates the effects of the treatments on the

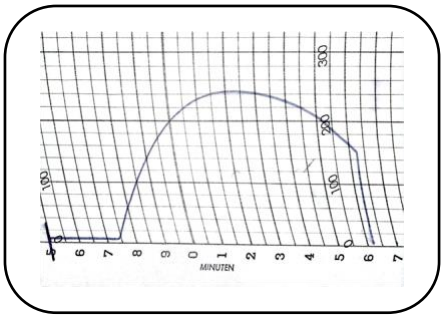
quality of dough. The results in Table 5 show that quinoa flour affects the quality of the dough. The addition of quinoa flour up to 25% in dough had an enhancer effect on dough quality while the higher concentrations of quinoa flour showed defects in quality. Energy values of the dough decreased by adding either quinoa flour than the control sample.
The rheological results showed that the addition of quinoa flour up to 25 and 50% have potentialities to be used in supplementing flour for manufacturing biscuits. On the other hand, increasing the supplementation will have deleterious effects on dough quality. However, it is well known that weak flours are suitable for manufacturing biscuits. These results are in agreement with **Moawad *et al.*** (Moawad et al., 2018)**.**



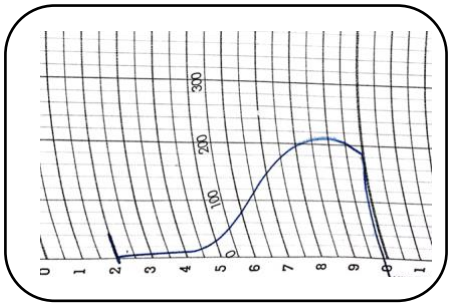
100%Wheat flour (72%)



75%Wheat flour+25% quinoa flour



50%Wheat flour+50% quinoa flour



75% quinoa flour +25% Wheat flour

Figure 3. Effect of wheat flour substitution by quinoa flour with different levels on extensograph dough characteristics**.**

3.6. Quality attributes of biscuit treatments

3.6.1. Physical characteristics

Physical characteristics of biscuits substituted with quinoa flour including density, hardness (texture) and color are shown in Table (4). Density was slightly increased by increasing the amount of quinoa compared to control sample. It was increased from 0.45(g/cm3) of control biscuits compared to 0.59, 0.63 and 0.68 (g/cm3) for biscuits samples substituted with 25, 50 and 75% of quinoa flour biscuits respectively. Concerning to hardness, it was increased gradually by increasing quinoa flour in treated biscuits. The hardness rose from 16.87N of untreated biscuits to 18.28, 20.18 and 22.23N for biscuits samples with 25, 50 and 75% of quinoa flour, respectively. The highest density and textures values of treated biscuits may be attributed to dietary fiber. The results are in accordance with those obtained by Bilgiçli and Ibanoglu (Bilgiçli & İbanoğlu, 2015) and

Nisar et al. (Nisar et al., 2017), they reported that the hardness of bread and cookies increased as increasing quinoa proportion.

Treated biscuits color evaluated by Hunter parameters L*, a*, and b* and the obtained results are shown in Table (4). The results declared that biscuits enriched by quinoa had lower values of lightness (L*) and yellowness (b*). Lightness (L*) was slightly decreased by increasing the amount of quinoa compared to the control sample. It decreased from 28.55 of control biscuits to 28.36, 27.24 and 26.34 of enriched biscuits by 25, 50 and 75% quinoa, respectively. Yellowness (b*) was slightly decreased by increasing the amount of quinoa compared to the control sample. It decreased from 66.32 for control biscuits compared to 65.43, 64.62 and 63.23 for biscuits samples substituted with 25, 50 and 75% quinoa flour biscuits respectively. a* values (redness), it was increased gradually by increasing of the level of quinoa flour in biscuits samples. Concerning to a* values (redness), it raised from 3.47 of untreated biscuits to 3.82, 4.83 and 5.25 for enriched biscuits by 25, 50 and 75% of quinoa r respectively. These results in agreement with those of Atef et al. (Atef et al., 2014) who reported that increasing the percentage of adding quinoa meal to wheat flours, led to a decrease in the values of lightness (L*) and yellowness (b*) slightly in all fortified samples. Also, Thejasri et al. (Thejasri et al., 2017) reported that the addition of quinoa flour to wheat flour led to a decrease in the lightness of gluten-free biscuit samples and they approved that to the higher value of protein in quinoa flour compared to wheat flour.

Table 4. Physical characteristics of biscuits substituted with quinoa flour

Physical property	1	2	3	4
Density (g. cm^{-3})	0.45±0.01^{c}	0.59±0.01^{b}	0.63±0.01^{b}	0.68±0.01^{a}
Hardness (N)	16.87±0.69^{d}	18.28±0.42^{c}	20.18±0.75^{b}	22.23±0.87^{a}
L*	28.55±0.14^{a}	28.36±0.05^{a}	27.24±0.09^{b}	26.34±0.34^{b}
a*	3.47±0.04^{c}	3.82±0.34^{b,c}	4.83±0.11^{b}	5.25±0.11^{a}
b*	66.32±0.04^{a}	65.43±0.24^{b}	64.62±0.62^{b,c}	63.23±0.18^{c}

1=100% wheat flour, 2=75% wheat flour+25% quinoa flour, 3=50% wheat flour+50% quinoa flour and 4=25% wheat flour+75% quinoa flour.

3.6.2. Chemical composition of supplemented biscuits

The influence of adding quinoa flour on the chemical constituents of biscuits showed in (Table 5). The obtained results declared that biscuits with quinoa flour treatments were higher in moisture, protein, fiber, ash, fat and minerals (Ca, Fe and Zn) contents but lower in Available carbohydrate content than the untreated biscuits. Moisture content increment could be explained by the high fiber content found in quinoa flour compared to wheat flour 72% allowing more absorption and retention of water. The addition of quinoa flour increased the contents of protein by 8-20%, fat by 2- 9%, fiber increased 2 to 4 times, iron by 4-20%, zinc by 28-43%, calcium by 14-21%, phosphor by 8-20%, while the decrease in energy is not significant. On the other hand, carbohydrate content was reduced by 6-15%. Such effects are due to the inherent content of quinoa flour used which contributes to the component's content of the resultant biscuits. The chemical composition of the control biscuit was in agreement with those reported by Youssef (Youssef, 2015) he reported that the wheat biscuit contained 3.75% moisture, 9.11% protein, 1.00% fiber, and 77.19% total carbohydrates. Also, Brito et al. (Brito et al., 2015) reported that quinoa flour let to increase ash and Fiber content and decreases the total carbohydrate content compared to the biscuit produced from wheat flour as a control.

Table 5. Chemical composition of supplemented biscuits

Parameter	1	2	3	4
Ash (%)	0.53±0.03^{d}	0.73±0.02^{c}	0.85±0.04^{b}	1.04±0.06^{a}
Moisture (%)	4.6±0.27^{c}	5.9±0.07^{b}	6.17±0.05^{b}	7.18±0.09^{a}
Protein (%)	8.6±0.38^{c}	10.03±0.16^{b,c}	10.97±0.1^{b}	12.09±0.16^{a}
Fat (%)	17.61±0.09^{c}	17.92±0.11^{c}	18.28±0.09^{b}	19.39±0.34^{a}
Fiber (%)	0.87±0.05^{c}	2.08±0.1^{b}	2.83±0.17^{b}	3.39±0.29^{a}
Carbohydrate (%)	67.7±0.55^{a}	63.29±0.4^{b}	60.88±0.34^{c}	56.9±0.56^{d}
Iron mg/100g	7.81±0.34^{b}	8.16±0.06^{b}	8.75±0.05^{a,b}	9.18±0.03^{a}
Calcium mg/100g	126.36±1.8^{d}	148.38±1.5^{c}	159.69±0.39^{b}	161.56±0.43^{a}
Zinc mg/100g	6.14±0.13^{c}	7.02±0.34^{b}	7.41±0.07^{a}	7.79±0.17^{a}
Phosphor mg/100g	292.98±1.9^{a}	267.24±0.54^{b}	245.12±1.1^{c}	236.00±0.93^{d}
Energy (K.cal)	464.2±1.5^{a}	456.3±0.5^{b}	453.82±1.1^{c}	452.43±0.41^{c}

1=100% wheat flour, 2=75% wheat flour+25% quinoa flour, 3=50% wheat flour+50% quinoa flour and 4=25% wheat flour+75% quinoa flour.

Table 6. Sensory evaluation of supplemented biscuits

Sample	**Appearance (9)**	**Texture (9)**	**Color (9)**	**Taste (9)**	**odor (9)**	**Over all acceptability**
1	8.33±1.04^{a}	8.33±1.11^{a,b}	8.46±1.06^{a}	8.33±1.34^{b}	8.46±1.36^{a}	7.86±1.35^{b}
2	8.68±0.6^{a}	8.68±0.6^{a}	8.62±0.61^{a}	8.75±0.57^{a}	8.81±0.54^{a}	8.5±0.81^{a}
3	8.62±0.6^{a}	8.56±0.62^{a}	8.31±0.94^{a,b}	8.5±0.63^{a}	8.62±0.5^{a}	8.56±0.51^{a}
4	7.75±0.68^{b}	8.01±0.63^{b}	7.93±0.92^{b}	7.87±0.71^{c}	8.12±0.8^{a}	8.00±0.73^{b}

1=100% wheat flour, 2=75% wheat flour+25% quinoa flour, 3=50% wheat flour+50% quinoa flour, and 4=25% wheat flour+75% quinoa flour.

3.6.3. Sensory evaluation of biscuits:

The influences of quinoa flour substitution on sensory characteristics (appearance, color, taste, flavor and overall acceptability) of control and treated biscuits are illustrated in Table (6). It can be observed that all evaluated sensory attributes were positively affected by adding quinoa flour. The obtained results indicated that, no significant differences in color, taste, odor, texture and appearance between the control sample and the biscuit samples containing 25-50% quinoa flour. While that the sample contained on 75% quinoa flour to the lowest value from color, taste, odor, texture and appearance. Also, no significant differences in color between the samples containing 25-50% quinoa flour (8.62 and 8.56 respectively) and the control sample biscuit (8.46). While the sample contained 75% quinoa flour to the lowest value (7.93). Concerning odor and texture, the obtained results indicated that no significant difference between the control sample and sample (2) contained 25% quinoa flour plus 75% wheat flour and sample (3) contained 50% quinoa flour plus 75% wheat flour in the same characteristics.

Moreover, no significant difference between the biscuit sample (2) produced from 25% quinoa flour plus 75% wheat flour and the biscuit sample (3) contained 50% quinoa flour

plus 50% wheat flour in appearance character, whereas the biscuit sample (3) produced from 50% quinoa flour plus 50% wheat flour recorded the highest value of overall acceptability (8.56) followed by the biscuit sample (2) contained 25% quinoa flour plus 75%wheat flour (8.5) and according to the results of Thejasri et al. (Thejasri et al., 2017), they studied the effect of quinoa flour on the quality of biscuits with different substitution levels to wheat flour and they reported that the produced biscuit recorded a high score concerning the taste and overall acceptability and the biscuit was almost same to the control sample.

3.7. Some nutrient facts the biscuits enriched by 50% quinoa seed flour:

Some of the nutrient facts of 100-gram biscuits enriched by 50% quinoa were shown in Table (7). Compared to the daily recommendation for adults (19+ years) as reported in recommendations from the Committee on Medical Aspects of Food Policy (COMA) and the Scientific Advisory Committee on Nutrition (SACN), 100 grams of enriched biscuits covered about 18.28% of carbohydrates, 18.84% fat, 19.44% of proteins and 17.8% energy. With regard to minerals and vitamins, it covered the requirements of iron and vitamins B1 and Folate. While it covered about 22.81% of calcium, 42.6% of phosphorus and 44.56% of zinc, 62% Thiamin, 30.76% Riboflavin, 50% Pyridoxine, and only 14.19% of Niacin.

Table 7. Some nutrient facts of biscuits content at 50% quinoa flour+50% wheat flour.

Parameters	3	Recomidaion19-64	% recovery
Protein (%)	10.79	55.5	19.44
Fat (%)	18.28	97	18.84
Carbohydrate (%)	60.88	333	18.28
Energy (K.cal)	445.08	2500	17.8
Iron mg/100g	8.75	8.7	100.1
Calcium mg/100g	159.69	700	22.81
Zinc mg/100g	7.41	9.5	44.5
Phosphor mg/100g	245.12	550	42.6
B1 mg/100g	0.9	0.8	110
B2 mg/100g	0.4	1.3	30.76
B3 mg/100g	2.2	15.5	14.19
B6 mg/100g	0.7	1.4	50
Folate mg/100g	0.3	0.2	150

3=50% wheat flour+50% quinoa flour, B_1.Thiamin, B_2. Riboflavin, B_6 Pyridoxine, and B_3.Niacin.

4. Conclusions

Biscuits are a favorite meal among many people all over the world. Many nations throughout the world, notably Egypt, utilize it for school feeding meals, military, and during times of crises. Quinoa has grown in popularity as a functional food due to its high protein, fat, fiber, vitamin, mineral, amino acid, and phytochemical content. As a result of its high level of protein, fat, minerals, dietary fiber, and healthful components, quinoa flour has various useful components that make it excellent for enhancing cereal products. The study's findings obviously demonstrated that adding quinoa seed flour to biscuits in amounts between 25 and 50 percent can increase their nutritional content without significantly changing their physical characteristics or other organoleptic characteristics. Quinoa is touted as a functional and nutritive alternative to wheat that may be

incorporated into daily meal plans to support leading a healthy lifestyle.

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Declarations:

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and materials

The current manuscript includes all of the data produced and examined for the study, and the associated authors have no objections regarding the data's and materials' accessibility.

Competing interests (Conflict of interest)

The authors assert that they have no competing interests.

Authors' contributions

M. R. E.: analyzed and interpreted the data of the work; performed lab experiments, analyzed and interpreted the data of the work, and prepared the original manuscript; **S. M. A:** supervised and reviewed the manuscript; **I. E. E.:** performed lab experiments, analyzed and interpreted the data of the work, and prepared the original manuscript; **A. A. E.:** supervised and reviewed the manuscript; performed lab experiments, analyzed and interpreted the data of the work, and prepared the original manuscript.

Funding

The authors received no external financial support for this study's practical part, authorship, and publication.

Acknowledgment

The authors extend their sincere gratitude to Professor Dr. Valentina Pavlova, Professor of Chemistry and Biochemistry at the Faculty of Technology and Technical Sciences, University "St. Kliment Ohridski" Bitola (UKLO), Republic of North Macedonia, Veles, and to Dr. Vesna Karapetkovska Hristova, Associate Professor of Biotechnology at the Faculty of Biotechnical Sciences (FBS), University "St. Kliment Ohridski" Bitola, Macedonia, for their valuable assistance in reviewing the manuscript. Special appreciation is also extended to the reviewers and the academic editorial office for their meticulous evaluation and insightful feedback, which significantly enhanced the quality of the manuscript.

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

PRODUCTION OF DEEP-FRIED WHEAT CHIPS USING PURPLE WHEAT FLOUR: PHYSICOCHEMICAL, TEXTURAL, SENSORY PROPERTIES AND OPTIMIZATION

Şemsettin Kaya[1], Ferhat Yuksel[2]✉

[1]*Gumushane University, Faculty of Engineering and Natural Science, Food Engineering Department, 29100 Gumushane, Turkey*

[2]*Nigde Omer Halisdemir University, Bor Faculty of Health Science, Nutrition and Dietetics Department, 51700 Nigde, Turkey*

✉*fyuksel@ohu.edu.tr (F.Yuksel)*

https://doi.org/10.34302/crpjfst/2024.16.3.16

Article history:
Received:
May 3rd, 2024
Accepted:
September 19th, 2024

Keywords:
Deep-Fried Wheat Chips; Purple Wheat Flour; Textural and Sensory Analyses and Optimization.

ABSTRACT

The aim of this study was to develop alternative and healthy deep-fried chips by fortifying wheat chips with purple wheat flour (PWF). The effect of process variables (PWF content: 0-100%, frying temp. and time: 170-190 °C and 40-60 s) on the physicochemical, textural, instrumental color, sensorial properties, and optimization of the chips were investigated using response surface methodology. The levels of PWF in the chips receipt dramatically reduced oil uptake (by 23%), whereas the protein content of chips increased of the end products. The instrumental hardness of the enriched chips increased while sensory firmness decreased with increasing PWF. Generally, the chips enriched with PWF received the highest overall acceptability score by the panelists. Higher desirability (optimization results) and the process variables were determined as a 0.73 and 92.72 g/100g PWF, 170 °C, 40 s, respectively. These findings show that PWF can be easily incorporated into products that involve deep-fried chips.

1.Introduction

Currently, snack foods are becoming an important component of many diets. Many age groups, particularly children, consume with them for enjoyment. (Kayacier et al. 2014). There are many various kinds of snack foods, such as chips, cookies, crackers, and granola-type bars. Snack foods comprise of many different types including chips, cookies, and crackers. While they are primarily consumed in between meals, there has been a significant increase in their consumption during main meals. Snack foods are generally known as unhealthy due to their high energy density (Hartmann et al. 2012; Durmaz and Yuksel, 2021). According to statistics, the global chips market is expected to expand at a compound annual growth rate (CAGR) of 3.92% between 2020 and 2028, reaching US$ 43.8 billion by that time (Potato Chips Market, 2024). Excessive energy consumption is related to obesity, which is acknowledged as a risk factor for numerous illnesses, including cancer, diabetes, and heart disease (Romieu et al. 2017). Consumers demand healthy foods. Therefore, the snack industry has studied on the development of healthy snack foods to meet consumer's demand. Recently, most research has been conducted on the addition of functional food components to snack foods, particularly chips. For example, Rababah et al. (2012) studied the fortification of corn chips with broad bean flour, chickpea flour, or isolated soy protein. Yüksel et al. (2015) studied the fortification of wheat chips with barley flour.

Rogalski & Szterk (2015) researched enrichment corn chips with linseed oil. Germinated soybeans were used to create a healthy snack chip by Maetens et al. 2017.

Purple wheat is a hybrid of ancient Abyssinian wheat (*Triticum aethiopicum*) and spelt (*Triticum dicoccum*). Purple wheat has recently become very popular due to its important nutritional properties. In general, flour and bakery products are the top consumption areas (Gamel et al. 2019a). Some of the nutritional and physical properties of purple wheat flour are as follows; 3.0% fat, 13.77% protein, 8.4% dietary fiber, 73.10% hectoliter, 21% gluten, 46 glycemic index, 339 TEAC/100 g total antioxidants, 169.61-177.47 mg GAE/100 g total phenolics (Yu & Beta 2015). One of the most effective qualities of purple wheat is the purple color pigments and anthocyanins in the grain structure. The total anthocyanin content of purple wheat can vary from 96 μg/g (Abdel-Aal et al. 2006) to 230 μg/g (Liu et al. 2010). Purple wheat has already taken its place in the food industry in terms of its content, nutritional efficiency, and bioactive components; and it is thought to be used in the production of products with richer content (Ficco et al. 2016).

In the literature, there is no available study on the fortification of chips with purple wheat flour. The aim of this study was to enrich wheat chips with purple wheat flour to present a new healthy snack for the snack industry. Response surface methodology was applied to evaluate the effect of process variables (purple wheat flour content, frying time, and frying temperature) on the physicochemical properties, textural, instrumental color, and sensorial properties of the enriched wheat chips.

2. Materials and methods

2.1. Materials

Wheat flour, purple wheat flour, frying oil (corn oil), and salt were obtained from a local market (Gumushane, Turkey). Wheat flour comprised 12.9 g $100g^{-1}$ moisture, 11.1 g $100g^{-1}$ of protein, 2.5 g $100g^{-1}$ of dietary fibre, 2.9 g $100g^{-1}$ of fat, and 0.55 g $100g^{-1}$ of ash. Purple wheat flour comprised 13.0 g $100g^{-1}$ moisture, 60.8 g $100g^{-1}$ carbohydrate, 15.0 g $100g^{-1}$ of protein, 8.5 g $100g^{-1}$ of dietary fibre, 2.0 g $100g^{-1}$ of fat, and 0.2 g $100g^{-1}$ of salt.

2.2. Preparation of the chips

Table 1. The coded and uncoded values of design

	Coded Values				**Uncoded Values**	
Samples	A: Frying temperature (°C)	B:Frying time (s)	C: PWF (g/100g)	A: Frying temperature (°C)	B:Frying time (s)	C: PWF (g/100g)
1	0.00	0.00	0.00	180.00	50.00	50.00
2	1.00	0.00	1.00	190.00	50.00	100.00
3	0.00	0.00	0.00	180.00	50.00	50.00
4	0.00	-1.00	-1.00	180.00	40.00	0.00
5	-1.00	0.00	-1.00	170.00	50.00	0.00
6	-1.00	0.00	1.00	170.00	50.00	100.00
7	0.00	0.00	0.00	180.00	50.00	50.00
8	1.00	-1.00	0.00	190.00	40.00	50.00
9	0.00	1.00	1.00	180.00	60.00	100.00
10	-1.00	1.00	0.00	170.00	60.00	50.00
11	-1.00	-1.00	0.00	170.00	40.00	50.00
12	1.00	0.00	-1.00	190.00	50.00	0.00
13	0.00	1.00	-1.00	180.00	60.00	0.00
14	0.00	-1.00	1.00	180.00	40.00	100.00
15	1.00	1.00	0.00	190.00	60.00	50.00

*WF/PWF= 100/100 (w/w). The salt was added as a 1 g/100g for every design point.

Table 1 shows the experimental design. In accordance with the experimental design, mixtures containing WF and PWF in various ratios were prepared, and homogenized for 5 min. After the addition of water (50 mL), the dough was kneaded using a dough mixer for 10 min (Kitchen Aid Professional 600, MI, USA). The resultant dough was rested for 30 min in a plastic wrap. The thickness of the dough was adjusted to 1 mm (Rondo, Doge SS0635, Switzerland), and the chips shape was obtained using a special mold. The prepared chips were fried in an oil bath with a temperature (temp.) controller (Mikrotest, Turkey). The prepared chips are shown in Fig 1. After deep-frying, the chips were cooled on a paper towel.

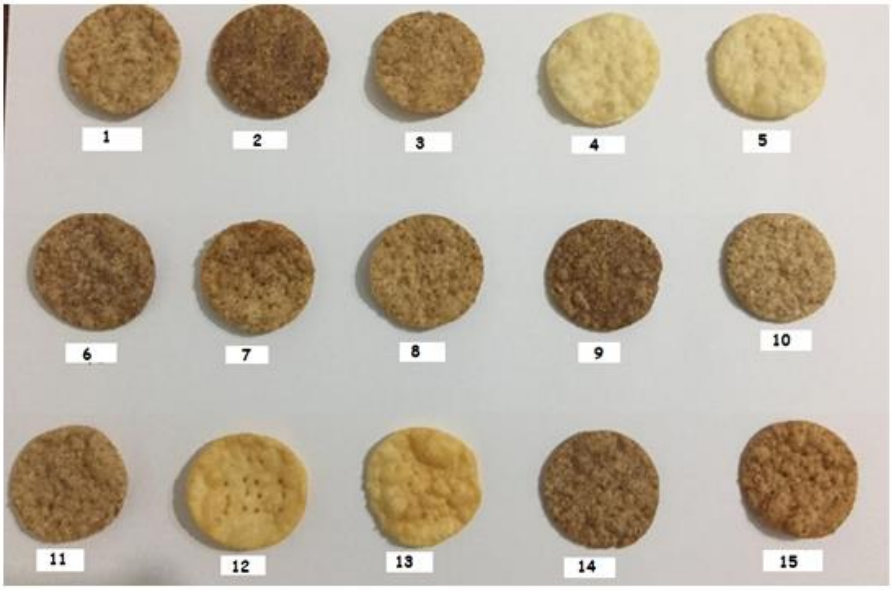


Figure 1. The produced chips according to the design.

2.3. Physicochemical properties

The dry matter contents of the chips sample were determined by the oven drying method using an oven (Nuve FN 120 Turkey). With the use of a furnace (Protherm PLF115M, Turkey) and the dry burning method, the ash content of the chips was determined. To determine the fat content of the sample, a Soxhlet extractor was used (Buchi B-811, Switzerland). The water activity (a_w) was measured using a a_w measurer (Decagon, USA). The protein content of the chips samples was designated by the Kjeldahl method (AOAC 2000).

2.4. Textural properties

The texture analyzer equipped with a Kramer shear cell attachment (HDP/KS 5) (TA.XT Plus, England) was used to determine the hardness of the samples. 3 g chips were inserted into the cell, and the blade was set to 5 cm/min. The highest force necessary to break the chips sample was established from the deformation curve. Ten replications were carried out.

2.5. Color properties

A Lovibond colorimeter (Lovibond, England). was used to determine the instrumental color properties (L*, a*, and b*).

2.6. Sensory properties

Twenty semi-trained panelists used a 9-point scale to evaluate the chips samples (1: undesired and 9: desired). These panelists were selected from students and lecturers of the Food Engineering Department. The color, crispness, taste/odor and overall acceptability of the samples were evaluated. The samples were randomly coded with 3-digit numbers before being served to the panelists. Drink water was

given to the panelists to clean their lips between sampling.

2.7. Statistical analysis and modeling

For modeling the processing variables (PWF, frying temp. and time) in the current investigation, a 3-factor-3 level Box Behnken experimental design (Box & Behnken, 1960) with three replicates at the center point was selected, and predictive regression models were built (Table 1). The second-order polynomial equation of function Xi as stated below, was fitted for each response analyzed:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_{ii}^{\ 2} + \sum_{\substack{i=1 \\ i<j}}^{3} \sum_{j=1}^{3} b_{ij} X_i X_j \quad (1)$$

where Y is the estimated response; b0, bi, bii, bij are constants. Xi, Xii and Xj are processing variables. All analyses were conducted using uncoded values, and as Table 1 illustrates, the maximum number of tests that could be run was 15. As shown in Table 1, the experimental combination was applied in triplicate at the model's center point on the first, third, and seventh runs. Design Expert Statistical Package Software (Version 7.0.0.a Stat Ease Inc. Hennepin, MN, USA) was used for the computational work, including 3D surface plots, in order to conduct the response surface analysis. The Design Expert was used to determine the optimization of the original design based on sensory evaluations.

3.Results and discussions

Response surface methodology was used to examine the impact of the process factors (PWF concentration, frying time, and frying temperature) on the physicochemical, instrumental color, and sensory qualities of the PWF-enriched wheat chips. In addition, the optimization of results was applied. To investigate the main and interaction effects of the variables on the analyzed parameters, a three-factor and three-level Box-Behnken design (Table 1) was used.

3.1 Physicochemical and color properties

The content and variance analysis (F values) of dry matter, ash, a_w, oil, protein, hardness of wheat chips enriched with PWF are tabulated in the Tables 2 and 3, respectively. The maximum and minimum dry matter contents of the chips samples were 99.91 and 96.94 g/100g, respectively. The highest dry matter content was determined as a 99.91 g/100g with a PWF level of 100 g/100g at 180 °C frying temperature for 60 s. Only the frying temperature showed a significant effect on the dry matter content. The dry matter content was significantly and positively impacted by the linear term of the frying temperature ($p<0.05$). This regression model effectively predicts the dry matter content of the chips sample dependent on the PWF, frying temperature, and frying duration due to its maximum coefficient of determination (R2 = 0.87, Table 4). Moisture reduction in deep-fried products is generally expected. This is because the moisture in the samples evaporates away from the product with high heat during frying (Kayacier et al. 2014). Similar findings were reported by Durmaz & Yuksel (2021) and Ayustaningwarno et al. (2020), who observed an increase in the dry matter content of chips.

The higher ash content of the wheat chips enriched with PWF could be explained by the higher dietary fiber content of the PWF. The PWF (8.5 g $100g^{-1}$) had approximately 4 times dietary fiber content than the WF (2.5 g $100g^{-1}$) and so the ash content of the sample was affected by the dietary fiber content of flours. Only PWF showed significant effect on the ash content. The PWF linear term has a significant suitable impact on the ash content. ($p<0.05$, Table 3.). For the evaluation of ash content, the coefficient of determination was determined to be suitable (R2 = 0.66, Table 4). Similar findings were reported by Gamel et al. (2019a), who observed an increase in the ash content of the bars and crackers fortified with the PWF. It can also be said that the salt content used in the production of chips increases the ash content of the samples.

Table 2. Physicochemical, textural, and instrumental color contents of the samples.

Samples	Dry matter (g/100g)	Ash (g/100g)	Water activity (a_w)	Oil (g/100g)	Protein (g/100g)	Hardness (kg)	L*	a*	b*
1	99.26±0.03	1.62±0.09	0.08±0.00	31.94±2.17	8.14±0.08	18.45±2.49	49.73±2.12	7.36±0.46	23.72±1.38
2	99.20±0.25	1.67±0.03	0.07±0.00	35.86±2.01	9.39±0.61	22.48±3.80	44.73±0.85	9.37±0.37	23.38±0.67
3	99.74±0.31	1.00±0.00	0.07±0.02	33.30±3.77	8.06±0.40	19.41±2.63	49.84±2.48	7.75±0.55	24.83±0.72
4	97.08±0.12	1.05±0.07	0.20±0.01	39.05±3.06	7.34±0.08	17.20±2.59	67.95±1.72	1.27±0.36	24.90±1.10
5	96.94±0.95	1.36±0.31	0.17±0.02	37.62±2.33	7.66±0.80	18.39±1.81	66.21±1.67	0.97±0.32	23.69±0.93
6	97.85±0.07	1.86±0.01	0.14±0.00	34.88±2.46	8.46±0.80	26.14±3.67	43.30±1.87	7.57±0.53	19.69±1.08
7	99.29±0.04	1.05±0.01	0.06±0.01	30.58±0.79	7.18±0.16	24.85±2.88	46.40±1.36	7.96±0.17	23.69±0.59
8	99.81±0.01	1.55±0.23	0.07±0.00	36.65±0.08	8.14±0.24	24.57±3.07	48.54±1.32	8.81±0.54	25.81±1.23
9	99.91±0.09	1.67±0.12	0.06±0.00	35.71±1.70	7.34±0.16	38.96±4.51	37.19±2.88	8.63±0.45	21.28±1.69
10	98.99±0.04	1.21±0.21	0.07±0.02	37.56±1.41	5.29±0.40	20.15±2.61	53.17±1.62	5.99±0.49	22.45±1.41
11	98.32±0.15	1.02±0.00	0.13±0.00	29.48±1.74	8.46±0.40	39.61±3.56	51.12±1.68	6.68±0.85	21.66±1.30
12	99.44±0.10	1.09±0.05	0.07±0.00	37.15±0.16	8.06±0.20	20.00±2.69	56.50±2.67	6.41±0.95	29.26±1.98
13	99.35±0.01	1.10±0.09	0.07±0.00	40.73±3.68	5.64±0.24	26.63±2.45	58.67±1.60	6.01±0.56	33.42±1.56
14	98.93±0.07	2.08±0.11	0.09±0.00	32.55±2.68	9.26±0.40	26.27±1.78	46.86±1.04	7.64±0.65	20.19±1.42
15	99.81±0.08	1.67±0.10	0.06±0.00	36.93±2.96	8.62±0.16	32.39±3.41	45.03±1.10	9.47±0.21	27.19±0.68

Table 3. Variance analysis (F values) results of the effect of process variables on changes in the physicochemical, textural, and instrumental color properties of samples

Source	Dry matter (g/100g)	Ash (g/100g)	Water activity (a_w)	Oil (g/100g)	Protein (g/100g)	Hardness (kg)	L*	a*	b*
A	14.36*	0.30	9.67*	1.85	9.03*	0.081	4.15	21.87**	14.86*
B	5.81	3.313E-003	9.07*	6.47	19.09**	0.38	4.80	4.29	6.24
C	3.61	7.50*	4.15	8.97*	15.85*	3.44	68.63**	45.60**	32.18**
AB	0.35	0.010	0.72	4.52	12.76*	5.12	0.71	0.48	0.032
AC	1.00	0.014	0.33	0.16	0.27	0.19	2.86	3.49	0.32
BC	1.25	0.43	3.37	0.16	0.043	0.074	3.454E-003	3.71	4.97
A^2	1.22	0.20	0.68	1.82	2.45	0.19	0.058	0.057	0.19
B^2	0.19	0.12	8.554E-003	4.06	4.80	4.82	0.053	0.020	0.44
C^2	6.16	1.17	5.70	10.86*	0.47	0.029	4.44	11.73*	0.12
Model	3.76	1.07	3.72	4.12	7.27*	1.59	9.51*	10.16**	6.60*
Lack of fit	0.13	0.54	0.06	0.31	0.57	0.19	0.20	0.06	0.09

A: Frying temperature, B: Frying time, C: PWF. *p<0.05, **p<0.01

The water activity of the wheat chips ranged from 0.06 a_w to 0.20 a_w (Table 2). The highest a_w content was determined as a 0.20 a_w with a PWF level of 0.0 g/100g at 180 °C frying temperature for 40 s while the lowest a_w content was determined as a 0.06 a_w with a PWF level of 100.0 g/100g at 180 °C frying temperature for 60 s. The frying temperature and time had a significant effect on the a_w content. The linear term of the frying temperature and time had a significant positive effect on the a_w content ($p<0.05$, Table 3.). This regression model effectively predicts the a_w content of the wheat chips sample dependent on the PWF, frying temperature, and time due to its high coefficient of determination (R2 = 0.87, Table 4). The most important effect on the results of a_w in deep-fried products is said to be moisture content contained in the end product (Yuksel et al. 2020). In this context, considering that the moisture content of the samples is between 3.09 g/100g and 0.09 g/100g, it is seen that the main reason why the a_w results of the samples are found to be quite low.

The PWF showed a significant effect on both oil contents. The highest oil content was determined as a 40.73 g/100g with a PWF level of 0.0 g/100g at 180 °C frying temperature for 60 s while the lowest oil content was determined as a 29.48 g/100g with a PWF level of 50.0 g/100g at 170 °C frying temperature for 40 s. The linear and quadratic term of the PWF content had a significant effect on the oil content of the samples ($p<0.05$). This regression model effectively predicts the oil content of the wheat chips sample based on the PWF, frying temperature, and time due to its high coefficient of determination (R2 = 0.88, Table 4). In deep-frying, mass and heat transmission occur simultaneously. As water from the food evaporates as water vapor, heat is transported from the frying oil to the food's surface, and some of the evaporated water is replaced by the oil as the food absorbs it (Kaplan, et al. 2021). In this study, the oil level in the final chips sample decreased considerably (~ 23%). The major reason for the decreased oil level with the increase of PWF in the recipe of samples might be that PWF behaves as a coating material, which occasioned the formation of lesser pores, and thus less oils were taken up during the frying process. Similar findings were reported by Durmaz & Yuksel (2021) and Mayo-Mayo et al. (2020), who observed a decrease in the oil content of chips and tortillas fortified with potato peel powder, roselle, and mango peel powder.

The maximum protein content was determined as a 9.39 g/100g with a PWF level of 100.0 g/100g at 190 °C frying temperature for 50 s, whereas the minimum protein content was observed to be 5.29 g/100g with a PWF level of 50.0 g/100g at 170 °C frying temperature for 60 s. The linear term of the PWF, frying temperature, and frying time content had a significant effect on the protein content of the samples ($p<0.01$, $p<0.05$ Table 3). In addition, the interaction term of the frying temperature and time content had a significant effect on the protein content of the samples ($p<0.05$ Table 3). This regression model effectively predicts the protein content of the samples based on the PWF, frying temperature, and time due to its high coefficient of determination (R2 = 0.92, Table 4). In this study, the amount of protein in the final chips sample increased considerably. The main reason for the increased protein amount with the increasing of PWF in the recipe of samples is that PWF (15.0 g/100g) has a higher protein content than WF (11.1 g/100g). In the literature, the protein contents of PWF were found to be; 10.3 g/100g protein (Gamel et al. 2019a), 10.3 g/100g protein (Saini et al. 2020), and 11.5 g/100g protein (Abdel-Aal et al. 2018). Gamel et al. (2019b) reported that the protein content of functional foods enriched with purple wheat flour was in the range of 7.3–18.8 g/100 g and that the protein content of bar, crackers, breads, pancakes and, porridge samples was affected by the purple wheat flour content.

The hardness of the wheat chips enriched with PWF ranged from 17.20 kg to 39.61 kg (Table 2). The process factors had no obvious impact on the hardness content ($p>0.05$). The highest hardness value was obtained with a flour

level of 50 g/100g at 170 °C for 50 s, whereas the lowest hardness content was observed with 0.0 g/100g with a PWF level of 50.0 g/100g at 180 °C frying temperature for 40 s. The response model's R2 score was 0.74, indicating that it explained 74% of the variability in hardness. Texture is one of the most important attributes that determine, the consumer acceptance of deep-fried foods such as chips. The hardness properties of samples are key parameters that describe the texture of fried foods. In the literature, the instrumental hardness contents of corn chips, wheat chips and wheat chips samples were found as follows; 7.8-17.9 kg (Rababah et al. 2012), 13.48-27.27 kg (Yuksel et al. 2015) and 7.54-35.37 kg (Cevik et al. 2022), respectively. These results in the literature are similar to the instrumental hardness results in our study.

Table 4. Results of model equality and determination coefficients.

Analyze	***Model***	***R^2***
Dry matter	$Y=-43.90+1.38A+0.25B+0.17C-0.002AB-0.0006AC-0.0006BC-0.003A^2+0.001B^2-0.0003C^2$	*0.87*
Ash	$Y=25.43-0.27A-0.02B+0.003C-0.0002AB+0.00004AC-0.002BC+0.0008A^2+0.0006B^2-0.00008C^2$	*0.66*
a_w	$Y=6.04-0.05A-0.03B+0.007C-0.0001AB+0.00001AC-0.00005BC+0.0001A^2+0.00001B^2-0.00001C^2$	*0.87*
Oil	$Y=311.44-3.61A+1.71B-0.33C-0.02AB+0.0007AC+0.0007BC+0.01A^2+0.02B^2+1.26C^2$	*0.88*
Protein	$Y=205.68-1.91A-1.13B-0.03C+0.009AB+0.0003AC-0.0001BC+0.004A^2-0.006B^2+0.00007C^2$	*0.92*
Hardness	$Y=1235.03-8.31A-19.12B+0.5C+0.1AB-0.002AC+0.001BC+0.014A^2+0.1B^2-0.0002C^2$	*0.74*
*L**	$Y=185.63-1.30A+1.86B-1.33C-0.01AB+0.005AC-0.0002BC+0.004A^2-0.0004B^2+0.001C^2$	*0.94*
*a**	$Y=17.81-0.35A-0.37B+0.53C+0.003AB-0.001AC-0.001BC+0.001A^2-0.001B^2-0.001C^2$	*0.95*
*b**	$Y=-131.39+1.54A-0.51B+0.27C+0.001AB-0.001AC-0.003BC-0.003A^2-0.006B^2-0.0001C^2$	*0.92*
Color	$Y=52.30-0.25A-0.73B-0.12C+0.005AB+0.001AC-0.0002BC-0.0002A^2-0.002B^2+0.00008C^2$	*0.64*
Hardness	$Y=166.45-1.34A-1.40B-0.12C+0.008AB+0.0007AC-0.00006BC+0.002A^2-0.001B^2+0.00007C^2$	*0.77*
Taste/Odor	$Y=112.01-0.89A-0.78B-0.1C+0.005AB+0.0006AC-0.0002BC+0.00015A^2-0.002B^2+0.00004C^2$	*0.86*
Oiliness	$Y=-34.90+0.73A-0.77B-0.17C+0.005AB+0.0009AC-0.00056BC-0.0028A^2-0.0014B^2-0.00012C^2$	*0.56*
Overall acceptability	$Y=30.26-0.0078A-0.74B-0.11C+0.006AB+0.00067AC-0.000017BC-0.00096A^2-0.0034B^2-0.000095C^2$	*0.73*

* A: Frying temperature, B: Frying time, C: PWF.

The mean values of the instrumental color properties of wheat chips samples are shown in Table 2. The PWF content and frying temperature showed significant effect on the color properties. The PWF content had a significant effect on the L*, a* and b* values, whereas the frying temperature had a significant effect on a* and b* value ($p<0.01$, $p<0.05$, Table 3). The maximum L* content was determined as a 67.95 with a PWF level of 0.0 g/100g at 180 °C frying temperature for 40 s, whereas the minimum L* content was observed to be 37.19 with a PWF level of 100.0 g/100g at 180 °C frying temperature for 60 s. The linear term of the PWF content negatively impacted the L* value ($p<0.05$). The response model's R2 value was 0.94, indicating that it explained 94% of the variability in L*. The a* value varied from 0.97 to 9.47 (Table 2). The linear term of the PWF and frying temperature positively affected the a* value ($p<0.01$). Also, the quadratic term of the PWF positively affected the a* value ($p<0.05$). The highest a* content was determined as a 9.47 g/100g with a PWF level of 50.0 g/100g at 190 °C frying temperature for 60 s while the lowest a* content was observed to be 0.97 with a PWF level of 0.0 g/100g at 170 °C frying temperature for 50 s. This regression model effectively predicts the a* content of the samples based on the PWF, frying temperature, and time due to its high coefficient of determination (R2 = 0.95, Table 4). The highest b* content was determined as a 33.42 with a PWF level of 0.0 g/100g at 180 °C frying temperature for 60 s while the lowest b* content was observed to be 19.69 with a PWF level of 100.0 g/100g at 170 °C frying temperature for 50 s. The linear term of the PWF and frying temperature negatively impacted the b* value ($p<0.01$, $p<0.05$). This regression model effectively predicts the b* content of the samples based on the PWF, frying temperature, and time due to its high coefficient of determination (R2 = 0.92, Table 4).

PWF negatively impacted the L* and b* values of the wheat chips. The purple color of the PWF could reduce the L* and b* values of the wheat chips. The frying temperature positively impacted the redness and yellowness of the wheat chips enriched with PWF. Caramelization and Maillard reactions could be connected to these findings. This non-enzymatic browning reaction (Caramelization and Maillard) leads to the formation of brown-colored complexes in foods, including carbohydrates. In addition, higher temperatures accelerate the caramelization reaction (Kaplan et al. 2021). Similar findings were reported for potato chips (Lee and Pangloli et al. 2014) and corn tortilla (Rojas-Molina et al. 2020).

3.2. Sensorial Properties

The color, firmness, and taste/odor, oiliness and overall acceptability of the wheat chips enriched with PWF were evaluated by the panelists. The process variables had no apparent impact on the color or oiliness. The linear term of the frying temperature and time negatively affected the taste/odor content ($p<0.05$, Table 5). Also, the interaction term of frying temperature and time significantly affected the firmness, taste/odor, and overall acceptability ($p<0.05$, Table 5). Otherwise, the PWF content showed no significant effect on the sensory properties ($p>0.05$, Table 5). The color score ranged from 5.60 to 7.40 and the maximum color value was determined as a 7.40 with a PWF level of 50 g/100g at 180 °C for 50 s, whereas the minimum color score was observed to be 5.60 with a PWF level of 50 g/100g at 170 °C for 60 s (Fig.2). The firmness score decreased with increasing process variables and, the hardness content of wheat chips samples varied from 5.20 to 7.67 (Fig.2). The highest firmness score of the sample was obtained with PWF 50 g/100g at 170 °C for 40 s. The taste/odor score ranged from 4.67 to 7.00 and the maximum taste/odor score was determined as a 7.00 with a PWF level of 50 g/100g at 170 °C for 40 s while the minimum taste/odor score was observed to be 4.67 with a PWF level of 50 g/100g at 180 °C for 50 s (Fig.2). The oiliness score varied from 4.47 to 6.87 and the lowest oiliness score was determined 4.47 with a PWF level of 50 g/100g at 170 °C for 60 s. The overall acceptability score varied from 5.07 to 7.20. The highest score

(7.20) was obtained using a PWF level of 50 g/100g at 170 °C for 40 s. The response models' R2 values ranged from 0.56 to 0.86, indicating that the models explained 56-86% of the variability in the sensory qualities.

Generally, the most common color of the chips product is golden yellow (Ozcan et al. 2021). In chips prepared with PWF, this color cannot be observed due to the color pigments from the PWF. However, this situation did not cause any negative situation in the panelists, and the colors of the chip samples prepared with PWF were liked. Similar findings were reported by Kumari et al. (2020) and Pasqualone et al. (2015), who observed an increase in the color score of chapatti and biscuits fortified with PWF. Crispness is a desirable sensory parameter in chips products (Durmaz & Yuksel 2021). The sensory hardness values showed that the increasing amount of PWF in the formulation did not negatively affect the friability of the final product. For this reason, it has been observed that chips products produced with PWF can be easily consumed by consumers. No spices other than salt were used in this study. The taste and odor of the chips products originated from PWF and WF. The frying conditions also affected the taste and odor. It was understood that the use of PWF in the production of chips did not cause a negative situation for the panelists and that it could easily be used in the production of chips. Similar findings have been reported for breads (Janeckova et al. 2014) and chapatti (Kumari et al. 2020).

Table 5. Variance analysis (F values) results of the effect of process variables on the changes in the sensory properties of the chips samples

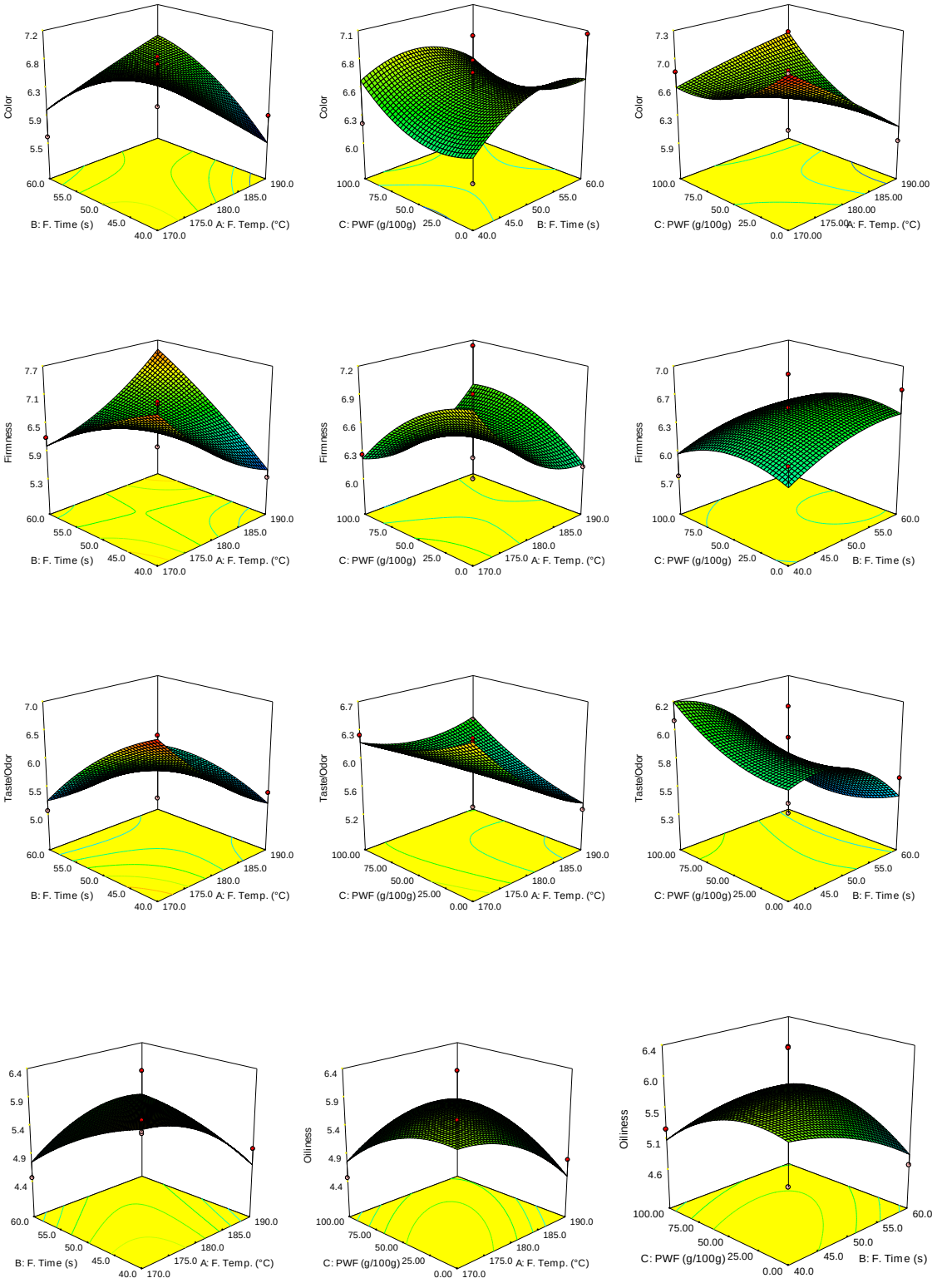
Source	Color	Firmness	Taste/Odor	Oiliness	Overall acceptability
A	1.10	0.45	7.69*	0.43	1.29
B	0.12	0.66	6.61*	0.31	0.017
C	0.089	0.28	0.38	0.012	7.746E-004
AB	4.26	11.95*	9.84*	2.02	6.92*
AC	1.83	2.03	3.11	1.62	2.14
BC	0.17	0.018	0.48	0.68	1.549E-003
A^2	4.798E-003	0.90	0.78	0.66	0.16
B^2	0.61	0.17	1.21	0.16	2.03
C^2	0.59	0.46	0.35	0.67	1.00
Model	0.99	1.89	3.41	0.71	1.47
Lack of fit	2.06	1.27	0.27	1.31	0.70

A: Frying temperature, B: Frying time, C: PWF. *p<0.05, **p<0.01

Table 6. Results of optimization.

Samples	Frying temperature (°C)	Frying time (s)	PWF (g/100g)	**Desirability**
1	170.00	40.00	92.72	**0.730**
2	170.00	40.00	91.30	0.730
3	170.12	40.00	92.20	0.726
4	170.04	40.00	99.94	0.726
5	170.20	40.00	91.23	0.724
6	170.00	40.00	67.54	0.704

* Values of 0.70 and above were taken for desirability.


Color
7.2
6.8
6.3
5.9
5.5
60.0
55.0
50.0
45.0
40.0
B: F. Time (s)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Color
7.1
6.8
6.6
6.3
6.0
100.0
75.0
50.0
25.0
0.0
C: PWF (g/100g)
40.0
45.0
50.0
55.0
60.0
B: F. Time (s)
Color
7.3
7.0
6.6
6.3
5.9
100.0
75.0
50.0
25.0
0.0
C: PWF (g/100g)
170.00
175.00
180.00
185.00
190.00
A: F. Temp. (°C)
Firmness
7.7
7.1
6.5
5.9
5.3
60.0
55.0
50.0
45.0
40.0
B: F. Time (s)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Firmness
7.2
6.9
6.6
6.3
6.0
100.0
75.0
50.0
25.0
0.0
C: PWF (g/100g)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Firmness
7.0
6.7
6.3
6.0
5.7
100.0
75.0
50.0
25.0
0.0
C: PWF (g/100g)
40.0
45.0
50.0
55.0
60.0
B: F. Time (s)
Taste/Odor
7.0
6.5
6.0
5.5
5.0
60.0
55.0
50.0
45.0
40.0
B: F. Time (s)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Taste/Odor
6.7
6.3
6.0
5.6
5.2
100.00
75.00
50.00
25.00
0.00
C: PWF (g/100g)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Taste/Odor
6.2
6.0
5.8
5.5
5.3
100.00
75.00
50.00
25.00
0.00
C: PWF (g/100g)
40.0
45.0
50.0
55.0
60.0
B: F. Time (s)
Oiliness
6.4
5.9
5.4
4.9
4.4
60.0
55.0
50.0
45.0
40.0
B: F. Time (s)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Oiliness
6.4
5.9
5.4
4.9
4.4
100.00
75.00
50.00
25.00
0.00
C: PWF (g/100g)
170.0
175.0
180.0
185.0
190.0
A: F. Temp. (°C)
Oiliness
6.4
6.0
5.5
5.1
4.6
100.00
75.00
50.00
25.00
0.00
C: PWF (g/100g)
40.0
45.0
50.0
55.0
60.0
B: F. Time (s)

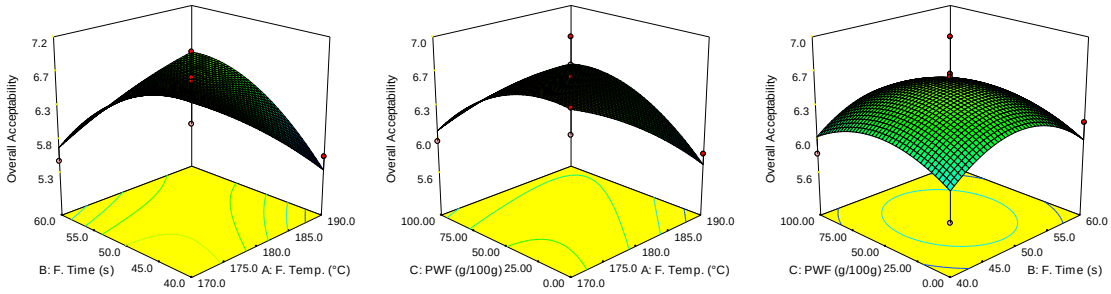


Figure 2. Three-dimensional representation of the sensory properties of chips samples

3.3. Optimization

The optimization results for wheat chips enriched with PWF are shown in Table 6. In addition, the change in the level of desirability according to the formulation variables for chips production conditions and the optimization data for the chips sample with the highest desirability are given in Table 6. The highest desirability value obtained because of the optimization made by considering the most liked points by the panelists was determined as 0.73. The flour formulation and production conditions to be used in the production of chips with the highest desirability are as follows: 92.72 g/100g PWF, 170 °C and 40 s. In addition, all other production conditions with desirability values of 0.70 and above are shown in Table 6. According to the optimization results, it was determined that the level of desirability increased as the content of PWF increased in the chips formulation, whereas the level of desirability decreased as the frying temperature and time increased.

4. Conclusions

PWF was added to the wheat chips to provide a nutritious snack. The addition of PWF into the chips sample formulation reduced the oil absorption of the resultant product. In addition, the protein content of chips samples increased with the addition of PWF. Higher and lower sensory scores were given for the enriched wheat chips by the panelists at the following process variables: 92.72 g/100g PWF, 170 °C, 40 s and 67.54 g/100g PWF, 170 °C, 40 s, respectively. Wheat chips enriched with PWF may find an opportunity to be commercialized as a healthy snack and alternative.

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Acknowledgment

The authors would like to thank the Food Engineering Department of Gumushane University for laboratory analyses of the samples. We would also like to thank the Food Engineering Department of Nigde Omer Halisdemir University for textural analysis of the samples.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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EVALUATING THE IMPACT OF NON-GLUTEN MODIFICATIONS ON BREAD QUALITY: A STRUCTURAL EQUATION MODELING APPROACH

Budianto[1]✉

[1]*Chemical Engineering, Al-Kamal Institute of Science and Technology, Jakarta, Indonesia*
✉*budianto_delta@yahoo.com*

https://doi.org/10.34302/crpjfst/2024.16.3.17

Article history:
Received:
September 3rd, 2024
Accepted:
October 1st , 2024

Keywords:
Acylation;
Glycosylation;
Innovation Process;
The Maillard Reaction;
Phosphorylation.

ABSTRACT

While modifications for low-gluten bread production have been extensively reported, including the utilization of Non-Gluten Components (NGC), Natural Hydrocolloids (NH), and Chemical Modification Reaction (CMR). Implementing this modification remains challenging and has yet to meet the expectations for bread quality (BQ). This research aims to propose a measurement model for assessing the effectiveness of modifications (NGC, NH, and CMR) on BQ. This research involved 45 bread companies in Indonesia, and the three modifications were attempted for application. Structural Equation Modeling (SEM), with the Partial Least Square (PLS) approach, was employed for analysis. The findings indicate that the modified (NGC, NH, and CMR) variables did not directly contribute to a positive effect on BQ (T stat. <1.65; p >0.05). However, when mediated by Research and Development (R&D) the three modifications showed a positive impact on BQ, with respective contributions of 19.2% (NGC), 14.6% (NH), and 12.8% (CMR). R&D had a fairly strong influence (f^2>0.35), and 28.2% (R^2) of its indicators were understood by NGC, NH, and CMR. The model's suitability was deemed satisfactory, with SRMR<0.07; GFI>0.36; and NFI>0.9. The original contributions of this research lie in providing practical recommendations for the widespread application of modified variables and proposing conceptual a framework for gluten-free bread modification with R&D mediation.

1.Introduction

Gluten is a complex compound characterized by high allelic polymorphism that gives Specific protein codes for glutenin and gliadin. The nature of gluten is stable in binding heat so that it helps in improving the texture, taste and roasting (Biesiekierski, 2016). Gluten is one of the most common causes of gastrointestinal symptoms and triggers a potentially autoimmune disease *Celiac disease, Glutenataxia, Dermatitis herpetiformis* (Biesiekierski, 2016). These conditions encourage research related to modifications of gluten substitutes that do not cause disease. A healthy lifestyle will encourage the manufacture of gluten-free bread with characteristics similar to bread in general (high gluten bread).

Efforts to make gluten-free bread include the use of Non-Gluten Component ingredients (NGC), Natural Hydrocolloid (NH) and Chemical Modifications Reaction (CMR) by involving Research & Development (R&D) in the innovation process. There is a lot of previous literature related to the manufacture of gluten-free bread with a variety of methods and analysis. Referring to the previous literature review, the use of NGC, NH, and CMR is only a recommendation in the modification of gluten-free bread. There is no study that has tested the effect (NGC, NH, and CMR) on bread quality (BQ).

The aims of this study were: a) to measure the effect of the modified variables (NGC, NH, and CMR) on BQ in the bakery industry. b) provide practical recommendations so that modification efforts can be widely accepted and applied in industrial areas.

1.1. Non-Gluten Component (NGC)

The gluten character Elastic has similarities to starch in the form of thick liquid and is very suitable for textural expansion (Balaghi et al., 2011). Starch is a polysaccharide derived from seeds, fruit, plant extracts, seaweed and microorganisms (Meybodi et al., 2015). Starch in the bread formula as a texture softener, and appearance. Substitution of rice flour is more acceptable as a substitute for wheat flour (Navarro & Araya, 2015)

Dietary fiber in seeds, fruit, and vegetables can be an alternative to gluten in wheat flour (Gan et al., 1989). Dietary fiber can replace the function of gluten based on dietary studies using gluten-free bread when compared to normal bread (Grehn et al., 2001). The addition of dietary fiber can help the stability of the dough so as to produce an even crumb, smooth texture, and sharp and even brown coloring (Gallagher et al., 2004).

Whey protein has a mesoscopic character that helps in retaining CO_2 gas produced by fermentation in bread, slows the movement of water in the bread so that the crumb is soft (Lazaridou et al., 2007). The addition of whey protein can increase water absorption thereby helping in the stretch setting of the dough. Which affects the Milllard browning reaction and preferred caramelization over other additives. N-ethyl maleimide (NEM) Whey protein can block thiol agents resulting in nearly eight times the volume of bread and an increase in rheological properties (van Riemsdijk et al., 2011).

The combination of flour and fermented water is the solution to get gluten-free bread which is rich in vitamins, iron, folate and dietary fiber (Zannini et al., 2012). Sourdough is a combination of flour and water that has undergone a fermentation process with lactic acid bacteria (LAB) and yeast (Gobbetti et al., 2018). The gluten network plays a role in slowing water transfer and maintaining CO_2 gas produced during the fermentation process, although the use of fermentation delays the starch retrogradation process and the cessation of gluten-free bread (Sabanis & Tzia, 2011). Biological acidification, amylolytic and proteolytic activity of cultures are the main mechanisms involved in delayed retrogradation (Rojas et al., 1999). Some LAB do not have amylolytic activity, so to get gluten-free bread with a long shelf life, it is recommended to use sprouted grains (Rojas et al., 1999). The addition of sourdough culture to gluten-free bread can improve the celiac immune system by producing peptides containing proline/glycine through proteolytic activity (Rollán et al., 2005). These conditions make gluten-free bread a functional food because of the ability of LAB to produce exopolysaccharides, especially fructooligosaccharides (Schwab et al., 2008)

Referring to the description above, the research hypotheses are: **H1**: NGC has a direct positive effect on BQ; **H2**: NGC has a direct positive effect on R&D

1.2. Modification of gluten protein chemical reaction (CMR)

The structural and functional linkages seen in food and non-food systems are the main areas of chemical alteration of gluten proteins. Protein side chain chemical modification can enhance: a) nutritional quality as a result of necessary amino acid availability, denaturation, and protein matrix degradation. b) functional characteristics (amount of emulsifier, foaming) c) physical state (texture). Abedi & Pourmohammadi (2020) made careful observations regarding: (i) when an essential amino acid (lysine) is derivatized, the Maillard reaction and base degradation are carried out to produce lysinoalanine compounds. (ii) the presence of chemical residues from the modification results (the amount of lysine is quite low) for the modification reaction (de Jongh & Broerse, 2012). This allows the optimal use of gluten for various purposes in the food

and non-food industries. The hypotheses formed are: **H3**: CMR has a direct positive effect on BQ; **H4**: CMR has a direct positive effect on R&D.

The indicators used for the CMR variable are:

Phosphorylation : An alcohol and a carboxylic acid react to produce the esterification reaction, which requires heat as a source of energy and the acid as a catalyst (Robertson et al., 2014). Phosphorylation is an esterification process brought on by urea and phosphoric acid treatment at temperatures higher than 130^{o}C. Phosphorylated gluten can absorb around 200 times its own weight in water because urea can limit hydrogen bonding and decrease the stability of secondary connections (Robertson et al., 2014). It is believed that esters derived from the hydroxyl groups in the gluten amino acids, tyrosine, serine, and threonine, are responsible for the increased absorptivity of water. Because the esterification step requires dehydration events, the negatively charged phosphate group makes the protein polymer chains oppose one another, increasing water absorption (Robertson et al., 2014).

Glycosylation: Glycation, which is based on glycoconjugates between polysaccharides and proteins, is also known as the Maillard reaction. Protein NH2 residues are reduced to sugar or polysaccharide reducing groups by perlecovalent bonds during glycolysis (Abedi & Pourmohammadi, 2020). The protein molecule's negative charge rises and the base level falls as a result of this circumstance. The glycated protein's isoelectric pH subsequently shifts in the direction of a higher pH. Carbohydrates with more hydroxyl groups and negative charge have altered solubility, water retention, foaming characteristics, antioxidant activity, and stability in hot environments(J. Liu et al., 2012).

Deamidation: Through the process of first conversion, the amide groups present in glutamine and asparagine are transformed into carboxylic groups, resulting in the formation of glutamate and aspartic acid. Carboxylic acids are preferable for protein deamidation due to their superior suitability. Carboxylic acid reactions are preferable for deamidation as they effectively hydrolyze peptide bonds (Liao et al., 2016). The deamidation of carboxylic acids allows for a controlled level of protein hydrolysis and enhances the features and organoleptic properties (Y. Liu et al., 2018). (iii) HCL produces an uncontrolled degree of protein hydrolysis and a large number of isomerization of amino acids and dichloropropanol (Liao et al., 2010) (iv) antioxidant activity accelerates the deamidation of gluten (Qiu et al., 2013) (v) improved nutrition due to digestibility and conversion power to low molecular weight hydroxylates (Cui et al., 2013). (vi) the total amount of essential amino acids and lysine in digestion increases. (vii) carboxylic acid (acetic acid) can be efficient due to increased solubility of functional characteristics (Liao et al., 2010; Qiu et al., 2013) (viii) Deamidated gluten is effective in reducing celiac (Qiu et al., 2013)

Acylation is a chemical modification of the amino group turn into amide (Majzoobi & Beparva, 2014) because acylation groups react with -amino groups (arginine and lysine), aliphatic hydroxyl groups (serine and threonine), and nucleophilic groups, such as phenolic (tyrosine). However, the reactivity of lysine -amino group is higher than others (Majzoobi et al., 2017). Functional features of acylated proteins have increased (water absorption, foaming capacity, emulsion properties, water holding capacity due to: breaking of hydrogen bonds, electrostatic repulsion, dissociation of high molecular weight proteins, less protein-protein interactions, but more protein-water and reduce the content of high molecular weight glutenin, gliadin and glutin (Abedi & Pourmohammadi, 2020).

1.3. Natural Hydrocolloids (NH)

The use of materials that have elastic characteristics such as gluten. Salehi (2019) succeeded in classifying NH based on the findings of previous studies. (i) *Xanthan gum* is widely found in bread making because it increases the volume, and carboxyl methyl cellulose is used as a gluten substitute. (ii) *Guar gum* is a natural latex added to bread dough with a certain level of texture and crumb. (iii)

Carrageenan Gum is a seaweed extraction with low viscosity so it is used for texture improver (Rosell et al., 2001) carrageenan function to reduce firmness and increase the volume of baked bread. (iv) *Methylcellulose* (MC) is widely used in the bakery industry because of its properties (soluble in water, high molecular weight, forms a thickener in water systems) (Sanz et al., 2005). (v) *Carboxy methyl cellulose gum* is a natural hydrocolloid for long shelf life through moisture retention and prevention of syneresis (Ozkoc & Seyhun, 2015), this type is able to provide a barrier layer during heating which causes water and oil loss (vi) *methyl propyl hydroxyl / cellulose gum* is used to improve taste, thickener, and gives a melt sensation in the mouth (Turabi et al., 2008). (vii) *Locust Bean Gum* is added to minimize breakage due to brittle texture. The addition of ingredients ranging from 1-3% can add volume, the crumb is softer (Angioloni et al., 2008). Various kinds of natural hydrocolloids can be used such as balangu seed gum, wild sage seed gum, basil seed gum and crispy seed gum.

The description helps in making research hypotheses, **H5**: NH has a direct positive effect on BQ and **H6**: NH has a direct positive effect on R&D

2. Materials and methods

2.1.Materials

The data were taken from 45 bakery companies spread throughout Indonesia. Observations were made for 15 months (March 2022 – June 2023). Respondents were represented by employees of the Research & Development (R&D) division because the research data was in the form of trial results of NGC, MRC, and NH raw materials in the bread-making process and required data analysis. Research data in the form of qualitative analysis (organoleptic) and quantitative analysis (results of laboratory analysis, such as: gluten content, water content, viscosity, etc.).

We gathered information from respondents regarding the ease of application of ingredients for bread making. The scale we used was as follows: 1. Very difficult to apply; 2. Difficult to apply; 3. Neutral; 4. Easy to apply; 5. Very easy to apply.

Qur R&D variable used the following assessment: 1. Very unexpected; 2. Unexpected; 3. Neutral; 4. expected; 5. Highly expected.

Our "BQ" variable refers to: 1. Very dislike; 2. Dislike; 3. Neutral; 4. Liked; 5. Very liked. However, for gluten content, we used laboratory analysis results with the following criteria: 1. (>3%); **2**.(2.1-3%); **3**.(1.1-2.1%); 4. (0.1-1.1%), and 5 (0%).

2.2.Methods

2.2.1. Determination of Variable Indicators

The selection of indicators using Principal Component Analysis (PCA) with SPSS software is carried out to determine indicators of various activity items to be carried out evaluated. The function of PCA is basically to reduce several variables into new variables or new dimensions which are the result of indicator extraction (Budianto et al., 2022). The choice of PCA was because the indicators used were relatively new so there was no reference regarding the indicators used in the variables (budianto; Kusmardini, 2021).

We focus on the equations related to identifying and selecting the principal components that explain most of the variance in the data. The following are the key steps along with the corresponding equations:

$$X = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1p} \\ x_{21} & x_{22} & \dots & x_{2p} \\ \vdots & \vdots & \ddots & \vdots \\ x_{n1} & x_{n2} & \cdots & x_{np} \end{bmatrix} \quad \text{.....(1)}$$

X is the original data matrix with dimensions $n \times p$, where: n is the number of observations (rows in the matrix), and p is the number of variables or indicators measured (columns in the matrix). x_{ij} is an element of the X matrix, which represents the value of the j-th variable (column) for the i-th observation (row). Thus, x_{ij} represents the observed data for variable j in the i-th observation.

Covariance indicates the direction of the relationship between variables, whether they move together (positive), in opposite directions

(negative), or have no linear relationship (values close to zero)

$$C = \frac{1}{n-1} X^T X \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

C: Covariance Matrix; X is the original standardized data matrix; X^T: the transpose of the X matrix.

Eigen decomposition to obtain eigenvalues and eigenvectors

$$Cv = \lambda v \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

v: Eigenvector, representing the linear combination of the original variables that define the principal component. λ: Eigenvalue, representing the variance explained by the principal component.

The percentage of variance explained by each principal component:

$$\mathrm{VE}_k = \frac{\lambda_k}{\sum_{j=1}^{p} \lambda_j} \text{ X } 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

VEk: Explained Variance k; λk : Eigenvalue of the k-th principal component; *p*: Total number of principal components.

New indicators based on factor score values are calculated based on the contribution of each variable in the main component, and then used as new indicators.

$$Factor\ Score_i = \sum_{k=1}^{m} PC_k \text{ x } W_k \ldots (5)$$

Factor Score$_i$: The factor score for the i-th observation, used as a new indicator; PC_k: The *k*-th principal component that has been calculated; W_k: The weight or coefficient for the k-th principal component used in the formation of the factor score; m: The number of principal components considered (usually those with the largest eigenvalues

2.2.2. Statistical Analysis

Test the analysis to see how much influence the relationship between variables. Testing the effect of variables using Structural Equation Modeling (SEM), with Partial Least Square (PLS) approach with Smart PLS software version 6.0. Validity test using cross loading value > 0.6 and the value of Square Root of Average Variance Extracted (AVE) > 0.50.

$$AVE = \frac{\sum_1^n \lambda_i^2}{n} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

λ_i is the factor loading of the i-th indicator, and *n* is the number of indicators. An AVE greater than 0.5 is generally considered to indicate good convergent validity

Reliability test with Cronbach's Alpha value > 0.6, Composite Reliability > 0.7. Structural model testing by accommodating all construct variables formulated in hypothesis testing. All standard parameters refer to Hair et al. (2011)

$$CR = \frac{\sum_1^n \lambda_i^2}{\sum_1^n \lambda_i^2 + \sum_{i=1}^n \theta \mathrm{i}} \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

CR:composite reliability, Where θi is the error variance of the i-th indicator. Additionally, Cronbach's Alpha is also used to measure reliability, which is formulated as follows:

$$\alpha = \frac{n}{n-1} (1 - \frac{\sum_{i=1}^n \sigma_{\epsilon i}^2}{\sigma_X^2} \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

$\sigma_{\epsilon i}^2$ is the error variance of the *i*-th indicator, and σ_X^2 is total variance of construct X

Coefficients Q^2 and f^2

The Q^2 coefficient is used to measure the predictive relevance of the model, which is formulated as follows:

$$\mathrm{Q}^2 = 1 - \frac{SSE}{SST} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

SSE:Sum of Squared Errors, and SST: Total Sum of Squares.

Meanwhile, the f^2 coefficient measures the effect size of the exogenous variable on the endogenous variable

$$\mathrm{f}^2 = \frac{\mathrm{R}^2_{\mathrm{included}} - \mathrm{R}^2_{\mathrm{excluded}}}{1 - \mathrm{R}^2_{\mathrm{included}}} \ldots\ldots\ldots\ldots\ldots\ldots (10)$$

2.2.3. Model testing

Model testing was conducted using SEM-PLS with Smart PLS software to assess the influence of variables and test hypotheses. Model adequacy was assessed based on Hair et al.(2011) for SRMR (<0.07), Chi-Square, NFI (>0.9), and Goodness of Fit index (>0.36) values.

$$SRMR = \frac{1}{p(p+1)/2} \sum_{i<j}(\sum_{ij} - S_{ij} \ldots (11)$$

$$\text{Chi} - \text{Square Test} = (N-1)(\sum - \sum(\theta))^{T}((\sum - \sum(\theta))^{-1} \ (12)$$

$$GFI = 1 - \frac{\sum(S_{ij} - \sum_{ij})^2}{\sum S_{ij}^2} \ldots\ldots\ldots\ldots\ldots (13)$$

2.2.4. Research Framework

The research framework can be seen in Fig. 1, the variables NGC, MRC, and NH as independent variables, the R&D variable as the mediating/intervening variable and BQ as the dependent variable.

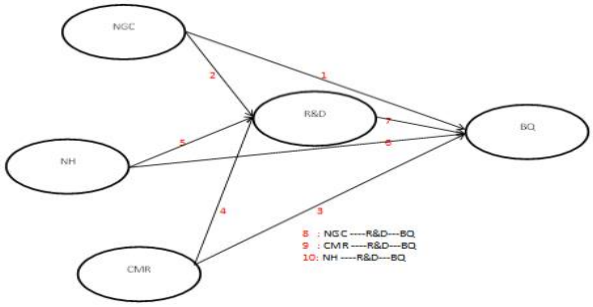


Figure1. Research Framework

3. Results and discussions

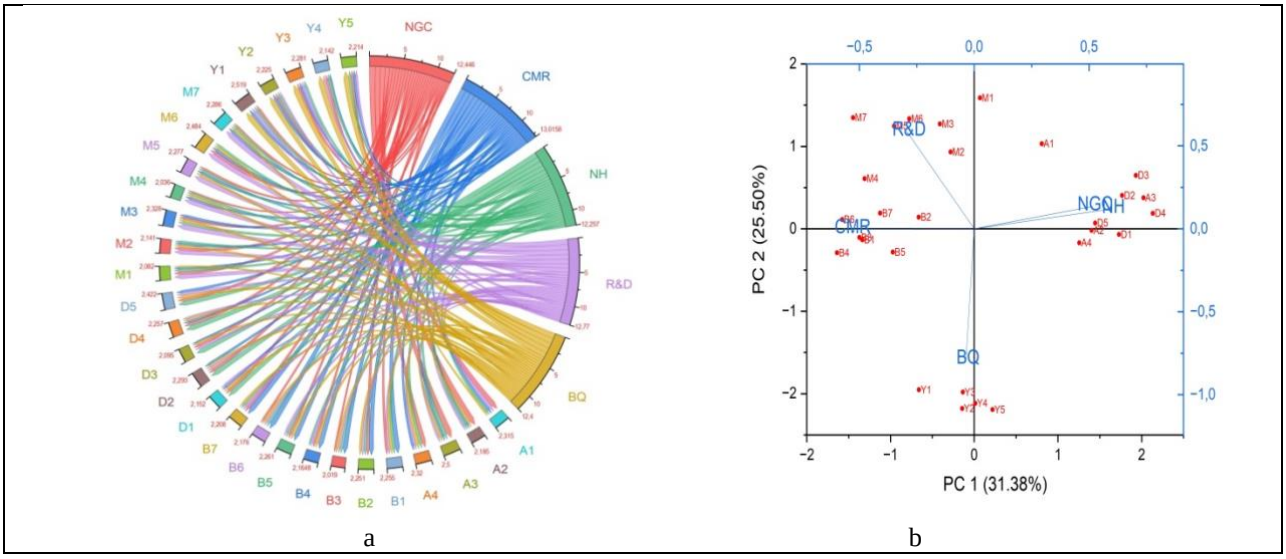
3.1. Principal Component Analysis (PCA)

Data categorization was performed using numerical representations (Table 1) and visualizations (Fig.2) based on the chord diagram and PCA. Referring to Table 1, the indicators of NGC were: A1, A2, A3, and A4. CMR indicators include: B1, B2, B3, B4, B5, B6, and B7. NH indicators consist of D1, D2, D3, D4 and D5. Indicators for the R&D variable include: M1, M2, M3, M4, M5, M6, and M7. Meanwhile, indicators for BQ comprise: Y1, Y2, Y3, Y4, and Y5.

Table 1. Determination of indicators using Principal Component Analysis (PCA)

Indicator	Code	Rotation Method: Varimax with Kaiser Normalization VARIABLE (* significant p<0.05)				
		NGC (1)	**CMR** (2)	**NH** (3)	**R&D** (4)	**BQ** (5)
Starch	A1	**0,754**	0,432	0,356	0,487	0,286
Food fiber	A2	**0,778**	0,375	0,358	0,298	0,376
Whey protein	A3	**0,873**	0,398	0,475	0,369	0,385
Fermentation (flour + water)	A4	**0,634**	0,365	0,472	0,382	0,467
Phosphorylation	B1	0,347	**0,743**	0,389	0,387	0,389
glycosylation	B2	0,452	**0,658**	0,396	0,391	0,354
Glycosylation (gluten+glucose)	B3	0,257	**0,672**	0,385	0,361	0,344
Glycosylation (Gluten + lactose)	B4	0,367	**0,753**	0,298	0,358	0,388
Glutamic acid deamidation	B5	0,392	**0,685**	0,389	0,374	0,421
Deamidation of carboxylic acids	B6	0,298	**0,779**	0,395	0,382	0,322
acylation	B7	0,438	**0,743**	0,352	0,364	0,311
Xanthan gum gum	D1	0,472	0,342	**0,658**	0,298	0,382
Guar Gum	D2	0,435	0,352	**0,745**	0,397	0,364
Carrageenan Gum	D3	0,389	0,267	**0,749**	0,392	0,298
Methylcellulose (MC)	D4	0,482	0,367	**0,763**	0,298	0,347
Methyl carboxy cellulose gum	D5	0,473	0,392	**0,684**	0,421	0,452

Existing product innovation	M1	0,481	0,298	0,374	**0,672**	0,257
Existing product evaluation	M2	0,459	0,338	0,334	**0,643**	0,367
Material composition evaluation	M3	0,378	0,372	0,443	**0,753**	0,382
Low price orientation	M4	0,238	0,435	0,344	**0,632**	0,387
Quality standards based on consumers	M5	0,349	0,389	0,364	**0,784**	0,391
Product innovation based on existing machines	M6	0,487	0,482	0,361	**0,768**	0,386
Material composition trial	M7	0,298	0,473	0,358	**0,793**	0,364
Gluten content	Y1	0,369	0,481	0,374	0,452	**0,843**
Form	Y2	0,382	0,459	0,382	0,257	**0,745**
Color	Y3	0,387	0,378	0,364	0,367	**0,785**
Smell	Y4	0,391	0,238	0,298	0,392	**0,823**
Taste	Y5	0,386	0,349	0,397	0,298	**0,784**



a b

Figure 2. Determination of indicators from the variables based on the correlation between indicators using the Chord Diagram (a), and the correlation of indicators based on PCA testing (b).

The chord diagram (Fig.2a) showed consistent findings, dividing the indicator item into 5 variables with nearly identical bandwidth (12.5). Additionally, we illustrated the indicator mapping using the biplot method with PCI (31.38%) and PC2(25.5%), which revealed identical indicators across the five tested variables.

3.2. Descriptive Statistics

The mean of the NGC variable was good (3.5-4) although it was found that A2=3.44 (table 2). The order of using NGC that was in great demand was A3, A1, A4 and the lowest was A2. The role of A2 was categorized as "adequate" in the process of making gluten-free bread, while other indicators (A3. A1, and A4) were categorized as good (widely used, easy to apply).

The mean of the CMR variable was bad (<3), it can be seen from B5 (2.6), B7 (2.8) and B6 (2.9) although found B1 (3.40), B3 (3.32), B2 (3.2) and B4 (3.20) The order of using CMR based on respondents' interests was B1, B3, B2, B4 B6, B7 and the lowest was B5 and B7 categorized as bad.

Table 2. Descriptive Statistics of Respondent Test Results.

Descriptive Statistics					
SIZE SCALE					
1. Very difficult to apply	2. Difficult to apply	3, Neutral	4. Easy to apply	5. Very easy to apply	
Variable	Statistic	Bootstrap[a]			
		Std. Deviation	Std. Error	95% Confidence Interval	
				Lower	Upper
Non-Gluten Component (NGC)					
Strach (A1)	3.7600	.52281	.1023	3.5200	3.9200
Food fiber (A2)	3.4400	.71181	.1398	3.1600	3.6800
Whey protein (A3)	3.8000	.40825	.0814	3.6400	3.9600
Fermentation (A4)	3.5200	.65320	.1227	3.2800	3.7600
Average	**3.6300**				
Modification of gluten protein chemical reaction (CMR)					
Phosphorylation (B1)	3.4000	.86603	.1662	3.0800	3.6800
Glycosylation (B2)	3.2000	.81650	.1642	2.8800	3.5200
Glycosylation (gluten+glucose) (B3)	3.3200	.85245	.1672	3.0000	3.6400
Glycosylation (Gluten + lactose)(B4)	3.2000	.81650	.1593	2.8800	3.4800
Glutamic acid deamidation(B5)	2.6000	.57735	.1126	2.4000	2.8400
Deamidation of carboxylic acids(B6)	2.9600	.61101	.1220	2.7200	3.2000
Acylation (B7)	2.8800	.60000	.1159	2.6400	3.1200
Average	**2.9100**				
Natural Hydrocolloids (NH)					
Xanthan gum gum (D1)	3.5600	.76811	.1526	3.2400	3.8400
Guar Gum (D2)	3.3600	.81035	.1570	3.0400	3.6400
Carrageenan Gum (D3)	3.6400	.63770	.1225	3.4000	3.8790
Methylcellulose (MC) (D4)	3.5600	.58310	.1205	3.3200	3.8000
Methyl carboxy cellulose gum (D5)	3.0800	.64031	.1234	2.8400	3.3590
Average	**3.4100**	0	0	105	105
SIZE SCALE					
1. Very unexpected	2. Unexpected	3, Neutral	4. Expected	5. Highly expected	
Research & Development (R&D)	Statistic	Bootstrap[a]			
		Std. Deviation	Std. Error	95% Confidence Interval	
				Lower	Upper
Existing product innovation (M1)	3.8000	.40825	.0832	3.6000	3.9600
Existing product evaluation (M2)	3.8400	.37417	.0716	3.6800	3.9600
Material composition evaluation (M3)	3.8400	.37417	.0730	3.6800	3.9600
Low price orientation (M4)	3.8000	.50000	.1010	3.5600	3.9600
Quality standards based on consumers (M5)	3.9200	.27689	.0550	3.8000	4.0000
Product innovation based on existing machines (M6)	3.7600	.59722	.1170	3.4800	3.9600
Material composition trial (M7)	3.8400	.37417	.0716	3.6800	3.9600
Average	**3.8300**				
SIZE SCALE					
1. Very dislike	2. Dislike	3. Neutral	4.Liked	5.Very liked	
1. gluten > 3%	2. gluten 2.1-3%	3. gluten 1.1-2.1%	4. gluten 0.1-1.1%	5. gluten 0%	
Bread Quality (BQ)	Statistic	Bootstrap[a]			
		Std. Deviation	Std. Error	95% Confidence Interval	
				Lower	Upper
Gluten content (Y1)	3.7600	.43589	.0833	3.6000	3.9200
Form (Y2)	3.8400	.37417	.0729	3.6800	3.9600
Color (Y3)	3.7200	.45826	.0896	3.5210	3.8800
Smell (Y4)	3.7200	.54160	.1084	3.4800	3.9200
Taste (Y5)	3.7200	.45826	.0896	3.5200	3.8800
Average	**3.7500**				

The mean of the NH variable was sufficient (3-3.5) it can be seen from, D2, D6) although there were some indicators that were worth >3 (D1, D3, D4) The order of using NH based on the respondents' interests was D3, D1, D4, D2 and the lowest was D5. The roles of D1, D3, D4 were categorized as "good" in the process of making gluten-free bread, while other indicators (D2, D5) are categorized as adequate.

The average R&D variable was good (3.5-4) it can be seen from, M1, M2, M3, M4, M5, M6, and M7. The order of the use of NH based on the respondent's interest was M5, M7, M2, M1, M4, M3 and the lowest was M6. The role of all indicators was categorized as "good" in the mediation process for making gluten-free bread.

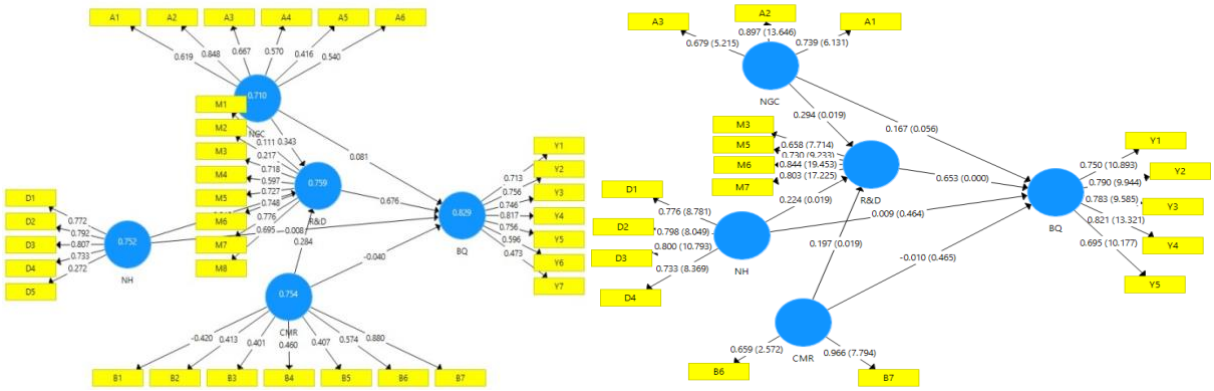
The mean of BQ variable was good (3.5-4). The order of the NH indicators based on the research results was Y2, Y3, Y4, Y5, and the lowest was Y1. The role of all indicators was categorized as “good” in the results of making gluten-free bread. Y1 scale determination was based on the gluten content that was still detected in the bread-making process (table 2).

3.3. SEM_PLS analysis

Determination of indicators using PCA helps in mapping based on the variables, although the indicator will be partially deleted by SEM PLS if the outer loading value is <0.6. The following are the results of the analysis using SEM PLS.

All indicator and their variables were subjected to bootstrap testing, and the loading factors of each indicator are depicted (Fig.3a). To ensure valid and reliable data, indicator loading factors > 0.55 were selected, while those below 0.55 had to be removed. The analysis outcomes are presented in Fig.3b.

Based on Fig.3b, the validity and reliability of the research model can be seen in table 3. Convergent validity refers to the extent to which indicators are able to measure the same variable, in this case, all indicators were able to measure their variables based on outer loading, AVE, and commonality.



a b

Figure 3. SEM_PLS analysis. Loading factor for all indicators (a), and Loading factor > 0.55 (b)

Table 3. Test the Validity and Reliability of the Research Model

Test	Parameter	Standard	Results
Convergent Validity	Factor loading (outer loading)	>0.55	0.659 -0.966
	AVE	>0.5	0.581– 0.684
	Commonality	>0.5	0.581 – 0.684
Discriminant Validity	Root Square AVE and Correlation latent variables	Root Square AVE > Discriminant validity	Root Square AVE> Discriminant Validity
	Cross Loading	>0.6	0.726 – 0.906
Reliability	Cronbach's Alpha	>0.6	0.614 – 0.827
	Composite Reliability	>0.7	0.807 – 0.878

Discriminant validity, which explained that the three variables in the model could be distinguished from one another, was demonstrated in this research model. The model's reliability also met the requirements based on Cronbach's Alpha, and Composite Reliability. All validity and reliability tests were met, so that further tests (variable influence tests) could be carried out.

Based on table 4, the accepted hypotheses were: H2, H4, H6, H7, H8, and H9. While the rejected hypotheses include: H1, H3, and H10. Exogenous variables (NGC, CMR, and NH) all did not have a direct positive impact on BQ, but were able to have a positive effect on BQ if mediated by R&D, this was because R&D had a strong effect of 69.2% (f^2) on BQ. Only H3 had a direct negative impact on BQ. The R&D variable was able to explain the exogenous variable by 28.2% (R2), while the BQ variable was 55.7%.

Table 4. The Effect of Variable Paths and model fit

Hypothesis	Paths	Coefficient (β)	T statistics >1.65	p Value < 0.05	f^2	Remark
1	NGC—BQ	0.167	1,541	0.062	0.039	(+) Not significant
2	NGC—R&D	0.294	2.104	0.018	0.079	(+) significant
3	CMR—BQ	-0.010	0.088	0.465	0.000	(-) not significant
4	CMR—R&D.	0.197	2.274	0.012	0.052	(+) significant
5	NH—BQ	0.009	0.086	0.466	0.000	(+) not significant
6	NH.—R&D	0.224	2.115	0.017	0.046	(+) significant
7	R&D---BQ	0.653	7.286	0.000	0.692	(+) significant
8	NGC—R&D--BQ	0.192	2012	0.022		(+) significant
9	CMR—R&D--BQ	0.128	2,153	0.016		(+) significant
10	NH—R&D--BQ	0.146	2002	0.023		(+) Not significant
f^2 : 0.02- 0.15 Weak Effect; f^2 : 0.15-0.35 Sufficient Effect ; f^2 : 0.35 Strong Effect						
R^2: R&D 0.282……BQ: 0.557						

Model fit	Standard	Saturated model	Estimated model
SRMR	< 0.07	0.054	0.054
Chi-Square	<60.76	51.261	51.261
NFI	> 0.9	0.908	0.908
GFI	>0.36	0.365	0.365

3.4. Model Fit

Throughout our analysis process, we ensured that the model we proposed satisfies several model fit indices.

We chose SRMR to assess how well our research model fits the patterns of relationships among variables in the data. A low SRMR value indicates that the model fits the data well. In this study, the SRMR met the criterion (<0.07).

Although Chi-Square is rarely used as an evaluation measure in PLS models, we employ this analysis to assess the quality of the model built from observational data. In this study, the model also met the criteria for the Chi-Square value (<60.76).

The NFI assesses the extent to which this research model fits the baseline model and lacks correlation between variables. It helps gauge the model's fit with the most basic patterns. In this study, the NFI of this research model was quite high (> 0.9).

The main focus of this research is to measure the effect of the modified variable (NGC, NH, and CMR) to BQ. If you look at the one-way analysis, the three variables were unable to exert a positive impact on BQ, two variables showed a not significant positive effect (NGC and NH), while one variable (CMR) had a negative impact. Referring to the value of one-way analysis, NGC was superior to NH and CMR. Looking at the results of the two-way analysis/mediation role, two variables were able to have a positive effect on BQ (H8 and H9) while H10 has not had a positive effect on BQ. These conditions indicate that the NGC variable was more influential than CMR and NH.

R&D has succeeded in carrying out its function as an intervening (mediation) variable. R&D activities were able to encourage activities in CNG, CMR, and NH through the evaluation of material composition (M3) for easy application and there was no overlapping of modification functions. R&D explores consumer desires for quality reference (M5), this will facilitate the innovation of bakery products based on consumer tastes. Innovations were carried out by R&D by utilizing the condition of the machine (M6) so that the flexibility of the production process would be achieved. Material composition trial (M7) was the main key along with the trial frequency, it will be easy to understand the character of each ingredient.

The next goal of this research was to recommend is practical so that modifications can have a positive effect on BQ. Digging up failure information in the application then evaluating and activating R&D practices in problem solving. The following are the constraints and the role of mediation (R&D) in this study.

NGC did not have a direct positive effect on BQ, because (i) the number of uses was very varied to the standard of friability, and the results of research trials showed that the use of 10-20% could increase the volume 8 times, this strengthens the findings Nunes et al. (2009). (ii) the addition of starch and dietary fiber causes a decrease in vitamins, iron and folate, this was anticipated by the addition of water then fermented with lactic acid bacteria, and the results of the study were in line with the findings Schwab et al. (2008).

CMR had a negative effect on BQ, it shows that there were still many obstacles in its application if it was not mediated. The constraints and the role of mediation were as follows: (i) Solubility in phosphorylation was unstable, the results of research trials showed protein solubility at pH>6. (ii) unstable molecular weight so the role of R&D must be to maintain hydrophilic substitution and the formation of non-disulfide cross-links between protein sub-units. (iii) the strength of the gel was not strong so it must control the reaction of the carbamide formation step, this causes the modified gel to become firm. The findings are supported by Robertson et al. (2014). (iv) understanding the digestibility of gliadin takes a long time, this understanding is broken by the results of R&D analysis through a simulation of stomach and intestinal digestion for 2 hours. This finding strengthens previous research (Xue et al., 2019). (v) protein solubility was very slow, so R&D made efforts to conjugate gluten hydroxylate with glucosamine and transglutamine. (vi) in the deamidation process, there was susceptibility to the addition of alkali, this condition cannot be avoided so efforts are made to use alkali for the generation of fibrous microstructures. This finding is in line with previous research (Li et al., 2018).

Table 5. Constraints and their solutions

Variable	Indicator	Constraints	The role of R&D
Non-Gluten Component (NGC)	Starch	- Addition of starch and dietary fibre leads to a decrease in vitamins. iron, and folate - Loss of dough elasticity - Dough is very crumbly - Dough is not sticky	- Addition of water for lactic acid bacteria fermentation process - Addition of 1-3% Xantan Gum - Addition of 3-5% egg - Addition of 5-10% corn starch
	Food Fiber	- Decreases the volume of the bread - Produces a rough texture - Other odours and flavours emerge	- Addition of 2-3% additives such as hydrocolloids - Improving texture with the addition of 1-2% binding agent (Xantan Gum) - Offsetting flavour and aroma with the addition of dietary fibre.

	Whey protein	- Coagulation during dough mixing - Shorter product life	- Addition of 1-4% emulsifier - Addition of 2-5% natural preservatives
Modification of gluten protein chemical reaction	Phosporylation	- Potential to alter the functional properties of the protein - Inconsistent texture	- Use of phosphate concentration of 0.2-0.5 mol/L - Reaction temperature 25^{o}C and neutral pH
	Glycosylation	- Not all reaction results are safe for consumption - Addition of sugar significantly affects texture and flavour	- Choosing ingredients that are gluten and allergen free - Reducing the amount of sugar in the ingredients
	Glycosylation (gluten+glucose)	- Increases the sugar content of the product - Unstable molecular weight	- Replacing glucose with plant-based sweeteners - hydrophilic substitution and formation of non-disulfide crosslinks between protein sub units
	Glycosylation (Gluten + lactose)	- Lactose allergy - Slow glycoprotein solubility	- Addition of almond milk or soya milk. - Conjugation of gluten hydroxylate with glucosamine
	Glutamic acid deamidation	Bitter taste of glutamate	The addition of 1% CH_3COOH can prevent excessive deamidation.
	Deamidation of carboxylic acids	- Damage to protein structure - Unhealthy trans fat content - susceptible to alkali addition	- Use of transglutaminase enzyme - Addition of vegetable fat (olive oil) - Use of alkali for the generation of fibrous microstructures
Natural hydrocolloids	Xantan Gum	- Organoleptic of bread changes depending on the type of NH - Has a high viscosity - Produces a thick texture in bread dough	- Addition of pectin - Reducing viscosity with the use of xantam gum only in the range of <10% - Addition of water <5%
	Guar gum	- Unstable at low pH - Unstable at high temperatures	Avoid manufacturing process at low pH and high temperature
	Carrageenan gum	- Has low viscosity at low temperatures	- Carragenan gum is preheated before mixing into the dough
	Methylcellulose	- Hydration takes a long time - Coagulation occurs	Longer stirring for homogeneity
	Metyl carboxy cellulose gum	- Degradation at low pH - Low dough binding capacity - Brittle if heated at high temperature (>160^{o}C)	- Mixing is done at neutral pH - Mixing with other binders

NH did not have a positive effect on BQ if it was not mediated by R&D. This was because (a) the organoleptic changes in bread, so it was necessary to add pectin to maintain the organoleptic. The findings of this study are in line with the findings of Roman et al. (2019). (b) the viscosity of the carrageenan extract was low, R&D offered another function as a texture improver and reduced firmness and to increase the specific volume of the baked product although it did not help maintain the general structure of the bread. Product diversification by utilizing the natural properties of carrageenan is also carried out by Rosell et al. (2001).

Table 5, maps the problem of gluten-free bread modification and practical recommendations for research. Practical recommendations refer to the problem of modifying gluten-free bread in the Indonesian bakery industry. This study also provides a conceptual framework related to the application model of gluten-free bread modification with a mediation.

Practical recommendations include re-evaluating the modification process with an emphasis on optimizing the use of NGC and NH and improving the solubility of CMR. Continuous efforts in research and development are needed to overcome the challenges faced, such as solubility and gel strength in CMR. This study provides a foundation for the development of higher-quality and innovative gluten-free bread products.

4. Conclusions

This study proposes a measurement model to evaluate the effectiveness of modification (NGC, NH, and CMR) on bread quality (BQ). The findings indicate that NGC and NH showed a non-significant positive impact on BQ, while CMR had a negative impact. R&D played a crucial role as a mediator, facilitating modification activities and addressing constraints to achieve the desired positive impact on bread quality.

Practical recommendations include re-evaluating the modification process with an emphasis on optimizing the use of NGC and NH and improving the solubility of CMR. Continuous efforts in research and development are needed to overcome the challenges faced, such as solubility and gel strength in CMR. This study provides a foundation for the development of higher-quality and innovative gluten-free bread products.

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Acknowledgment

I would like to thank all the respondents (R&D) in the food company who helped in the modification test of gluten-free bread making.