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QUALITY PARAMETERS IN KAYMAK PRESERVED AT DIFFERENT TEMPERATURES

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ABSTRACT

Kaymak is a high-fat dairy product produced from buffalo or cow's milk. Due to the high oil content, it can easily deteriorate under improper conditions. Therefore, the storage temperature is quite important for kaymak. In this study, which was conducted for this reason, some quality parameters were studied in kaymak samples stored in two different temperature degrees (A:4 ± 2°C; B:25 ± 2°C). According to the results of the analysis, total aerobic mesophilic bacteria and yeast/mold during storage increased to the level of 2.00 log cfu/g in group A; 3 log cfu/g in group B. The levels of proteolytic and lipolytic bacteria were found to have increased to 2 log cfu/g in both groups. While the PH values of the samples were 6.03 at the beginning, they decreased to 5.78 in group A and 5.67 in group B. Peroxide and malondialdehyde (MDA) values increased more in group B than in group A. While the L* value, which is the color parameters decreased in both groups, a* and b* values increased. In the organoleptic properties of kaymak samples were found acceptable until the 15th day in group A, 7th day in group B. Consequently, the production and storing conditions in producing an easily-perished product, kaymak, should be ensured optimally from the quality of raw materials.

1.Introduction

Kaymak is a taste unique to Turkey. Today, this taste takes its place in breakfasts and desserts. Kaymak is described as a cream that contains at least 60% milk fat by weight. "Afyon Kaymağı" is defined as a dairy product produced by boiling buffalo milk at 92°C for at least 2 minutes and cooling it by following its technique (TFC, 2003). Milk fat is the raw material of kaymak. Milk fat acts as a raw material in various dairy products and is effective on the nutritional value, taste-aroma, and physical properties of the product (Akalin et al., 2006; Kocaeli, 2009). Milk fat, which is a good source of energy, contains fat-soluble vitamins such as A, D, E, and K, essential fatty acids such as linoleic and arachidonic acids, medium-chain fatty acids, and especially conjugated linoleic acid (KLA) in its structure (Akalin et al., 2005; Anli & Gürsel, 2013). KLA, which is included in milk fat, is known to have effects such as reducing blood lipids, increasing metabolic rate, reducing the amount of body fat, strengthening immunity, reducing inflammation, increasing bone growth and muscle mass, and antiatherosclerotic and anticarcinogenic effects (Kurban & Mehmetoğlu, 2006; Seçkin & Baladura, 2011).

Kaymak can be exposed to microbial contamination in the production, storage, and marketing stages. The number and type of microorganisms of the product affect raw

materials, hygienic conditions at the production stage, storage conditions and the sensory quality of the product (Yilsay & Bayizit, 2002). Apart from these, the shelf life of kaymak differs from other milk fat-based products due to its excess moisture content. Additionally, the absence of a fermentation stage in kaymak production is also effective in the short shelf life of kaymak (Akalin et al., 2006). The shelf life of kaymak, a non-fermented product, is an average of one week, while the shelf life of other products is reported as 6-8 months (Sserunjogi et al., 1998).

This study researched the microbiological, physicochemical, and sensory properties of Afyon Kaymağı maintained at different temperatures during storage were investigated and revealed its shelf life.

2. Materials and methods
2.1. Materials
2.1.1. Samples of Kaymak

Kaymak samples were produced by using buffalo milk. Table 1 shows the values of raw buffalo milk which was used in the production of Kaymak. In kaymak production, buffalo milk was filtered first and taken into special containers, and it was subjected to heat treatment at 85°C. After heat treatment, it was rested for 3-4 hours and left for 18 hours at 4°C. After this stage, the layer of kaymak formed on the milk was cut and taken into special containers.

The kaymak samples produced were packaged in their original packaging (about 90-100 g) and divided into two groups. One group (A) was retained at 4C±2 °C and the other group (B) at 25±2 °C. From all samples, samples were taken for analysis on the 0th, 3rd, 7th, 12th, and 15th days. Five kaymak samples (n:5) were analyzed for each analysis.

Table 1. Characteristics of Buffalo Milk Used in Kaymak Production

pH	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	SCC (ml)	TBC (cfu/ml)
6.74±0,02	7.23±0,09	4.31±0,31	5.07±0,31	10.08±0,35	149,000±1000	760,000±1000

SNF: solids nonfat, SCC: somatic cell count, TBC: total bacteria count

2.2. Methods
2.2.1. Analysis of Buffalo Milk

Chemical properties of milk (Fat, protein, lactose, and SNF) were identified with Milk Analyzer MID - Infrared (MIRIS), Somatic Cell number was identified with Somatic Cell Counter (Chemometec SCC 100).

2.2.2. Color Measurement

L*, a*, b* values of kaymak samples were performed by using Hunter-Lab ColorFlex (A60-1010-615 Model Colorimeter, HunterLab, Reston, VA). Before measurements, the spectrocolormeter was calibrated with white and black reference colors. Hunter l*, a*, b * values were obtained with three different readings.

2.2.3. Water activity (a_w) measurement

The water activity (a_w) of kaymak samples was determined by using the Novasina brand (LabMASTER NEO, Switzerland) water activity measurement device.

2.2.4. pH measurement

The pH measurement of the samples was determined by using the Inolab brand (pH-7110, WTW, Germany) pH meter. The pH meter was calibrated with standard solutions 4.00 and 7.00 before measurement.

2.2.5. Microbiological analyses

The 10 grams of samples were added to 90 ml sterile peptone water with Tween80 and samples were homogenized for analysis. Then, serial dilutions were prepared from 1:10 diluted sample and sowing was done instead of the medium. In the samples, total aerobic mesophilic bacteria count (ISO, 2003),

proteolytic bacteria (Frank et. al., 1985), lipolytic bacteria (Smith & Alford, 1984), and yeast/mold count (ISO, 2008) were applied.

2.2.6. Number of peroxides

The number of peroxides in kaymak samples was determined according to the spectrophotometric method reported by Downey (1975).

2.2.7. Malondialdehyde (MDA)

Malondi-aldehyde (MDA) level in kaymak samples was determined according to the spectrophotometric method reported by Draper and Hadley (1990).

2.2.8. Sensory Analysis

Sensory analysis of kaymak samples was performed by evaluating the look, color, taste/flavor, and general liking of the samples by 10 volunteer expert panelists. The panelists made the evaluations by using the hedonic scale in the score range 1-3 (very bad - unacceptable), 4-5 (medium), 6-7 (good), 8-9 (very good).

2.2.9. Statistical analysis

The results of five kaymak samples (n:5) were evaluated for each analysis. In the results of the analysis, the difference between the kaymak samples which emerged during storage was determined by ANOVA, and the difference between the groups was determined by using the independent t-test.

3. Results and discussion

Table 2 shows the results of microbiological analysis determined during the storing process in the study. While the total number of aerobic mesophilic bacteria increased by approximately 2.00 log cfu/g during storage in group A, it increased by 3.00 log cfu/g in group B. In kaymak samples, the differences between the groups on the same days were found significant after the 3rd day (p<0.05). Çakmakçı and Hayaloğlu (2011) reported the total number of mesophilic bacteria in Ispir kaymak samples as 4.02 log cfu/g. An increase in proteolytic and lipolytic bacterial levels of 2.00 log cfu/g during storage was detected in both groups; differences were found between the two groups at the same time (p<0.05). In kaymak samples, while yeast/mold levels were found to increase by 2.00

log cfu/g in group A during storage; in group B, an increase of 3.00 log cfu/g was found. At high temperature (25±2°C), as expected in the stored samples, the bacteria number was found higher than the samples stored at 4°C. In a study, the average number of yeast/mold in kaymak samples was reported as 3.06 log cfu/g (Çakmakçı & Hayaloğlu, 2011). Microbiological differences between products can occur depending on the quality of the milk used in production, production, and storage conditions.

While the pH value in kaymak samples at the beginning of the storing was 6.03±0.07, it decreased by 5.78 in group A, and 5.67 in group B (Table 3). The differences between the two groups between the 3rd, 7th, 12th days were found significant (p<0.05). Akalın et al. (2006) reported the pH value in kaymak samples between 6.20-7.20, Çakmakçı and Hayaloğlu (2011) reported pH values in Ispir Kaymak between 6.50-6.76 in their studies. Additionally, Dereli and Şevik (2011) reported the pH value in kaymak samples, which were stored using a different packaging method, as 6.95 at the beginning, they reported it to be 5.84 on the 14th day. The pH values we obtained were found to be low compared to the results of other researchers. These differences may depend on factors ranging from raw material quality of products to production conditions. Additionally, increases in lactic acid bacteria levels due to microbial contamination levels and retention of cream can be effective. The water activity of the samples decreased during storage (p<0.05). While the water activity value decreased to 0.942 in group A; it decreased to 0.924 in group B (P<0.05) (Table 3).

Peroxide and MDA values, which are criteria for deterioration in kaymak, which is a product with a high oil content (60%), increased during storage in both groups (Table 3). Increases in group B were higher compared to group A, and the differences between the two groups were found significant starting from the 3rd day (p<0.05). The fact that group B was stored at high temperature compared to Group A was evaluated as the main factor. In other

studies, Dereli and Sevik (2011) reported that the peroxide value in kaymak samples increased from 0.11 to 0.25 from the beginning of storage to the end. Similarly, the study by Çön et al. (2000) found that the peroxide value increased (12,64 meq O2/kg) in the kaymak samples packed by vacuum at 18th day. However, Akalin et al. (2009) did not determine the peroxide value in the kaymak samples they collected.

Lipid oxidation is a major quality problem during the processing or storage of fats and fat-containing foods (Yang et al., 2002; Lampi et al., 1997). Differences between these characteristics can be caused by production and storage conditions, as well as due to long-term storage of products in unsuitable conditions.

Table 2. Result of microbial Analysis of Kaymak Samples during Storage (n:5)

Groups	Days	TAMB	Proteolitic Bacteria	Lipolitic Bacteria	Yeast/Mold
A	0	5.56 ± 0.24^c	4.67 ± 0.19^d	4.62 ± 0.07^c	2.07 ± 0.21^d
	3	$5.60 \pm 0.30^{c-x}$	$5.16 \pm 0.09^{c-x}$	$4.83 \pm 0.21^{c-x}$	3.13 ± 0.50^c
	7	$5.72 \pm 0.22^{c-x}$	$5.34 \pm 0.04^{bc-x}$	$5.60 \pm 0.30^{a-x}$	$3.76 \pm 0.15^{b-x}$
	12	$5.64 \pm 0.05^{b-x}$	$5.44 \pm 0.08^{b-x}$	$5.24 \pm 0.03^{b-x}$	$4.24 \pm 0.03^{a-x}$
	15	$6.86 \pm 0.07^{a-x}$	$6.00 \pm 0.05^{a-x}$	$5.35 \pm 0.05^{ab-x}$	$4.47 \pm 0.10^{a-x}$
B	0	5.56 ± 0.24^d	4.67 ± 0.19^d	4.62 ± 0.07^a	2.07 ± 0.21^d
	3	$5.15 \pm 0.14^{c-y}$	$5.50 \pm 0.10^{c-y}$	$5.24 \pm 0.03^{b-y}$	3.30 ± 0.04^c
	7	$5.39 \pm 0.06^{c-y}$	$5.82 \pm 0.19^{b-y}$	$6.48 \pm 0.11^{a-y}$	$4.46 \pm 0.28^{b-y}$
	12	$6.04 \pm 0.13^{b-y}$	$5.92 \pm 0.15^{b-y}$	$6.05 \pm 0.13^{c-y}$	$4.50 \pm 0.06^{b-y}$
	15	$7.20 \pm 0.06^{a-y}$	$6.22 \pm 0.03^{a-y}$	$6.21 \pm 0.04^{b-y}$	$5.07 \pm 0.04^{a-y}$

a–e Means in each column period in same group with different letters were significantly affected by storage (p < 0.05); x-y Means in each column in different between Kaymak groups were significantly at similar period (p < 0.05); A: 4°C±2, B: 25°C±2, TAMB: total aerobic mesophilic bacteria

Table 3. Result of Physico-Chemical Analysis of Kaymak Samples during Storage (n:5)

Groups	Days	pH	a_w	Peroxide	MDA
A	0	6.03 ± 0.07^a	0.952 ± 0.004^a	0.0718 ± 0.004^e	16.56 ± 0.44^e
	3	$6.04 \pm 0.05^{a-x}$	0.943 ± 0.003^b	$0.0936 \pm 0.002^{d-x}$	$19.33 \pm 1.70^{d-x}$
	7	$6.09 \pm 0.02^{a-x}$	$0.954 \pm 0.001^{a-x}$	$0.1408 \pm 0.004^{c-x}$	$31.42 \pm 0.86^{c-x}$
	12	$5.85 \pm 0.05^{b-x}$	$0.943 \pm 0.001^{b-x}$	$0.3201 \pm 0.005^{b-x}$	$40.60 \pm 2.08^{b-x}$
	15	5.78 ± 0.02^b	$0.942 \pm 0.002^{b-x}$	$0.3917 \pm 0.008^{a-x}$	$47.36 \pm 0.61^{a-x}$
B	0	6.03 ± 0.07^a	0.952 ± 0.004^b	0.0718 ± 0.0039^e	16.56 ± 0.44^e
	3	$5.88 \pm 0.06^{b-y}$	0.948 ± 0.002^b	$0.1282 \pm 0.0064^{d-y}$	$47.33 \pm 0.36^{d-y}$
	7	$5.85 \pm 0.04^{b-y}$	$0.962 \pm 0.002^{a-y}$	$0.1850 \pm 0.0027^{c-y}$	$64.99 \pm 0.75^{c-y}$
	12	$5.62 \pm 0.04^{c-y}$	$0.928 \pm 0.002^{c-y}$	$0.3780 \pm 0.0038^{b-y}$	$101.32 \pm 1.37^{b-y}$
	15	5.67 ± 0.06^c	$0.924 \pm 0.002^{c-y}$	$0.4502 \pm 0.0092^{a-y}$	$109.83 \pm 0.91^{a-y}$

a–e Means in each column period in same group with different letters were significantly affected by storage (p < 0.05); x-y Means in each column in different between Kaymak groups were significantly at similar period (p < 0.05); A: 4°C±2, B: 25°C±2

L*, a*, b* values in both groups varied during storage (Table 4). In both groups, while the L* value decreased, the values of a* and b* increased. Significant differences were found between L*, a*, b* values in both groups (p<0.05). Although L* values in groups A and B decreased compared to the beginning, the decrease in group B samples was greater (p<0.05). The a* and b* values of sample doubled, especially in group B. A decrease in the L* value between products and an increase in the values of a* and b* can be due to a change in the quality of products depending on the difference in storage temperature.

Table 4. Result of Color Analysis of Kaymak Samples during Storage (n:5)

Groups	Days	L	a	b
A	0	98.74 ± 0.13^a	-1.20 ± 0.05^b	6.62 ± 0.07^c
	3	$96.96\pm0.25^{b-x}$	$-1.42\pm0.03^{d-x}$	$7.21\pm0.03^{b-x}$
	7	96.52 ± 0.24^c	$-1.34\pm0.02^{c-x}$	$7.33\pm0.02^{ab-x}$
	12	$96.46\pm0.06^{c-x}$	$-1.30\pm0.07^{c-x}$	$7.43\pm0.27^{ab-x}$
	15	$96.12\pm0.07^{d-x}$	$-1.10\pm0.02^{a-x}$	$7.55\pm0.03^{a-x}$
B	0	98.74 ± 0.13^a	-1.20 ± 0.05^b	6.62 ± 0.07^e
	3	$96.36\pm0.27^{b-y}$	$-1.25\pm0.03^{b-y}$	$7.77\pm0.14^{d-y}$
	7	96.49 ± 0.19^b	$-1.23\pm0.04^{b-y}$	$9.51\pm0.03^{c-y}$
	12	$95.88\pm0.12^{c-y}$	$-0.58\pm0.02^{a-y}$	$13.21\pm0.03^{b-y}$
	15	$95.10\pm0.02^{d-y}$	$-0.56\pm0.02^{a-y}$	$13.65\pm0.08^{a-y}$

a–d Means in each column period in same group with different letters were significantly affected by storage (p < 0.05); x-y Means in each column in different between Kaymak groups were significantly at similar period (p < 0.05); A: 4ºC±2, B: 25ºC±2

Table 5. Result of Organoleptic Analysis of Kaymak Samples during Storage (n:5)

Groups	Days	Appearance	Color	Aroma	General
A	0	8.50 ± 0.55^a	8.83 ± 0.41^a	8.67 ± 0.52^a	8.67 ± 0.52^a
	3	$8.50\pm0.55^{a-x}$	$8.67\pm0.52^{a-x}$	$8.50\pm0.55^{a-x}$	$8.50\pm0.55^{a-x}$
	7	$7.83\pm0.41^{b-x}$	$7.83\pm0.41^{b-x}$	6.83 ± 0.41^b	$7.67\pm0.52^{b-x}$
	12	$7.33\pm0.52^{b-x}$	$6.83\pm0.75^{c-x}$	$6.67\pm0.52^{b-x}$	$6.83\pm0.41^{c-x}$
	15	$5.67\pm0.52^{c-x}$	$4.83\pm0.41^{d-x}$	$4.33\pm0.52^{c-x}$	$4.67\pm0.52^{d-x}$
B	0	8.50 ± 0.55^a	8.83 ± 0.41^a	8.67 ± 0.52^a	8.67 ± 0.52^a
	3	$6.67\pm0.52^{b-y}$	$6.67\pm0.52^{b-y}$	$6.50\pm0.55^{b-y}$	$6.83\pm0.41^{b-y}$
	7	$6.17\pm0.41^{b-y}$	$6.33\pm0.52^{b-y}$	6.17 ± 0.75^b	$6.33\pm0.52^{b-y}$
	12	$4.67\pm0.52^{c-y}$	$4.67\pm0.52^{c-y}$	$3.17\pm0.41^{c-y}$	$3.50\pm0.55^{c-y}$
	15	$3.33\pm0.52^{d-y}$	$2.67\pm0.52^{d-y}$	$1.17\pm0.41^{d-y}$	$1.17\pm0.41^{d-y}$

a–d Means in each column period in same group with different letters were significantly affected by storage (p < 0.05); x-y Means in each column in different between Kaymak groups were significantly at similar period (p < 0.05); A: 4ºC±2, B: 25ºC±2

When the organoleptic properties of kaymak samples were examined in both groups, differences depending on time and between groups were found (Table 5, p<0.05). Additionally, while the general scores of the products were acceptable until the 15th day, they were found unacceptable after the 7th day in group B. Images of kaymak samples are shown at during the experimental period days (Picture 1).

A: 4ºC±2, B: 25ºC±2
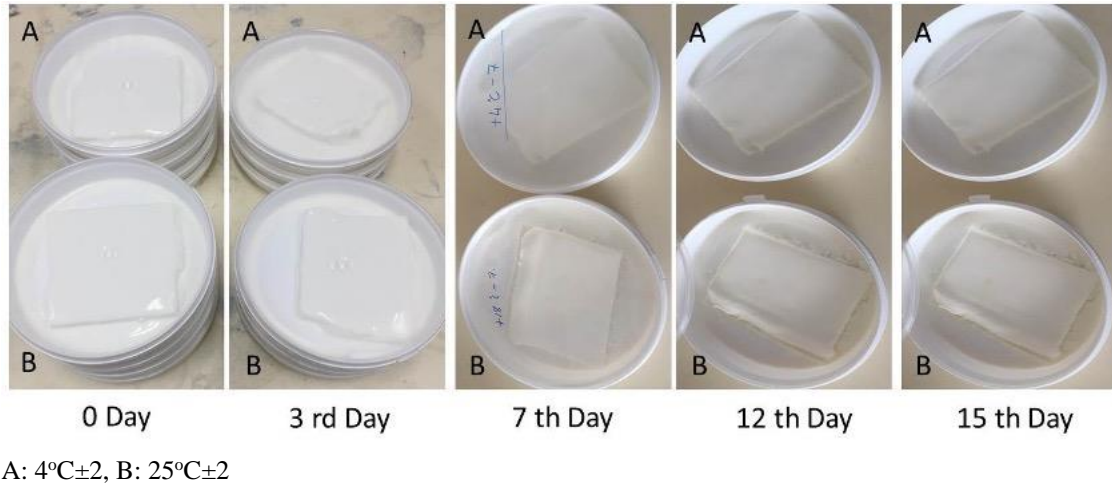
Picture 1. Kaymak samples at during the experimental period

4. Conclusions

The study examined the quality properties of kaymaks stored at different temperatures. As a result, it is seen that high temperature can have negative effects when storing kaymaks with a high oil ratio. But in a production to be carried out under appropriate and hygienic conditions, it was found that the products could have a 7-day shelf life when the is storage made at 25ºC±2, and 15-day shelf life at 4ºC±2 without affecting the consumer preferences and until the days specified (7-15). Therefore, the production and storage conditions starting from the quality of the raw material must be ensured optimally in kaymak, which is an easily perishable product. In order to extend the shelf life of cream, studies should continue with different natural antioxidant and antimicrobial compounds. In addition, the use of quality raw materials should be the first priority in obtaining quality products.

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POTENTIAL OF LENTIL POLYPHENOLS FOR ANTIOXIDANT, ANTIBACTERIAL, AND ANTIFUNGAL PROPERTIES (CORAL VARIETY)

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ABSTRACT

The objective of this study is to evaluate the content of total polyphenols, flavonoids and anthocyanins, to estimate the antifungal, antibacterial and antioxidant activity of the polyphenols extracted from the whole grains of dry *Coral* lentil, and to highlight the influence of cooking in water on the quantity and quality of phenolic compounds. This variety seems to be rich in phenolic compounds with a positive impact of cooking in water on the total polyphenol content. Concerning flavonoids and anthocyanins, cooking seems to have exerted a negative effect. An increase in antioxidant activity after cooking the grains was recorded. The results of the antibacterial activity showed that the most sensitive strains were the Gram-positive strains. The extract that showed maximum inhibition was raw coral.

For antifungal activity, *Alternaria* strains seem to be the most sensitive. Phenolic extracts from raw grains were found to be very active; at the concentration of 2mg/ml and phenolic extracts from cooked grains were found to be very active; at the concentration of 1mg/ml. The *Penicillium* sp strain has a random growth which prevented us from calculating their inhibition rates. The highest value of antifungal index 100 is marked for the phenolic extract of cooked coral lentil followed by raw coral lentil. The phenolic extract of cooked Coral lentil showed fungistatic activities on both strains at 1mg/ml and fungicidal activity on *Penicillium,*sp at 2mg/ml. The phenolic extract of raw Coral lentil showed both fungicidal and fungistatic activity on *Alternaria,*sp and fungistatic activity on *Penicillium,*sp at 2mg/ml.

1. Introduction

A consensus has recently begun to form on the importance of pulses from an agronomic, nutritional, economic, and territorial perspective. Actions to increase knowledge of the importance of pulses in family nutrition are critically needed given the shift towards more diversified and high-quality diets. 2016 has been designated as the International Year of Pulses by the United Nations, offering a rare chance to recognize the key contributions made by this plant family and how to strengthen them (Sieglinde *and al.,* 2018).

Legumes are regarded as a good source of polyphenols, however the amounts vary greatly between genera and species (Singh *and al.,* 2017).

Several research have characterized the phenolic makeup of Lens culinaris seeds, demonstrating the distinct differences between the compounds that make up the coat and the cotyledons. The lentil seed coat is extremely rich in catechins, procyanidin dimers and trimers,

and, in smaller concentrations, quercetin, myricetin, lutein, and apigenin glycosides, according to (Dueñas *and al.,* 2002).

The cotyledon mostly comprises low quantities of hydroxybenzoic and hydroxycinnamic acids. The cotyledon had two trans-p-coumaric acid esters, p-coumaroylmalic acid, and p-coumaroylglycolic acid, whereas the seed coat included the stilbene trans-resveratrol-5-glucoside (Djabali *and al.*, 2021).

Lentils had not previously been linked to these compounds. Although cooking is required in order to ingest dried lentils, little research has been done on how cooking affects phenolic component quantity and profile as well as antioxidant, antibacterial, and antifungal activities. It is in this context that the main objectives of this study are set.

2. Materials and methods
2.1. Plant material

The dried lentils (Lens culinaris, Variété Corail) are locally produced and are characterised by an orange colour (Figure 1). They were purchased from a point of sale of dried vegetables for human consumption in the wilaya of Jijel (eastern Algeria).

Figure 1. Photograph of the lens variety studied.

2.2. Method
2.2.1. Preparation of the lens samples

Two batches of lentils were separated. The first batch consisted of 100g of dry (uncooked) lentils ground to powder (< 250 μm).

The second batch consisted of 100g of lentils cooked by boiling in a stainless steel pot with 600 ml of distilled water. The cooking was done covered to reduce the surface area in contact with the open air.   The factors used in the cooking process (temperature and duration)

were chosen with reference to preliminary analyses. The cooked lentils were drained, freeze-dried and ground to powder (< 250 μm).

The resulting powders from both batches were stored in hermetically sealed glass vials and deposited in the dark to avoid any deterioration of the samples.

2.2.2. Extraction and determination of polyphenolic compounds
2.2.2.1. Extraction

The extraction of total polyphenols from raw and cooked samples was carried out according to the protocol proposed by Mujica *and al.* (2009) , 1g of the powder from each sample was solubilised in 25ml of methanol acidified with 0.1% 2N HCl. The mixture was left for 2h at room temperature and then centrifuged at 1800g for 15min. The residue is re-extracted with 25ml of methanol and centrifuged again. At the end, the supernatants are combined and the dry extract is recovered after dry evaporation (variable time/45°C).

The extraction yield is calculated by the following formula (Falleh *and al.,* 2008),

$$R(\%) = (M \text{ extract}/ M \text{ sample}) \times 100 \textbf{ (1)}$$

2.2.2.2. Determination of total polyphenols

Total polyphenols were determined colorimetrically using the Folin-Ciocalteu method according to Djabali *and al.* (2020). The concentration of total polyphenols is calculated from the regression equation of the calibration curve established with gallic acid based on previous tests, and is expressed in µg gallic acid equivalent per gram of extract (µg GAE/g extract).

2.2.2.3. Determination of flavonoids

The aluminium trichloride (ALCL3) method was used for the determination of flavonoid content (Bakchiche and Gherib, 2014). 1.5ml of phenolic extract (1mg /ml) was added to an equal volume of ALCL3 solution (2%). The mixture was shaken vigorously and the absorbance was read at 430 nm after 30 minutes of incubation at room temperature. The flavonoid content was expressed as mg

quercetin equivalent per g sample (Huang *and al.,* 2004).

2.2.2.4. Determination of anthocyanins

The anthocyanin content was estimated by spectrophotometer using the differential pH method, using two buffer solutions, potassium chloride (pH 1.0; 0.2 M) and sodium acetate (pH 4.5; 0.4 M) (Lee *and al.,* 2005).

The absorbance of the extract was measured at 510 and 710 nm and then deduced by equation,

A= ([A510-A 710) pH 1,0-- [A510-A 710) pH 4,5)

$$(2)$$

The anthocyanin content was determined according to the equation,

C = A. MW. FD. 4000 / E   **(3)**

C, anthocyanin content (g ECy-3-Glu/g powder) ;

A, absorbance

MW, molecular weight of cyanidin-3-glucose (449.2g/mol);

FD, dilution factor ;

E, Molar extinction coefficient (26900) of cyanidin-3-glucose.

2.2.3. Evaluation of total antioxidant capacity

The total antioxidant capacity of the phenolic extracts was evaluated by the phosphomolybdenum method (Prieto *and al.,* 1999). A 0.3 ml volume of each phenolic extract (1000 ppm) was mixed with 3 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes are incubated at 95°C for 90 minutes. After cooling, the absorbance of the solutions is measured at 695 nm against the blank. The total antioxidant capacity is expressed as milligram ascorbic acid equivalent per gram of dry matter (mg EAA/ g DM).

2.2.4. Determination of antimicrobial activity

The antibacterial activity was evaluated on four bacterial strains, Escherichia coli, Pseudomonas aerogenosa ATCC27853, Staphylococcus aureus ATCC29213 and Lactobacillus sp.

These strains were provided by the medical analysis laboratory BAKIEWA wilaya of Jijel.

The antifungal activity was tested on two fungal strains, *Penicillium sp* and *Alternaria sp.*The selected strains were chosen either for their capacity to contaminate foodstuffs or for their pathogenicity. These strains were provided by the microbiology laboratory of the University of Jijel.

2.2.4.1 Testing for antibacterial activity

The antibacterial activity of the extracts was determined by the agar diffusion method (Choi *and al.,* 2009). The bacteria to be tested were plated on petri dishes containing nutrient agar to obtain a young culture of the bacteria and isolated colonies. From these plates and with the help of a platinum loop, a few well isolated and perfectly identical colonies are taken and placed in 5ml of sterile physiological water. The bacterial suspension is well homogenised, and the optical density read at 625 nm is justified as (0.08 - 0.10). This density measured at 625 nm is assumed to be equivalent to 108 CFU/ml (Mohammedi, 2006). The inoculum is adjusted either by adding culture if it is too low or sterile physiological water if it is too high. Inoculation should take place within 15 minutes of inoculum preparation.

2.2.4.1.1. Preparation of phenolic extract concentrations

Dimethylsulphoxid (DMSO) is the preferred solvent for recovery of phenolic extracts recommended by most authors. It has been shown to have no potent inhibitory power (Toty *and al.,* 2013). Based on preliminary tests, one gram of solution with concentration ranging from 0.25mg /ml to 2mg/ml was prepared in DMSO. The discs are made from Wattman paper with a diameter of 6mm ($0.28cm^2$ surface area) by die cutting. Then, these discs are put in a test tube, autoclaved, and stored at room temperature (the test tube is hermetically sealed).

20 ml of the supercooled nutrient agar is poured into Petri dishes. After solidification of the culture medium, 100µl of the bacterial suspension to be tested is spread on the surface. The discs impregnated with the different

extracts, are then gently placed on the surface of the agar. The Petri dishes are first left for 1 h at room temperature for pre-diffusion of the substances, before being incubated at 37°C in the oven for 24 h (Mohammedi, 2006).

On removal from the oven, the absence of microbial growth is indicated by a translucent halo around the disc, identical to sterile agar, whose diameter is measured; (including the 6mm disc diameter). Antibacterial activity is determined by measuring the diameter of the inhibition zone around each disc.

2.2.4.1.2. Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is the lowest concentration for which there is no growth visible to the naked eye after an incubation time of 18 to 24 h. It was determined by observing the growth-induced cloudiness of the test germs in each tube (Toty *and al.,* 2013).

2.2.4.1.3. Determination of bacteriostatic and bactericidal activity

A sample from the zone of inhibition is transferred to a tube containing heart-brain broth which is then incubated at 37 °C for 18 hours. The tubes are examined with the naked eye, a cloudy medium indicates bacteriostatic activity; while a clear medium indicates bactericidal activity of the extract (Laouer *and al.,* 2003).

2.2.4.2 Testing for antifungal activity

2.2.4.2.1. Determination of the inhibition rate

Phenolic extracts solubilised in DMSO were successively diluted to 0.25-0.5-1-2 mg/ml. These concentrations were chosen on the basis of preliminary tests. 1 ml of each phenolic extract, of each concentration, is added to tubes containing 19 ml of sterile potato dextrose agar (PDA) medium, still liquid. The mixture is homogenised and brought to 45°C (Subrahmanyam *and al.,* 2001). It is then immediately poured into 90 mm petri dishes (20ml/plate) (Satish *and al.,* 2010). After agar solidification, the petri dishes are divided into two parts (corresponding to the number of strains to be tested) and inoculated with a mycelial disc, 6 mm in diameter taken from the young culture of the fungus. The PDA without

extract served as a control for each strain (Khallil, 2009).

The final phenolic extract (CF) concentrations used were calculated from the following equation (Mohammedi, 2006).

$$CF = Ci/20 \qquad (4)$$

With,

CF, final concentration of the phenolic extract in lml of PDA;

Ci, initial concentration of phenolic extract solubilised in DMSO.

The strain was incubated for 7 days at 30°C (Mohammedi, 2006). The percentage inhibition of mycelial growth, compared to the control, was calculated by the following formula ,

$$PI(\%) = (A\text{-}B)/A \times l00 \qquad (5)$$

Where,

PI(%), Inhibition rate expressed as a percentage;

A, Diameter of colonies in the "positive control" dishes;

B, Diameter of colonies in the boxes containing the grain extract (Bajpai *and al.,* 2010).

2.2.4.2.2. Determination of the antifungal index

The concentration that inhibits 100% mycelial growth is expressed as the antifungal index (AI l00). The AI l00 values were calculated graphically, where the abscissa is represented by the concentration of the phenolic extract and the ordinate by the percentage of inhibition of mould growth (Chang *and al.,* 2008).

2.2.4.2.3. Liquid dilution method

This technique consists of two steps, the first to determine the minimum inhibitory concentrations (MICs) and the second to determine the fungicidal (FC) and fungistatic (FCS) concentrations.

2.2.4.2.4. Determination of minimum inhibitory concentrations

After sporulation of the selected fungal strain, the spores of the young culture are recovered by adding 10 ml of sterile distilled water under agitation (Solis-Pereira *and al.,* 1993). Afterwards, the absorbance of the fungal suspension is evaluated at 625 nm; in order to

standardise the spore suspension at 106 spores/ml (Hossain *and al.,* 2008). It is estimated that an absorbance between 0.08 and 0.1 corresponds to a concentration of l06 spores/ml (Braga *and al.,* 2007). Phenolic extracts from raw and cooked lentils, solubilised in DMSO, were added to potato dextrose broth (PDB) at a rate of lml in 9 ml. The PDB is then diluted successively beforehand to give the dilutions 0.25-0.5-1-2 mg/ml. These concentrations were chosen after preliminary tests.

A 10 µl spore suspension of the fungal strain to be tested was inoculated into test tubes containing PDB medium at different concentrations; these tubes are incubated for 7 days at 30°C. In parallel, one tube containing PDB medium was inoculated only with the fungal spore suspension, to serve as a control. The minimum concentrations for which no obvious growth was observed are defined as minimum inhibitory concentrations (Bajpai *and al.,* 2008).

2.2.4.2.5. Determination of fungicide and fungistatic concentrations

For the tubes in which no growth is observed, the experiment is continued in petri dishes. Each dish containing 20ml of sterile

PDA is inoculated with 1 µl of each tube showing complete inhibition of fungal growth. Growth is monitored for 1 to 4 days at 30°C. When there is no growth resumption; the concentrations are called fungicidal (CF) (Zarrin *and al.,* 2010) and the concentrations for which growth occurs are called fungistatic (CFS) (Bajpai *and al.,* 2008).

2.5. Statistical processing

For each parameter, the means plus or minus the standard deviation of three trials as well as the graphical representations were carried out by Excel 2007. The results obtained were processed by analysis of variance (ANOVA) using the IBMSPSS2016 software at a significance level of 0.05.

3.Results and discussions
3.1. Contents of polyphenolic compounds

The contents of polyphenolic compounds in raw and cooked lentils are recorded in table 1.

Table 1. Polyphenolic compound contents of raw and cooked lentils

Polyphenolc compound	Raw lentils	Cooked lentils
total polyphenol (mg GAE/g)	2.53±0.2[a]	2.62±0.19[a]
Flavonoids (mg QAE/g)	1.40±0.024 [a]	0,13± 0.01 [b]
Anthocyans (g ECy-3-Glu/g)	4.08±0.11[a]	2.35±0.04[b]

Online, the same letter means no significant difference (p≤0 .05).

3.1.1. Total polyphenol content

It should be noted that cooking did not have a significant impact on the total polyphenol content obtained, despite a slight increase compared to the raw state. Referring to the bibliography, variable polyphenol contents in raw lentils have been reported. The values recorded in the present study are significantly different from those cited by Ladjal Ettoumi and Chibane (2015) (6.21 µg EAG mg -1 DM), Zhang and al. (2015)(5.04 - 7.02 µg GAE/mg DM for red lentils and between 4, 56 and 8.34

µg GAE mg-1 DM for green lentils) and those reported by Djabali *and al.* (2020) (95.51±4.01 and 172.36 ± 2.78 µg EP mg $^{-1}$DM the *Ibla* and *Metropole* lentil varieties respectively.

The higher amounts of polyphenols recorded after cooking may be due to a facilitated solubilisation and extraction of these compound after tissue embrittlement by the thrmic treatment. Dewanto *and al.* (2002) attribute this increase to the facilitated release of phenolic compounds previously bound to cellular constituents during cooking.

In addition to cooking, there are other factors that can influence the total polyphnol content. Zielinski (2002) explains that variation in phenolic content is attributed to many factors including genotype, agronomic practices, maturity level at harvest, storage locations and post-harvest climatic and geographical conditions. Similarly according to Hegedűsová (2015), the difference in total polyphenol content could be due to the ecotype, the geographical region where the plant grows and even the method of extraction of these compounds.

3.1.2. Flavonoid content

Raw lentils recorded a mean flavonoid content of 1.40±0.024 mg QAE/g. A significant decrease in the amount of flavonoids is marked after cooking. Indeed, referring to the literature, lentils are not only an excellent source of macronutrients, but also contain phytochemical compounds, which can be classified into phenolic acids, flavanols, flavonols, soyaponins, phytic acid and condensed tannins (Yanping *and al.,* 2012). Profound changes in phenolic equipment also occur when plant organs are subjected to technological processes to transform them (bleaching, cooking, etc.) (Macheix *and al.,* 2005).

Kebe (2014) explains these losses by a migration mechanism in the cooking water, mainly for cafeoylquinic acid and small molecules of flavan-3-ols.

3.1.3. Anthocyanin content

Cooked lentils had a significantly lower average anthocyanin content than raw lentils. Richardson and Finley (1985) report that temperatures above 100°C can cause the degradation of most of the anthocyanins.

The values obtained in the present study are different from those reported by Oomah *and al.* (2011) (0.20 mg ECy-3-Glu/g for red lentils). Through these results, it appears that the anthocyanin content varies significantly between varieties. Horbowics *and al.* (2008) add as other factors the extraction solvent, the extraction method, the standard used, the temperature and light, the cultivation methods (grafting, fertilisation,...) or more broadly the production methods (open field, soilless,...).

3.2. Total antioxidant activity

The total antioxidant activity values recorded for raw and cooked lentils are significantly different. They are equivalent to 9±0.24 mg AAE/ g DM and 11.67±0.088 mg AAE/ g DM respectively. Cooking positively affected the antioxidant activity.

Our results are in agreement with those of Gharachorloo *and al.* (2012) who showed a significant increase in antioxidant activity of lentils after cooking.

Throughout the literature search, very large differences are noted regarding this correlation.

Some studies showed a good correlation between IC_{50} and polyphenol and flavonoid content, while other studies did not (Athamena *and al.,* 2010; Mariod *and al.,* 2010).

On the other hand, it is well established that antioxidant activity is positively correlated with the structure of polyphenols. Generally, polyphenols with a high number of hydroxyl groups present the highest antioxidant activity (Heim *and al.,* 2002) due to their power to donate more atoms to stabilize free radicals (Torres de pinedo *and al.,* 2007). Thus, the antioxidant effect is not only dose-dependent but also structure-dependent (Rodriguez-Bernaldo *and al.,* 2009).

3.3. Antimicrobial activity
3.3.1. Antibacterial activity

The results of the antibacterial activity are shown in Table 2.

From these results, it appears that the phenolic extracts of raw and cooked lentils have a good antibacterial activity against the four tested strains. On the other hand, whatever the bacterial strain considered, phenolic extracts are active when concentrated.

Several in vitro and in vivo studies have been focused on the evaluation of the antimicrobial properties of phenolic extracts. At present, this effect is certain and has been demonstrated by numerous experimental studies. Studies of the inhibitory power of

flavonoids on bacterial growth have shown that many flavone compounds (apigenin, kaempferol and others) have a significant effect on different gram-negatif (*Escherichia coli)* and gram-positif (*Staphylococcus aureus*) bacterial strains (Ulanowska *and al.,* 2007).

Table 2. Diameters of the inhibition zones (mm).

Concentration of the phenolic extract (mg/ml)		Bacterial strains			
		E. coli	*P. aeroginosa*	*Lactobacillus*	*S. aureus*
Lentils cooked	2	24 ± 0.2	26 ± 1	25 ± 0.25	31.2 ± 0.12
	1	12 ± 0.52	24.5± 0.5	15 ± 1.2	2 0 ± 0.1
	0.5	11.5± 1	15 ± 1.5	12 ± 0.33	18.5 ± 0.5
	0.255	10 ± 2.1	7 ± 0.1	11 ± 1.4	15 ± 1
Lentils raw	2	22.5±0.25	32.5 ± 2.5	40 ± 0.5	21 ± 0.2
	1	11 ± 0.3	25.5 ± 1.5	27 ± 0.1	16.5 ± 1.5
	0.5	10 ± 1.2	23 ± 1	19 ± 0.1	12 ± 0.5
	0.255	9 ± 3	6.5 ± 0.3	12 ± 0.3	8 ± 0.2

Polyphenols, especially flavonoids and tannins, are known to be toxic to microorganisms. The mechanism of toxicity may be related to the inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins (Cowan, 1999).

Table 3. Minimum inhibitory concentration

Strain bacterial	Extract phenolic	Concentration (mg/ml)					
		2	1	0.5	0.25	0.125	0.0625
E. coli	*Raw lentils*	-	-	-	CMI	+	+
	Cooked lentils	-	-	-	+	+	+
P. aeroginosa	*Raw lentils*	-	-	CMI	+	+	+
	Cooked lentils	-	-	CMI	+	+	+
L. aureus	*Raw lentils*	-	-	-	-	CMI	+
	Cooked lentils	-	-	-	CMI	+	+
S. aureus	*Raw lentils*	-	-	-	-	CMI	+
	Cooked lentils	-	-	-	CMI	+	+

(+), cloudy, (-) does not change.

3.3.1.1. Minimum inhibitory concentration

Table 3 shows the results of the MIC estimation. It can be seen that the phenolic extracts have a good susceptibility against all bacterial strains following the recorded MIC values (0.125- 0.5mg/ml)

From these results we can see that all strains were completely inhibited at 0.5mg/ml, however at 0.0625mg/ml all strains were resistant.

3.3.1.2. Determination of bacteriostatic and bactericidal activity

The results of the bacteriostatic and bactericidal effect are shown in Table 4.

These results show that the effect of the different phenolic extracts on the different strains is bacteriostatic. On the other hand, it is bactericidal for high concentrations which shows a dose-response antibacterial activity.

Table 4. Bacteriostatic and bactericidal concentrations

Strain bacterial	Extract phenolic	Concentration of phenolic extract (mg /ml)			
		2	1	0.5	0.25
E. coli	Raw lentils	-	+	+	+
	Cooked lentils	-	+	+	+
P. aeroginosa	Raw lentils	+	+	+	+
	Cooked lentils	+	+	+	+
Lactobacillus	Raw lentils	-	-	+	+
	Cooked lentils	-	+	+	+
S. aureus	Raw lentils	-	-	+	+
	Cooked lentils	-	+	+	+

(+), cloudy, (-) does not change.

3.3.2. Evaluation of the antifungal activity of polyphenolic extracts
3.3.2.1. Determination of the inhibition rate

The measurement of the inhibition rates (%) allowed us to classify the fungal strains according to their degree of sensitivity to each concentration tested in figures 02 and 03.
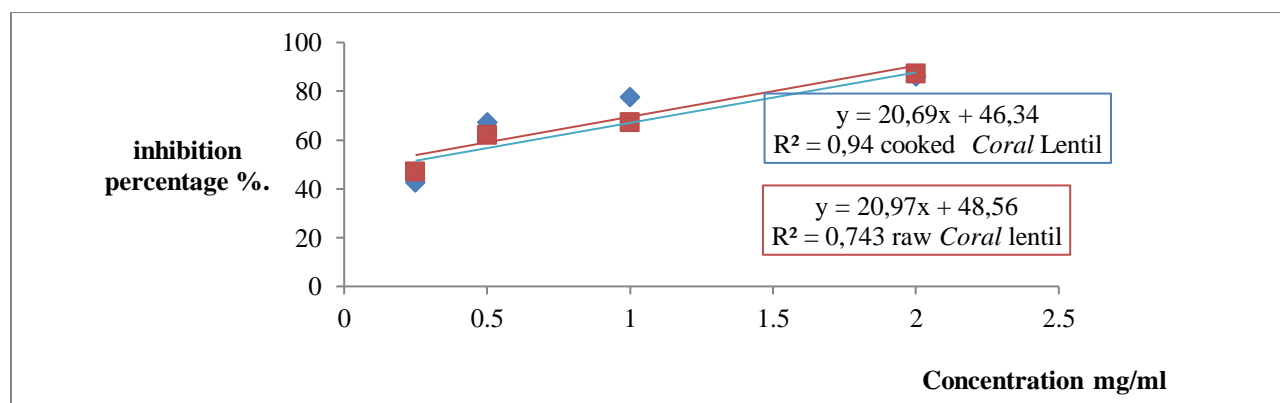

$$y = 20{,}69x + 46{,}34$$
$$R^2 = 0{,}94 \text{ cooked } \textit{Coral} \text{ Lentil}$$

$$y = 20{,}97x + 48{,}56$$
$$R^2 = 0{,}743 \text{ raw } \textit{Coral} \text{ lentil}$$

Figure 2. Inhibition rates of phenolic extracts of cooked and raw lentils against *Alternaria sp.*
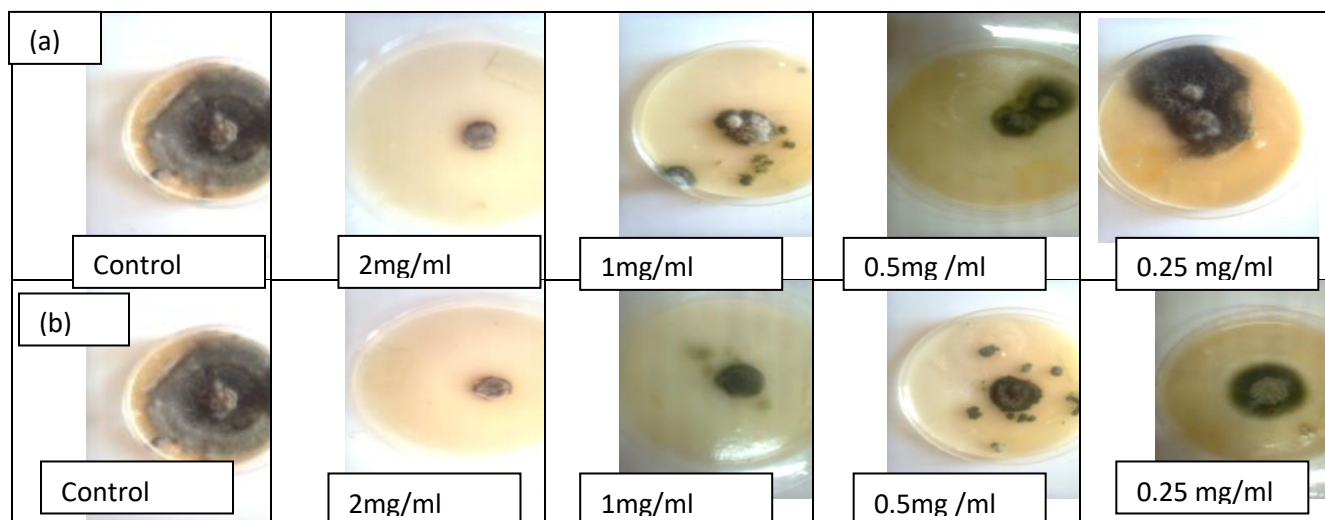
Figure 3. Inhibition effect of phenolic extracts against *Alternaria sp*. strain (a), Cooked lentils, (b), Raw lentils

The phenolic extract of raw coral lentil was found to be very active; at the concentration of 2mg/ml and at the concentration of 1mg/ml for the phenolic extract of cooked coral lentil. Both phenolic extracts were found to be active at concentrations of 1mg/ml and 0.5mg/ml (inhibition rate 50-74%).

The two phenolic extracts at a concentration of 0.25mg/ml are moderately active (inhibition rate 25- 49%).

The *Penicillium sp* strain has a random growth which prevented us from calculating their inhibition rates, the results obtained are shown in Figure 4.
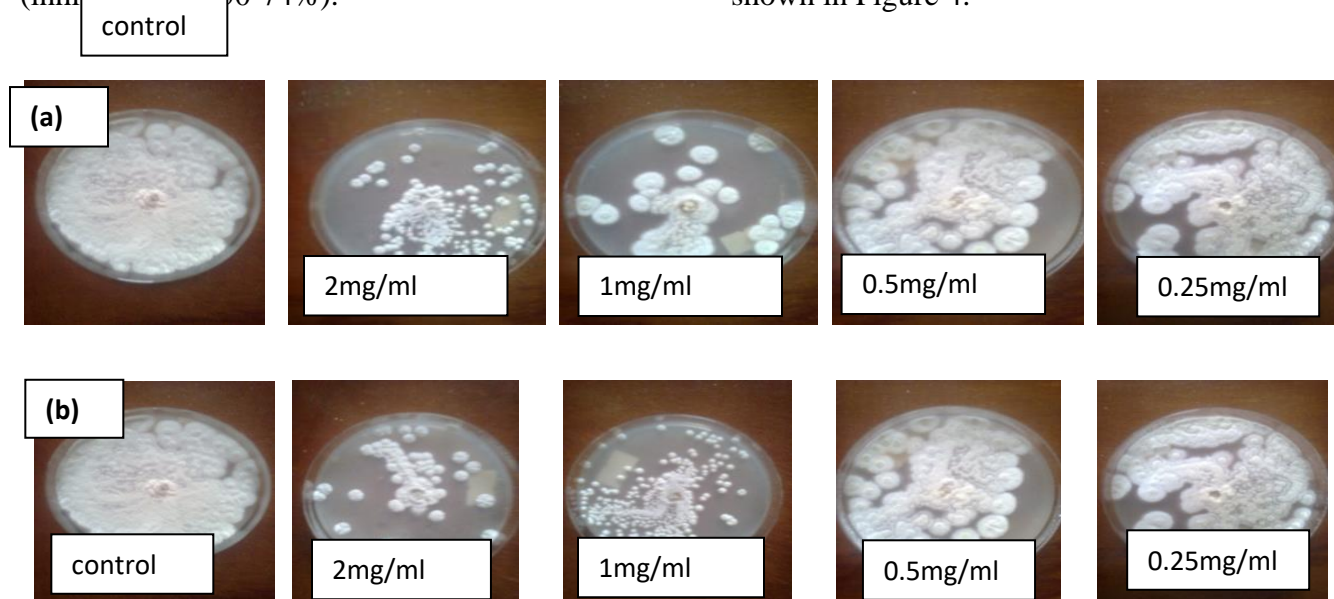

Figure 4. Inhibition effect of phenolic extracts on *Penicillium sp.* (a), Cooked *Coral* Lentil (b), Raw *Coral* Lentil.

3.3.2.2. Determination of the antifungal index (AI$_{100}$)

The IA$_{100}$ determined graphically are summarised in Table 5. The *Alternaria sp* strain tested does not have the same IA$_{100}$, it varies according to the variety and the concentration of the phenolic extract used. The highest IA$_{100}$

value is marked for the phenolic extract of cooked lentils. The IA_{100} of the strains belonging to the genus *Penicillium* was not determined because of the dispersion of the spores, therefore we could not measure their diameters.

Table 5. Antifungal indexes (IA100) of phenolic extracts of lentils

Fungal strains	A I_{100} (mg/ml)	
	Phenolic extract cooked *Coral* lentil	Phenolic extract Coral lentil Raw
Pénicillium sp	ND	ND
Alternaria sp	2.59	2.45

ND, not determined

3.3.2.3. *Minimum inhibitory concentrations (MIC)*

The minimum inhibitory concentrations (MICs) of the lentil phenolic extracts in liquid medium are presented in Table 6. The phenolic

extract of cooked lentils showed a MIC of 1mg/ml on both genera (*Alternaria sp* and *Penicillium*). The phenolic extract of raw lentils showed a MIC of 2mg/ml on the genus *Penicillum* sp and 0.5mg/ml on the genus *Alternaria sp.*

Table 6. Minimum inhibitory concentrations (MICs) of phenolic extracts from lentils

Strain	MIC of *Coral* lentil phenolic extracts (mg /ml)	
	cooked	raw
Pénicillum sp	1	2
Alternaria sp	1	0.5

3.3.2.4. *Fungistatic and fungicidal concentrations*

The fungistatic (CFS) and fungicidal (CF) activities are presented in Table 07.

Table 07. Fungistatic (CFS) and fungicidal (CF) activities in mg/ml of lentil phenolic extracts on both strains.

Strain	Phenolic extract cooked *Coral* lentil		Phenolic extract of raw *Coral* lentil	
	(CFS)	(CF)	(CFS)	(CF)
Alternaria ,sp	1	2	0.5	2
Penicillium,sp	1	ND	2	ND

ND, not determined

The phenolic extract of cooked lentils revealed fungistatic activities on both strains at 1mg/ml and fungicidal activity on *Penicillium sp.* at 2mg/ml.

Regarding the phenolic extract of raw lentils, subcultures revealed both fungicidal and fungistatic activity on *Alternaria sp.* (CFS=0.5mg/ml and CF=2 mg/ml) and fungistatic activity on *Penicillium sp.* at 2mg/ml.

Benmeddour *and al.* (2014) reported that the inhibitory action on moulds may be due to the formation of hydrogen bonds between the hydroxyl group of the phenolic compounds and the active sites of target enzymes. Cushnie and Lamb (2005) found that phenolic compounds showed inhibitory activity against *Penicillium digitatum* and *Penicillium italicum.*

According to Vicente *and al*. (2003), natural compounds are classified according to their mechanisms of action, covering inhibitors of cell wall component synthesis, lipid synthesis and protein synthesis.

4. Conclusions

Polyphenols are compounds of interest, the content of which is highly influenced by internal and external factors, including heat treatment. In this study, the applied heat treatment had a different influence on the content of polyphenols, flavonoids and anthocyanins. The antibacterial activity of the phenolic extract against four bacterial strains was demonstrated by the disk diffusion method. The zones of inhibition indicate that all strains are sensitive to phenolic extracts. The MICs obtained indicate that the phenolic extract showed good susceptibility against all bacterial strains. For antifungal activity, the measurement of inhibition rates (%) allowed us to classify the fungal strains according to their degree of sensitivity to each concentration tested. The MICs obtained vary according to the fungal genera. The subcultures carried out after obtaining the MICs made it possible to observe varied activities of phenolic extracts on the two strains.

It is desirable to continue this study with the quantitative and qualitative characterisation of the polyphenolic profile, the use of other extraction methods, the study of the effect of other cooking methods in order to draw more reliable conclusions.

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Acknowledgment

DEVELOPMENT AND QUALITY ASSESSMENT OF NEWLY DEVELOPED ONION SAUCE

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ABSTRACT

A sauce can be defined as a flavorful liquid, usually thickened, used to season, liquid, usually thickened, used to season, flavor and enhance other foods. Onion is widely cultivated, second only to tomato, and is a vegetable bulb crop known to most cultures and consumed worldwide. Even yet, long-term storage of onions in bulk can result in significant losses of up to 25%-30%. The present study aimed to develop and standardize the Onion sauce, assess its sensory, nutrition, texture, physicochemical and microbial analysis for a period of 60 days. Two types of Onion sauce were prepared with the variations in the ingredients. The sample A had the highest overall acceptability score (8.10) in sensory attributes. The moisture value of newly developed onion sauce was found to be 80.28g. The onion sauce contains low amount of fat 0.14g. The product contains very less content of sodium content (245.04g). The viscosity of onion sauce was identified to be a type of non-newtonian fluid with pseudoplastic behaviour. Physicochemical analysis indicated that the TSS and the titratable acidity values (21.90 °Brix and 1.20%) of newly developed onion sauce (21.90 °Brix) was found to be acceptable and was within the FSSAI limits for sauce. The color of onion sauce was good and brighter in color. The microbial results shows that the product had very low microbial colonies, no pathogenic organisms and no yeast and mold count on the 0th day, 30th day and 60th day of storage.

1.Introduction

Sauces, dressings and mayonnaises are commonly used in the everyday life of many consumers. They are usually packed in easy to use, small, disposable containers of different shapes, made of light materials and thus could be regarded as convenience foods. Sauces improve the taste of food (Sikora et al., 2008). A sauce can be defined as a flavorful liquid, usually thickened, used to season, flavor and enhance other foods (The Culinary Institute of America,2006). Sauces are savoury concoctions and mostly are liquid, fluid enough or thick. The word derives from the Latin salsus, meaning "flavored with salt."(Constable and Daniels,1983). Sauce is a beloved complementary food and is one of the flavoring ingredients often used in various foods. The main functions of a sauce are to enrich food, improve its flavor and aroma, as well as the taste. Besides, adding moisture, texture and improving visual appeal of the food (Krystyjan et al., 2012).

Onion (Allium cepa L.) has been valued as a food and a medicinal plant since ancient times. It is widely cultivated, second only to tomato, and is a vegetable bulb crop known to most cultures and consumed worldwide (FAO, 2012). It is commonly known as "Queen of the kitchen"

due to its highly valued flavor, aroma, and unique taste. It has medicinal properties of its flavour compounds (Selvaraj,1976; Griffithsetal,2002). Onion is an extensively grown biennial bulb crop, with world production of 74,250,809 tonnes from an area of 4,364,000 hectares. China and India are the primary onion growing countries, followed by the USA, Egypt, Iran, Turkey, Pakistan, Brazil, the Russian Federation, and the Republic of Korea (FAO, 2012). Even yet, long-term storage of onions in bulk can result in significant losses of up to 25%-30%. As a result, onion processing maintains product stability while preserving its unique sensory character. Other products such as dehydrated goods, such as flakes, rings, granules, powder, and processed onions, such as frozen or canned onions, or onions in vinegar and brine, are becoming increasingly popular on the international market (Lawande, 2012). Onion is used throughout the year, for example in curries, in the form of spices, in salads, as a condiment, or cooked with other vegetables, such as boiled or baked. It is also used in different forms of processed food, e.g. pickles, powder, paste, and flakes, and it is known for its medicinal values (Pareek et al., 2018). Despite its primary usage as a food source, this plant has been shown to provide a wide range of health benefits.

Onion and its bioactive components have been shown to have antioxidant, antibacterial, anti-inflammatory, anticancer, cardiovascular protective, neuroprotective, and immunomodulatory activities in several studies (Ranjith and Kumar, 2022). Onions are considered a rich source of more than 25 different flavonols with quercetin and its derivatives being the most significant ones, primarily in the form of flavonols glucosides (Petropoulos et al., 2016). Therefore, this study focuses on developing a new product, onion sauce that ensures extended product shelf life and also provides a high level of convenience in preparation and use for the consumers.

2. Materials and methods
2.1. Materials

Major ingredients used in this preparation of Onion sauce were fully matured dry onions (*Allium. cepa L.*) and gooseberry (*Emblica officinalis Gaertn.*), whereas the minor ingredients were ginger (*Zingiber officinale*) and garlic (*Allium sativum L.*), dry red chili, clove, peppercorn, salt, sugar, apple cider vinegar and arrowroot powder. These ingredients were purchased from local market in Chennai.

2.2. Standardization and pre-preparation of ingredients

Two types of Onion sauce were prepared with the variations in the ingredients. Sample A was developed without tomatoes and sample B was developed with addition of small quantity of tomatoes (Table 1). Fresh onion bulbs were selected and the outer dry layers were peeled off manually by using a sterile knife. They were washed with plenty of chilled running potable water to remove surface contamination. Cleaned and fresh mature bulbs of onion were cut by a stainless-steel knife into rough pieces and light sautéed in pan and soaked in apple cider vinegar for 5 hours and kept in well covered container until use. Gooseberry, ginger and garlic were cleaned, washed, deskinned, weighed using weighing balance and chopped into fine pieces. The stems of the red chillies were removed and was soaked in hot water for 15 minutes. Spices such as clove and peppercorns were ground into fine powder. All the ingredients were then kept ready until processing.

Table 1. Standardization of Onion Sauce

Ingredients	Sample A	Sample B
Onion	100g	100g
Tomato	-	20g
Gooseberry	20g	20g
Dry red chilli	1.5g	1.5g
Ginger	2.5g	2.5g
Garlic	2.5g	2.5g
Grounded spices	5g	5g

Apple cider vinegar	20ml	20ml
Lemon juice	5ml	5ml
Arrowroot powder	10g	10g

2.2.1. Preparation of Onion Sauce

The sautéed, Onion, chopped gooseberry, ginger and garlic and, soaked red chillies were placed in a stainless-steel vessel with grounded spices such as clove and peppercorns were ground into fine powder and dry sautéed for 20 minutes without adding oil. The sautéed ingredients were cooled and placed in a food processor and crushed thoroughly to get a fine texture. The mixture was boiled for about 10 minutes and stirred frequently. Apple cider vinegar and lemon juice were added and mixed thoroughly. After the raw flavor disappeared arrowroot starch was added as a thickener, boiled for 5 minutes and mixed thoroughly to obtain sauce consistency. Sample A onion sauce was packed and sent to the laboratory for further analysis. In sample B, sautéed, onion, and chopped gooseberry, ginger, garlic and soaked red chillies and 20 g of blanched tomatoes along with grounded spices (clove and peppercorns) were dry sautéed. The mixture was grinded into fine texture in a food processor. After the sauce gets thoroughly cooked arrowroot starch was added, boiled for 5 minutes to obtain the final product. The finished products sample A and B was cooled and poured carefully into previously sterilized Aluminum laminated pouch and sealed using electronic sealing machine and stored in refrigerated ($6°C$). The shelf-life of onion sauce was analyzed at different time intervals (1st, 30th, 60th) days of storage.

2.3. Sensory Analysis of Onion Sauce

The sensory quality of the newly developed onion sauce was assessed by 50 panel of judges between the age group of 18-24 years. The panel members were invited to evaluate the overall quality of the two samples. They were briefed on the purpose of the study and they were instructed on the method of scoring. The sauce was evaluated using a nine-point score card. About 1 tablespoon of sauce was served and for palate cleanse, a cup of water was given. Same

panelists were asked to evaluate sample A and B on 9-point scale (1 = Dislike extremely to 9 = Like extremely). Descriptive qualities were attributed to each score as a guide for panel members. The onion sauce was evaluated for attributes like color, appearance, taste, aroma, flavor, texture, mouthfeel and overall acceptability.

2.4. Proximate analysis of Onion Sauce

The standardized sample A of Onion sauce was analyzed for proximate composition. Moisture content was estimated using standard method (AOAC, 2019). Energy value of onion sauce was calculated by the method given by FSSAI (2016). The macronutrients such as carbohydrate, protein and fat were estimated using standard methods given by (FSSAI,2016; FSSAI, 2015; AOAC, 2012). The total ash content was analyzed using the method given by AOAC. (1995). The sodium value was determined by using the standard method (AOAC, 2012).

2.5. Texture analysis

The texture parameters such as firmness, viscosity and consistency of the onion sauce was measured (Paula and Conti-Silva, 2014).

2.6. Physicochemical analysis

The physicochemical tests, such as pH, total soluble solids (TSS), % acidity, water activity and color for the onion sauce were analyzed.

2.6.1. pH

The pH of onion sauce was measured by a pH-meter, using standard method given by IS:3025 (Part11):1983.

2.6.2. Total soluble solid (TSS)

Total soluble solids is a measure of the density of all soluble solids. The TSS content was determined using a refractometer. The amount of soluble solids is the total of all dissolved solids. The total soluble solids was analyzed using standard method of IS 13815: 1993 / ISO 2173: 1978 Fruit and Vegetable Products. Determination of Soluble solid content – Refractometer method.

2.6.3. Titratable acidity

The percentage of acidity was estimated according to the method given by AOAC (2019). The total titratable acidity (TTA) was expressed as percentage.

2.6.4. Water activity

The water activity was analyzed and estimated using the method described by Pardo et al. (2004).

2.6.5. Color

The color was measured according to Krishnan and Prabhasankar. (2010) where, the values of surface color of sauce in terms of lightness (L*) and color (+a: red; -a: green; +b: yellow; -b: blue) were measured using Hunter Lab color measuring system (Color measuring Labscan XE system, USA). The test was done in triplicates and the average value was reported.

2.7. Shelf life

The microbiological tests performed were for enumeration of organisms, yeast and molds and pathogenic organisms such as Escherichia coli, following the methods described by Indian Standard (IS 5402 (Part 1):2021; IS 5403:1999 reaffirmed 2018). Microbial assay was carried out using pour plating method and Bacterial colonies were counted using a Plate count agar (PCA) colony counter. The Yeast and mold count was obtained by using standard method (IS 5403:1999 reaffirmed2018). The viable count was enumerated by using Yeast-Extract-Dextrose Chloramphenicol-Agar medium. The microbial analysis was conducted for 1st, 30th and 60th day of storage.

2.8. Packaging

The newly developed onion sauce was aseptically packed in double layered Aluminium laminated pouch immediately after the preparation of the sauce is done. The sauce was directly poured into the pouch and sealed using electronic sealing machine to reduce the contamination. The product was stored in low temperature at refrigerated storage at different intervals of time (1st, 30th, 60th).

3. Results and discussions
3.1. Sensory analysis

Table 2 and figure 1, presents the mean scores for the sensory attributes color, appearance, texture, aroma, taste and mouthfeel of onion sauce samples A and B. The mean scores of the sensory attributes color, appearance, texture, aroma, taste and mouthfeel of Sample A were found to be 8.32, 8.3, 8.28, 7.82, 7.9 and 7.96 respectively. The mean scores of the sensory attributes in sample B was found to be 8.28, 8.2, 8, 7.48, 7.56 and 7.7 respectively. The mean scores for all the attributes were higher in sample A when compared to sample B.

Table 2. Sensory Attributes of Onion Sauce

Attributes	Sample A (M. ± S.D)	Sample B (M. ± S.D)
Color	8.32±0.843704	8.28±0.70102
Appearance	8.3±0.839096	8.2±0.699854
Texture	8.28±0.881557	8±0.968904
Aroma	7.82±0.98333	7.48±0.952762
Taste	7.9±1.073807	7.56±1.033322
Mouthfeel	7.96±0.85619	7.7±1.035098
Overall acceptability	8.32±0.843704	8.28±0.70102

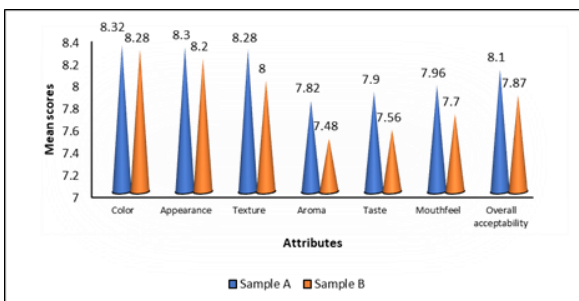M. = mean, S.D. = standard deviations

Figure 1. Mean sensory scores of Onion Sauce

However, statistical analysis showed no significant differences in the mean scores of sample A and B for all the attributes. The overall acceptability scores revealed that the sample A was more liked and preferred by the panelist as compared to the sample B. The higher overall acceptability could be due to the reason that tomatoes were not added in sample A, whereas in sample B tomatoes were added which masked the intense onion flavor in sauce.

The color of the sample A was brighter due to the addition of Kashmiri red chillies. Dried chilli is used primarily as a source of color, texture, and pungency, particularly in the production of crushed red pepper flakes, chilli powder, and chilli sauces. According to Fernandez et al. (2022), it was reported that the addition of cricket flour in barbeque sauces pointed to a more intense color when compared with sauce without cricket flour. While D'Heureux-Calix and Badrie (2004) showed that the color of sorrel (Hibiscus) sauces changed significantly (P < 0.05) as they became less dark and red during storage days. Similar results were also reported in another study conducted by Arefin et al. (2019). He identified that the color of Ready-to-use onion paste with preservatives (sodium benzoate, potassium metabisulphite and citric acid) changed to a slightly brown colour on 180 day of storage at ambient (AT) condition. The storage days did not show any effect on the color of the onion sauce after 60 days of storage. This may be due to the absence of chemical preservatives and addition of natural preservative such as apple cider vinegar and lemon juice which deepens the color of onion sauce.

The appearance of onion sauce was found to be good. The score of sample A was higher than sample B. There was a marginal increase in the score of sample A than the sample B. The most important attribute of any food is its color. The fine ground texture and the presence of bright red color of red chilli enhanced the appearance of onion sauce.

The highest mean score for taste was observed in sample A. The panelists liked the intense flavor of onions with the spiciness of red chilli. This suggests that the spiciness was more tolerable to consumers. Dried chilli is also valued for its contribution to flavour in chilli sauces and chilli powders. The flavouring principle is associated with volatile aromatic compounds and color (Peter,2001). The red chilli peppers are rich in antioxidant plant compounds that have been linked to various health benefits. According to Balaswamy et al. (2005), it was observed that the onion chilly chutney scored good in flavor and taste. It is recorded that the flavor of ready to use onion paste at fresh condition was ranked as good, which degraded to slightly off flavor from 5th and 20th days of storage at ambient and refrigerated condition respectively (Arefin et al.,2019).
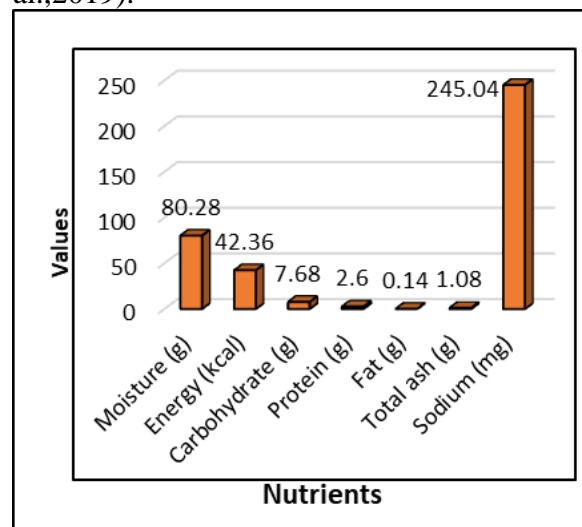
Consequently, the panelist rating for overall acceptability of sample A was higher than sample B. This was also in accordance with Arefin et al. (2019). When the onion was incorporated in hog plum sauce scored highest overall acceptability and was identified as best sample.

Durojaiye et al. (2003) and Gaffa et al. (2004) reported that storage at 4±10C refrigerated

temperature ensured maximum retention of chemical and sensory properties in comparison to storage at higher temperature. Similarly, the onion sauce was also preserved in refrigerated temperature 6°C which resulted in higher retention of chemical and sensory characteristics.

3.2. Proximate analysis

From the above table 3 and figure 2, it can be seen that the nutrient composition of newly developed onion sauce has an 80.28g of moisture. The energy value was found to be 42.36kcal. The Carbohydrate, protein and fat content was found to be 7.68g, 2.60g and 0.14. The total ash content was found to be 1.08g. The sodium content in 100g of onion sauce was 245.05mg.

Table 3. Nutrient composition of Onion sauce

Nutrients	Sample A
Moisture (g)	80.28
Energy (kcal)	42.36
Carbohydrate (g)	7.68
Protein (g)	2.60
Fat (g)	0.14
Total ash (g)	1.08
Sodium (mg)	245.04

Figure 2. Nutrient Composition of Onion Sauce

Moisture or water content is a measurement of the total water contained in a food product, usually expressed as a percentage by wet basis. Moisture is an important factor in food quality, preservation and resistance to deterioration. With regards to the moisture content, it was shown that 100g of onion sauce contains 80.28g moisture. The presence of onions and gooseberry in the product which contains 85% of moisture, contributed to the moisture content in sauce. The prominent water content and low calorie content of onion sauce makes it a healthy accompaniment.

According to Valladão et al. (2022) the moisture content of the red wine sauce and white wine sauce was found to be 83.72% and 85.36% respectively. Whereas, the moisture content of onion sauce was 80.28% and is found to be lower. The low moisture content in onion sauce resulted in prolonged shelf life and minimum microbial growth even at the 60th day of storage. Product having high moisture content has minimum shelf stability (Ayub et al., 2005).

The product had an energy value of 42.36 kcal/100 g. The energy content is very low in onion sauce. Low calorie content in onion sauce is due to the addition of vegetables such as onion, gooseberry and also spices that are added in sauce. Fruits and vegetables are indeed very low in calories, supplying only 9%. Most fruits and vegetables are very important in dietary planning because they provide the double benefit of being key components in the diet with a minimum level of calories.

According to Costa et al. (2017) the energy value of tucupi cream paste was found to be 153kcal, the energy values of commercial mustard pastes ranged between 44.66 to 66.7 kcal/100g and the commercial ketchups ranged from 112 to 138 kcal/100g. In another study Khedkar et al. (2019) reported energy amount of 441kcal in curry leaf chutney. In the present study, onion sauce contains significantly lower energy due to the use of only vegetable such as onion and gooseberry which are low in calories.

Macronutrients are the body's source of calories or energy to fuel life processes. With regards to the macronutrients in onion sauce, carbohydrate was found to be 7.68g, protein was found to be 2.60g and fat was found to be 0.14g. The carbohydrate value of newly developed onion sauce was identified to be low in content. According to IFCT(2017) the carbohydrate values of onion and gooseberry was revealed to be 9.56g and 4.39g respectively. The low carbohydrate values of onion and gooseberry resulted in low carbohydrate level. The addition of arrowroot and a negligible amount of sugar in sauce, explained the low carbohydrate level in sauce. With regards to the protein content, the addition of arrowroot starch significantly increases the amount of protein in onion sauce

2.60g. Apostol et al. (2020) reported that the addition of purslane leaves significantly increases the amount of protein in tomato sauce from 0.12 (%) to 1.83 (%). Whereas, in another study by Avinash and Madhav (2015), the protein content of tomato sauce enriched with bael was 0.13%. While comparing with these studies, the onion sauce contains significantly higher amount of protein which is (2.60%)/100g.

Sauce is generally considered as low fat product. However, onion sauce has very negligible amount of fat compared to tomato sauce which contains 0.62 ± 0.01g of fat (Akhtar et al., 2009). This could be due to the presence of onion and gooseberry in sauce which contains very low amounts of fat (0.08%) and (0.1%) respectively. Fruits contain less fat so their incorporation leads to decrease in fat content. However, the fat content of onion sauce is considered as low fat product which is less than 1.5g/100ml according to the FSSAI Regulations (2018).

The total ash content is the mineral content that are present in food product. Minerals play an important role in maintaining proper function and good health in the human body. The newly developed onion sauce contains 1.08g of ash content. The ash content of onion sauce was due to onions and gooseberry, which are rich in vitamins and minerals. The sodium content of onion sauce was 245.04mg.

The product has low calorie, low carbohydrate, and low fat. Hence, it could be a healthier choice for using onion sauce as an accompaniment or as bases in daily cooking and also it is made up of spices and condiments which are rich in antioxidants.

3.3. Texture analysis

The textural properties of the onion sauce are presented in Table 4 and figure 3. From the table 4 it is shown that firmness was found to be 321.10g, cohesiveness was found to be -167.17g and consistency of the sauce was found to be 374.63 g.sec respectively.

Table 4. Textural properties of Onion Sauce

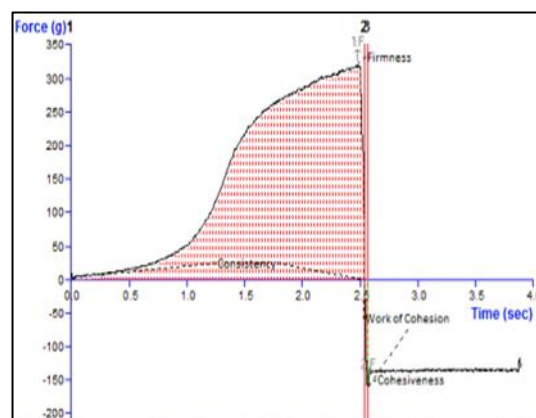| **Properties**	**Value**
Firmness	321.10g
Cohesiveness	-167.17 g
Consistency	374.63 g.sec

Figure 3. Texture analysis

3.3.1. Firmness

With regards to the firmness of onion sauce, the addition of arrowroot enhanced the texture and did not affect the firmness of sauce. The incorporation of gooseberry particles in protein matrix would have strengthened the binding during cooking. Fernandez et al. (2022) identified that, the increase in the addition of cricket flour concentration in the Barbecue sauce led to a higher firmness and adhesiveness due to the increased protein content of cricket flour.

3.3.2. Consistency

With regards to the consistency, the newly developed onion sauce had a good consistency and there was no change in consistency even after storage days. This could be due to the factor that arrowroot starch will gel when cooled and it is less likely that the cornstarch will break down when heated for long time. Arrowroot starch is popular for its high digestibility and medicinal properties. It possesses demulcent properties that soothes and protects irritated or inflamed internal tissues of the body and hence is given in bowel complaints (Mathew, 2007, Jyothi et al., 2009).

3.3.3. Viscosity

From the given figure 4, it can be seen that the viscosity of the onion sauce was found to be a type of non-Newtonian fluid with pseudoplastic characteristic due to the decrease in viscosity over shear rate time. The viscosity was thick and it was due to onion puree, sugar, gooseberry paste and also arrow root being used as thickener. It has been found that viscosity decreased with increase in shear force and rate. This revealed that onion sauce possesses a type of non-Newtonian liquid called pseudoplastic fluid where viscosity decreases as the shear rate increases. A non-Newtonian fluid is one whose properties are different from Newtonian fluids i.e., apparent viscosity changes with applied stress or forces (Barman et al., 2016). Similar results were reported by Costa et al. (2017) who detected the decrease in the steepness of the flow curves as the shear rate increases suggests the product behaved as a non-Newtonian fluid with pseudoplastic characteristic at the temperature range studied. This behavior is confirmed by the reduction in product viscosity as the shear rate increases (Schramm, 2000). In a study by Apostle et al. (2020) the same results were observed. The viscosity of purslane-rich sauces presents a type of non-newtonian liquid, which changes its viscosity under the action of shear force increase. It can be considered that the addition of purslane leaves leads to sauces with uniform and stable viscosity with specific appearance. According to Bortnowska et al. (2020), the steady rheological measurements showed that meat-based sauces behaved as non-Newtonian fluids and demonstrated pseudoplastic behavior. It was also explained that with increasing storage time the viscous nature of meat-based sauces was decreased, due to raise of fluidity. In the development of Kembayau fruit sauce the viscosity of the sauce decreased in long, but in small value.

This is due to low usage of xanthan gum in sauce. Syneresis that occurs in sauce will affect to the final viscosity of the sauce product (Khadijah and Hamdzah,1987).
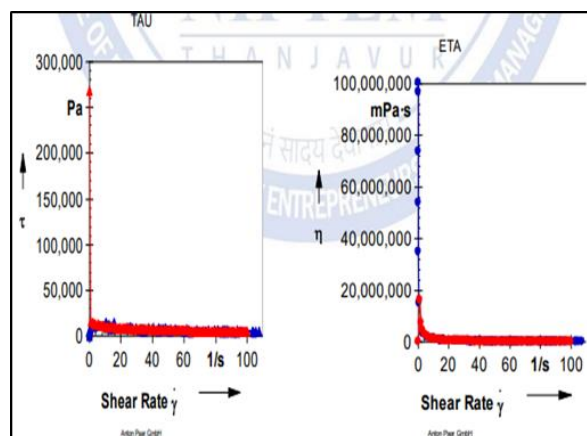
Figure 4. Viscosity (Results from NIFTEM, Thanjavur)

3.4. Physicochemical analysis

From table 5 and figure 5, it is shown that the newly developed onion sauce had a pH value of 3.68. The total soluble solid value of onion sauce was found to be 21.90°Brix, acidity was found to be 1.20% and water activity was found to be 0.96(aW) respectively.

Table 5. Physicochemical properties of Onion Sauce

Properties	Sample A
pH	3.68
Total Soluble Solid(°Brix)	21.90
Acidity (%)	1.20
Water activity (aW)	0.96

Figure 5. Physicochemical properties

3.4.1. pH

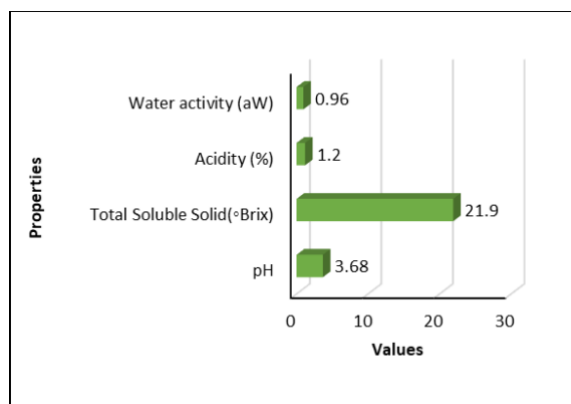Among the physicochemical properties analyzed, pH is very important because acidity influences the thermal processing conditions required for producing safe products. The pH value of onion sauce was found to be 3.68 and it is acidified foods.

According to FDA, Code of Federal Regulations. (1996) the acidified foods should have water activity greater than 0.85 and have a pH of 4.6 or below and these parameters must be maintained in all finished foods. Acidified foods are less likely to develop a quick microorganism growth, which means they might take longer to deteriorate. Although the sample was high in moisture content, which was reported in table 3 it also contained sugar, salt and acid. This reduces the water activity (aw) and inhibits the growth of disease causing microorganisms in the selected sample. To suit consumer acceptance, the pH was increased with the addition of apple cider vinegar which is natural preservative that works by raising the acidity of the food, which disables enzymes and kills bacteria that may cause spoilage. The obtained values were in accordance with a study conducted by (Rani and Banins. 1987), who found that the pH value for tomato ketchup ranged between 3.55 to 3.87. Whereas in another study, it was identified that the fresh onion paste pH value was 5.29 which was higher when compared to onion sauce developed in the present study.

3.4.2. Total soluble solids (TSS)

Results from table 5 showed that, the TSS content of newly developed onion sauce (21.90) was found to be acceptable and was within the FSSAI limits, where it stated that sauces/culinary pastes should not contain less than 15.0 percent of TSS. In comparison with jackfruit sauce which contains 34.5 to 41.8 °Brix (Ritthiruangdej et al., 2011), the TSS was found to be lower in onion sauce. While in another study it shows that the total soluble solid (TSS) of Kembayau fruit sauce was increased for 1°Brix during storage days. In general, total soluble solid of freshly prepared sauce will increase from 35 to 37°Brix. According to Aramouni et al. (2013) the absence of sugar in

the sugar free barbeque sauce resulted in a 65% reduction in solid content and also it is well known that the higher of the total solids the better will be the quality of the end product.

3.4.3. Titratable acidity

With regards to the titratable acidity, according to FSSAI standards for the sauces/culinary pastes should not have less than 1.2% of acidity and the onion sauce value was within the given limit (1.20%). According to Thakur et al. (2017), the titratable acidity of pomegranate chutney ranged from 1.11to1.98% which is more or less similar with the onion sauce acidity value. In comparison with acidity value of tomato sauce 1.54±0.03, onion sauce contains lower acidity value 1.20%. Scientific report stated that acidity value is influenced by sodium benzoate, vinegar, and citric acid (Rahman and Thajudin. 2015, Hussain et al., 2008)

3.4.4. Water activity

Water activity is usually controlled by the use of salt or sugar. The presence of salt and sugar concentration in the onion sauce may be sufficient to document control of water activity and commercial sterility. The water activity of onion sauce was found to be 0.96. According to FDA regulations (1984), most foods have a water activity above 0.95 and that will provide sufficient moisture to support the growth of bacteria, yeasts, and mold. Depending on various product characteristics this minimum level can be as high as 0.96 and the standard levels were maintained in onion sauce.

3.4.5. Color

From table 6, it can be seen that the color of onion sauce was found to be good and brighter in color. The l*a*b* values of onion sauce are 31.48, 20.21 and 33.09 respectively. The color of the onion sauce was observed to be intense red color. The addition of kashmiri red chillies in preparation of sauce lends a bright red color to sauce. Dried chillies contain capsaicin that has anti-inflammatory properties. Similarly in a study conducted by D'Heureux-Calix and Badrie. (2004), the color of the sorrel sauce

changed slightly from less dark to red due to the addition of sorrel(hibiscus) which is an excellent source of red color of food products (Esselen and Sammy, 1973). When apple cider vinegar and lemon juice was added, it deepens the color of the product.

Table 6. Color of the Onion Sauce

Sample	L*	a*	b*
Onion sauce	31.48	20.21	33.09

3.5. Shelf life

From table 7, we can see that the total plate count on first day was <1 cfu/g and on the 30th day it was found to be 6 cfu/g and after 60 days of storage it was found to be 12 cfu/g. There was marginal but insignificant increase in the number of colonies from 1st day to the 60th day of storage. There was no coliforms and pathogenic organisms found on the 1st, 30th and 60th day of storage. E. coli and pathogenic organisms were absent in the sample at the different days of storage. The yeast and mold count on the 1st, 30th and 60th day was <10 cfu/g. The table 7 indicates that there are very low microbial colonies, no E. coli and pathogenic organisms and also no yeast and mold. This shows that product had very low microbial colonies, no pathogenic organisms and no yeast and mold count on the 0th day, 30th day and 60th day of storage.

Microbiological contamination is a very important factor in determining the quality of food products. Microbiological tests were conducted for TPC (Total plate count), E.coli and yeast and mold count. These microorganisms are responsible for many food borne diseases. As per the FSSAI standards (2011) for thermally processed fruits and vegetables, the product should comply with TPC count of 1×10^2 and yeast and mould count of 50/g. There is no standard prescribed by the FSSAI for onion sauce. The quality of onion sauce was maintained due to the proper handling of product, storage condition and use of natural preservatives. The apple cider vinegar was added to increase the acidity and it is one of the best natural preservative. This inhibits the growth of spoilage and food microorganisms. Other ingredients such as salt and sugar could have also contributed to the preservative effect. According to FDA, change in the pH could transform a food into one which could support the growth of pathogens.

Onions were washed thoroughly using chilled water to reduce the risk of microbiological load. Park et al. (1998) reported that repeated washing of trimmed and cut pieces of onions by using chilled water resulted in a reduction of total microorganisms count. The product was processed and cooked in heat treatment namely boiling which kills or inactivates any bacteria active at the time, including E. coli and salmonella. The product was packed into double layered Aluminium laminated pouch in aseptic condition and kept in tight sterile air tight container at refrigerated temperature (6°C) during the study for extended shelf life.

This resulted in prolonged shelf-life and minimum microbial load in the product. Chilled foods stored at temperatures near 0 to 6°C have been reported to have longer shelf-life because of slower growth of psychrotrophs leading to delayed onset of spoilage (King and Cheethan, 2012). In fresh fruits and vegetables, low temperature does not necessarily stop enzymatic and non-enzymatic chemical reactions but instead slows their rates thereby leading to increased shelf-life in products (Kitinoja and Kader, 2002). Similar results were observed in a study conducted by Nkhata and Ayua (2018), the shelf-life of homemade tomato sauce was increased at low temperatures especially at 6°C. while in another study, Aramouni et al. (2012) identified that the TPC of all the treatments was below detectable levels (<10 CFU) in storage periods. This was due to the effect of pasteurization treatment. In onion sauce the yeast and mold count remained below the detectable levels during the storage days due to the storage of product in refrigerated temperature. It was also identified that sauces were preserved for longer period at refrigerated temperature than room temperature.

Table 7. Microbiological data of Onion Sauce during storage

Storage period	TPC (cfu/g)	Yeast and mold/g	Coliforms/g
0	<1	<10	ND
30	6	<10	ND
60	12	<10	ND

Preservatives are commonly used in almost all the processed food items in order to enhance the shelf life and maintain the product's quality. The shelf life of onion sauce was found to be shelf-stable due to the addition of natural preservative apple cider vinegar and dietary antioxidant lemon juice (ascorbic acid). It was found that the natural antioxidant ascorbic acid tends to delay, control, or inhibit oxidation and deterioration of food quality. The spices added in sauce (cinnamon and pepper) also could be a reason for prolonged shelf-life. According to Shahidi (2015), states that the natural antioxidant components of spices and herbs are great sources of antioxidants for food preservation.

The usage of apple cider vinegar as preservative on onion sauce shows effectiveness in terms of bacteria, yeast, and mold retardation. The apple cider vinegar was used due to its more conventional uses as a flavouring agent and as a food preservative to enhance the product shelf life. These results were in accordance with study conducted by Arefin et al. (2019), where it has been observed that, the bacterial load was lowest for onion paste treated with citric acid and potassium metabisulfite (KMS) stored at refrigerated temperature. In another study Balaswamy et al. (2005) reported that the total plate count (TPC) and yeast and mold count were within the permitted levels. It is also showed that there is a decreasing trend in the TPC and yeast and mold count during storage period due to low pH and presence of salt in onion-chilly chutney.

4. Conclusions

In the present century, people are living in a fast-paced environment. They are looking for quick, easy and convenient foods on-the-go, which can please their palate at the same time healthier. Onion is an important vegetable crop grown in India and forms a part of daily diet in almost all household facilities throughout the year. Due to over production in some seasons it leads to lot of wastage. This study has paved the way for commercial production of onion sauce by using appropriate processing and storage method to ensure longer shelf life and making it available in an easily accessible way for all the consumers. The production of newly developed onion sauce lends to retain the intense onion flavor and texture with a potential prolonged shelf life. The newly developed onion sauce may find its extensive use in the home as well as in catering industries and other establishments. It can be used as an accompaniment and dipping sauce, paste or base for preparation of curries and gravies, thickening agent for soups, gravies and also it enhances the nutritional value of the dish. The newly developed onion sauce, for its anticipated widespread use, may help to fill the needs of consumer's choice of convenient food product.

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EFFECT OF SUN DRYING ON PHYTOCHIMICAL QUALITY AND ANTIOXIDANT ACTIVITY OF FIVE FIG VARIETIES (*Ficus caricaL.*) FROM NORTH ALGERIA.

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ABSTRACT

The fig has been a typical fruit component of the health-promoting Mediterranean diet for a very long time. Due to its perishable and seasonal aspect, it must be kept in its dry form, which can be obtained by several drying processes, including sun-drying. It is known to provide many dietary elements and beneficial phenolic compounds that have good antioxidant properties. This study contributes to assess the impact of sun-drying on phytochemical quality and on the antioxidant activity of five fig varieties from Jijel region (North Algeria). The results show that drying has a positive effect on the content of various phytochemical compounds, in particular total polyphenols, which reached a value of 398.8 ± 2.39 mg AGE/100 g of dried fig, on the content of flavonoids (increase from 17.71 ± 0.55 to 22.96 ± 0.18 mg QE / 100 g of fig), and proanthocyanidines (from 0.65 ± 0.07 to 2.93 ± 0.17 mg CE/100 g of fig). On the other hand, a decrease in the content of anthocyanins (from 4.27 ± 0.13 to 1.59 ± 0.07 mg Q3GE/100 g of fig), and carotenoids (from 464.78 ± 1.74 to 140.96 ± 1.41 ug βCE/100 g of fig) is recorded. The *in vitro* evaluation of the antioxidant activity of extracts by DPPH free radical scavenging test, iron reduction, and H_2O_2 scavenging showed that dried figs have significant antioxidant activity dependent on polyphenols content.

1. Introduction

Fruits and vegetables containing high concentrations of bioactive compounds have attracted considerable interest over the past three decades, due to their potentially beneficial properties for health and their richness in antioxidant substances that play a major role in the prevention of various pathologies such as cancer, cardiovascular and neurodegenerative diseases that would be associated with oxidative stress (Bachir Bey *et al.,* 2017; Ercisli *et al.,* 2012).The latter is defined as a profound

imbalance in the equilibrium between pro-oxidants and antioxidants, which leads to irreversible cellular damage. The univalent reduction of oxygen results in the formation of reactive oxygen species (ROS) including free radicals (superoxide anion, hydroxyl radical), hydrogen peroxide, and singlet oxygen (Pincemail *et al.,* 1999; Xu *et al.,* 2017).

The ability of different fruits and vegetables to neutralize free radicals and restore oxidative balance *in vivo* is attributed to their richness in polyphenols, natural antioxidants with high antioxidant and cytoprotective potential (Akbari *et al.,* 2022). Among these fruits and vegetables, we stand out the fig. The fig is the fruit of the fig tree which belongs with the olive tree and the citrus fruits to the trilogy of the main fruit productions of Algeria. This importance is mainly linked to a multiplicity of uses and exchanges of genetic material, which led to its diversification and spread (Chouaki *et al*., 2006). It is considered as a "functional food", thanks to its richness in vitamins, essential minerals, dietary fibers, phenolic compounds, proteins and calories in large quantities (Zidi *et al*., 2020). However, they are seasonal and highly perishable due to their short shelf life of two days at room temperature (Sharifian *et al.,* 2012), and 7–10 days if stored at 0–2°C (Veberic *et al.,* 2008). Given the fact that they are highly perishable, which limits storage for long periods, and in order to increase potential markets, most of the production is intended for drying (Bouzo *et al.,* 2012). Once dried, figs can be stored for 6-8 months (Slatnar *et al.,* 2011).

Drying of fruits and vegetables is one of the oldest forms of food preservation. The major objective of drying agricultural products is to reduce the humidity to a level that allows safe storage for a long period of time. In Algeria, figs are traditionally dried by exposing them directly to the sun. This method of drying has advantages on the quality of these fruits. Drying leads to a reduction in weight and volume, thus minimizing the costs of packaging, storage and transport. Despite some drawbacks, sun drying is still used in several regions around the world. Solar energy is an important alternative source of energy and is preferred over other sources because it is inexhaustible, economical, non-polluting and renewable (Doymaz, 2005).

Studies dealing with fresh or dried figs in Algeria are few; especially since no study dealing with figs from Jijel region (North Algeria) has been undertaken. With this in mind, we are interested in studying the impact of sun drying on the phytochemical composition (content of polyphenols and various antioxidants) and in particular on the antioxidant activity of five varieties of figs (*Ficuscarica L.*) from Jijel region.

2. Materials and Methods
2.1. Plant material
In this study, five yellow varieties of fig were used in the fresh and dried states. The samples were collected in August 2020 from five different sites in Jijel city (North Algeria) namely: Chekfa, Ouled Askar, DjamaaBniHbibi, Milia and Mzayer.

Two separate batches were harvested from each variety. A batch of fresh harvested figs that were ripe, firm and healthy (not infected by insects) was intended for analysis in fresh state. The second batch intended for sun-drying, includes very ripe figs, with easily detachable peduncles.

For sun-drying process, fruits were uniformly arranged in sample trays in a single layer to be exposed to the sunlight, they were turned over each day. To avoid night humidity, the fruits were placed indoors during the. Sun-drying was completed within one week.

2.2. Extraction process
In the present study and after an optimization step carried out by several authors(Chan *et al.,* 2009;Uma *et al.,* 2010; Bey and Louaileche, 2015); acetone 70% was chosen for the extraction of phenolic compounds. For this, 6 grams of ground figs were mixed with 300 ml of acetone 70% (V/V). After 72 hours of stirring at ambient temperature, the mixture was filtered on filter paper and then centrifuged at 3000 rpm/20 min. The supernatant was recovered and then

stored at 4°C in dark and hermetically sealed bottles.

2.3. Quantification of antioxidants
2.3.1. Total phenolic contents

The total polyphenol assay was performed by colorimetry using the Folin–Ciocalteu reagent (Siham *et al.,* 2019). For the assay, a volume of 1 ml of Folin-Ciocalteu reagent (1/10) and a volume of 800 μl of sodium carbonate (7.5%) were added to 200 μl of extract. After 30 minutes of incubation in the dark, the absorbance was measured at 750 nm. The contents of phenolic compounds are determined by referring to a calibration curve established using gallic acid as a standard. The results are expressed in milligrams of gallic acid equivalent per 100 g of fig (mg AGE/100g) (Bey and Louaileche, 2015).

2.3.2. Flavonoids

The quantitative estimation of total flavonoids contained in the extracts was carried out using the aluminum trichloride (AlCl$_3$) method (Kosalec *et al.,* 2004). Briefly, 1 ml of the polyphenolic extract was added to an equal volume of a 2% AlCl$_3$ solution. The mixture was shaken vigorously, and the absorbance was read at 430 nm, after 10 minutes of incubation at room temperature. The amount of flavonoids in the extracts was determined using a calibration curve with quercetin as the standard. The results are expressed in milligram equivalent of quercetin per 100 g of fig (mg QE/100g).

2.3.3. Carotenoids content

The determination of the carotenoid content was carried out according to the method described by Sass-Kiss *et al.*(2005). 0.5 g of ground figs was added to 10 ml of the hexane/acetone/ethanol mixture (2/1/1). After 30 min of stirring the mixture was filtered and the upper phase was recovered (phase 1). Subsequently, 5ml of hexane was added to the lower phase for a second extraction and after 30 min of agitation, the upper phase was recovered (phase 2). The mixture of the two phases (1 and 2) was used for the determination of carotenoids by measuring absorbance at 430 nm. The results

are expressed in milligram equivalent of β-carotene per 100 g of fig (ug βCE /100g).

2.3.4. Antocyanins

The anthocyanin concentration was determined according to the procedure described by Ganjewala *et al.*(2008). An appropriate extraction was carried out: 1g of ground figs was mixed with 10 ml of a methanol/HCl mixture (V/V). After shaking for 10 min, the extract was centrifuged at 5000 rpm for 20 min. The supernatant was recovered and 5ml of it was mixed with 5ml of a methanol/HCl (V/V) mixture then the absorbance was measured at 530 nm. The results are expressed in mg of quercetin-3-glucoside equivalent per 100g of figs (mg Q 3-GE/100g), and are calculated by referring to the following formula:

$$C = A.MM.FD.1000/ ε.L. \tag{1}$$

Where:
• A: Absorbance;
• MM: Molar mass of quercetin-3-glucoside (478.3598 g/mol);
• FD: Dilution factor;
• L: Optical path;
• ε: Molar extinction coefficient of quercetin-3-glucoside (38000 mol.cm).

2.3.5. Proanthocyanidins assay

The proanthocyanidin content of fig extracts was estimated according to the method described by Porter *et al.* (1985) as developed by Nasseri *et al.* (2019). The assay consists of mixing of 200 μl of extract were added to 2 ml of iron sulfate. The mixture was then incubated at 95°C for 15 minutes. Absorbance was measured at 530 nm. The results are expressed in mg equivalent of cyanidin (CE) /100 g of fig, and are calculated by referring to the formula:

$$C=A.MM.FD.1000/ ε. L. \tag{2}$$

Where:
A: Absorbance;
MM: Molar mass of cyanidin (287.24g/mol);
FD: Dilution factor;
L: Optical path;
ε: Molar extinction coefficient (ε=34700mol.cm).

2.4. Antioxidant activities
2.4.1. DPPH free radical test

The evaluation of the scavenger effect of fig extracts on the DPPH radical is carried out according to the method described by Brand-Williams *et al.*(1995). Using this method, it possible to follow spectrophotometrically the kinetics of discoloration of radical DPPH of violet color at 517 nm.

For this, 100 μl of each extract were incubated with 2.9 ml of a methanolic solution of DPPH at 0.025 g/l. After an incubation period of 30 minutes, the absorbances at 517 nm were recorded. The anti-radical activity was estimated according to the following equation:

$$\text{Anti-radical activity (\%)} = [\{Ao-A1/Ao\}]x100. \quad (3)$$

Where:
Ao: Absorbance at 517 nm of the control (DPPH+Acetone 70%);
A1: The absorbance of the DPPH solution• in the presence of the extract.

2.4.2. Iron-reducing power test

This method is based on the ability of extracts to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The mechanism is known as an indicator of electron donor activity, characteristic of the antioxidant activity of polyphenols (Yıldırım *et al.,* 2001).

The reducing power of the samples was determined according to the method of Gulcin *et al.* (2002). This method consists of mixing 1 ml of the extract with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of a solution of potassium ferricyanide [$K_3Fe (CN)_6$] at 1% (m/v). The mixture was incubated at 50° C. for 30 min, then 2.5 ml of 10% trichloroacetic acid ($CCl_3 COOH$) are added to stop the reaction. The mixture was centrifuged at 3000 g for 10 min at room temperature. To 2.5 of the supernatant are added 2.5 ml of distilled water and 500 μl of iron chloride ($Fe Cl_3$) at 0.1%. The absorbance of the reaction medium was determined at 700 nm.

The increase in absorbance in the reaction medium indicates an increase in the reducing power of the extracts tested. The results are expressed in mg of ascorbic acid equivalent/100g of figs (Bougandoura and Bendimerad, 2013), referring to a calibration curve established with ascorbic acid.

2.4.3. Hydrogen peroxide scavenging test

The capacity of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*(1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 1.5 ml of the extract was added to 1 ml of the H_2O_2 solution.

The absorbance of the reaction mixture was measured at 230 nm against the blank containing phosphate buffer without H_2O_2. The percentage of hydrogen peroxide scavenging by the tested extracts was calculated according to the following equation:

$$H_2O_2 \text{ scavenging (\%)} = [\{Ao–Al/Ao\}] x100. \quad (4)$$

Where:
Ao: The absorbance of H_2O_2;
Al: The absorbance of H_2O_2 in the presence of the extract.

2.5. Statistical Analysis

Results were reported as mean ± standard deviation (three replicates); and the data were compared based on the values of the means. Differences between means were tested using the Tukey-Kramer HSD test (JMP version 7.0 software) with a significance level of 0.05.

3. Results and Discussion
3.1. Determination of phytochemical compounds
3.1.1. Total phenolic contents (TPC)

The total phenolic contents (TPC) of fig extracts varied significantly ($p < 0.05$) from 37.38 ± 1.54 to199.99 ± 1.72 mg AGE/100 g of fresh figs and from 80.47 ± 0.86 to 398.8 ± 2.39 mg AGE/100 g of dried figs (figure 1, table1)**.** These results showed clearly that a higher content of polyphenols was obtained in dried figs compared to fresh fig.
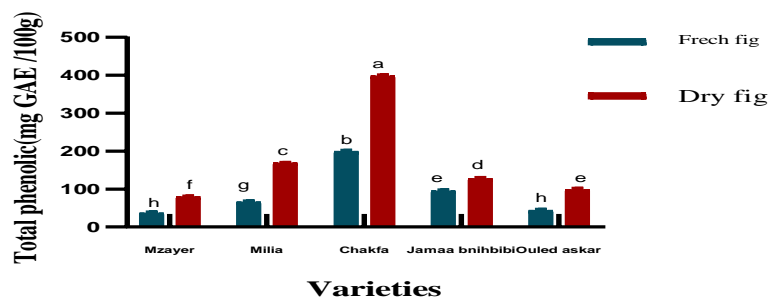
Figure1. Total phenolic contents of the fresh and dry fig varieties

The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).

Table1. Total phenolic contents of the fresh and dry fig varieties

Varieties	Total phenolic (mg AGE/100g)			
	Fresh		Dry	
Mzayer	37.38±1.54	80.47±0.86	37.38±1.54	80.47±0.86
Milia	66.09±1.84	169.36±0.81	66.09±1.84	169.36±0.81
Chakfa	199.99±1.72	398.80±2.38	199.99±1.72	398.80±2.38
DjamaaBni Hbibi	95.40±1.35	128.33±1.56	95.40±1.35	128.33±1.56
Ouled Askar	44.04±1.86	99.25±2.65	44.04±1.86	99.25±2.65

Fruit drying is an ancient food preservation technique that is still widely used today. In the literature, there are several reports on the effects of drying on phenolic compounds of various fruits including figs(Bachir Bey *et al.,* 2017; Manoj *et al.,* 2018; Arvaniti *et al.,* 2019).The findings in this study showed that sun drying has a positive effect on the polyphenol content of the five fig varieties studied. These results are consistent with those found by Manoj *et al*. (2018) who measured the total polyphenol content of fresh and dried figs from some varieties grown in India. Their results showed that the polyphenol contents in the extracts of fresh figs were 4.58 mg AGE/100 g of fig and 4.92 mg AGE/100 g of dried fig. In addition, in the research conducted by Slatnar *et al*. (2011) on a fig variety grown in Slovenia, a total phenol content of 7.49 mg AGE/100 g was reported in fresh fig fruits with a significant increase after drying (49.5 mg AGE/100 g).

Many studies report that the phenolic content can be related with drying method where

Lohani and Muthukumarappan (2015) reported that the phenolic compounds can be liberated by heat treatment, in this regard, the strong accumulation of phenolic in fig fruit after drying has been correlated with the hydrolysis of complex phenolic compounds such as tannins and lignins under the effect of the rise in temperature during drying and due to a release of other simpler and more numerous compounds (Al-Farsi *et al.,* 2005). Also, the drying can hasten the release of phenolic compounds bound to the membranes of heat-damaged cell organelles (Arslan and Özcan, 2010).

In a study by Vinson *et al.*(2005)on fresh and dried figs in California, conflicting findings were obtained. They found 486 mg AGE per 100 g of fresh figs and 320 mg per 100 g of dry figs (dry state). The same outcomes were reported by Bey *et al*.(2017)who examined three local Algerian fig varieties. They showed a decline in the amount of total phenols, which ranged from 107.08 to 181.06 mg AGE/100 g of fresh figs to

amounts between 30.81 and 40.91 mg AGE/100 g of dried figs. According to Shahidi(2004), drying can lead to polyphenol oxidase's oxidative breakdown of phenolic substances (PPO). Moreover, it may result in non-enzymatic degradation of phenolic compounds.

3.1.2. Flavonoids content (TFC)

Flavonoids are commonly provided by fruits and vegetables and had been reported to have multiple biological effects including anti-inflammatory, anti-allergic, anti-tumor,immuno-modulatory and antioxidant activities. They can also inhibit some enzymes such as lipoxygenase, xanthine oxidase,

phospholipase, etc…. which is directly responsible for to their great antioxidant capacity (Ullah *et al.,* 2020).

The total flavonoid contents (TFC) of five varieties of fig in the fresh and dried states were measured and compared as shown in table 2, figure 2. The results showed significant differences in flavonoid accumulation in the five fig varieties in fresh and dried states (p < 0.05).TFC in dry fig extracts was higher than that in fresh fig extracts in all varieties, with values varying from 13.39 ± 0.9 to 22.96 ± 0.18mg QE/ 100 g and from 10.06± 0.32 to 17.71±0.55 mg QE/100 g of fig, respectively.
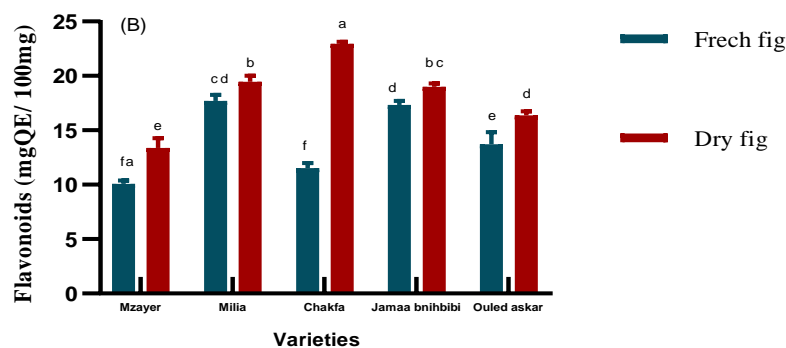

Figure 2. Flavonoid contents of the fresh and dry fig varieties
The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).

Table 2. Antioxidant contents of the fresh and dry fig varieties

Varieties	Flavonoid (mg QE /100g)		Proanthocyanidin (mg CE/100g)		Anthocyanin (mg Q3GE /100g)		Carotenoid (ug βCE /100g).	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Mzayer	10.06±0.32	13.39±0.9	0.37±0.04	2.90±0.23	3.24±0.23	0.69±0.03	158.78±0.77	51.88±1.54
Milia	17.71±0.55	19.45±0.57	0.72±0.08	2.75±0.14	4.27±0.13	1.44±0.06	237.78±0.88	84.07±0.53
Chakfa	11.54±0.45	22.96±0.18	0.31±0.01	1.32±0.03	3.07±0.10	1.26±0.02	176.23±1.39	81.75±1.68
Jamaa Bni Hbibi	17.32±0.36	19.00±0.29	0.93±0.03	2.04±0.02	3.44±0.01	1.59±0.06	336.00±1.88	140.96±1.41
Ouled Askar	13.72±1.1	16.39±0.35	0.65±0.07	2.93±0.17	2.30±0.09	1.28±0.04	464.78±1.74	130.75±1.27

TFC in dry fig extracts was higher than that in fresh fig extracts in all varieties, with values

varying from 13.39 ± 0.9 to 22.96 ± 0.18mg QE/ 100 g and from 10.06± 0.32 to 17.71±0.55 mg

QE/100 g of fig, respectively. In the comparison with the findings in this study, Bey and Louaileche (2015) reported a superior flavonoid content of 126.55 mg QE/100g in methanolic extracts of dried figs from Bejaia (Northern Algeria).

Previous studies have reported a decrease in flavonoid contents in dried fig, which is contradictory with our results (Kamiloglu and Capanoglu, 2015; Manoj *et al.,* 2018).This can be explained by accumulation of high levels of antioxidants in fruit skin, which is the part most exposed to sun during process. In this regard, the decrease in flavonoids after drying is not surprising, since these compounds act as UV filters, protecting certain cellular structures, such as chloroplasts, against the harmful effects of UV radiation (Treutter, 2006).

3.1.3. Proanthocyanidin contents

Numerous health advantages of proanthocyanidins (tannins) have been demonstrated, most notably their capacity to prevent cardiovascular disorders (Teixeira, 2002).

The proanthocyanidin contents (condensed tannins) of the analyzed fig samples showed significant differences between both fresh and dry states ($p < 0.05$). Indeed, in the fresh state, the content of condensed tannins varies between 0.31 ± 0.017 and 0.93 ± 0.031 mg CE /100 g of fig. After drying, contents ranging from 1.33 ± 0.03 to 2.93 ± 0.17 mg CE/100 g of fig are recorded (table 2, figure3). This increase can be explained by the rupture of the membrane of cell organelles by heat, so drying can accelerate the release of condensed tannins (Arslan & Özcan, 2010).

Figure 3. Proanthocyanidin contents of the fresh and dry fig varieties
The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).
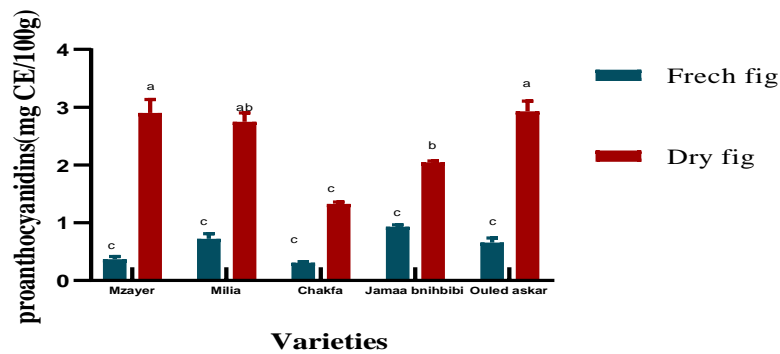

Figure 4. Anthocyanin contentsof the fresh and dry fig varieties
The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).

Figure 5. Carotenoid contents of the fresh and dry fig varieties

The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).
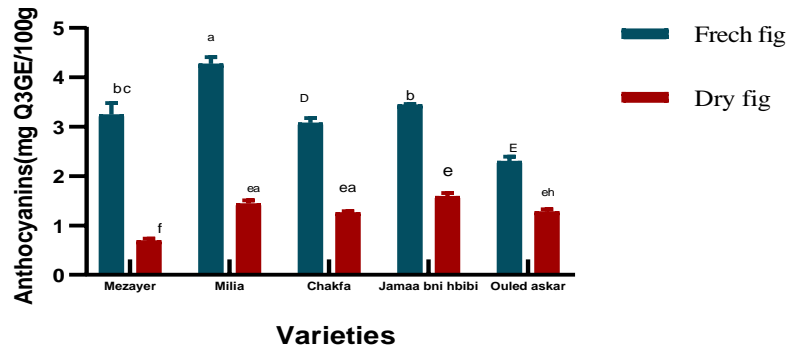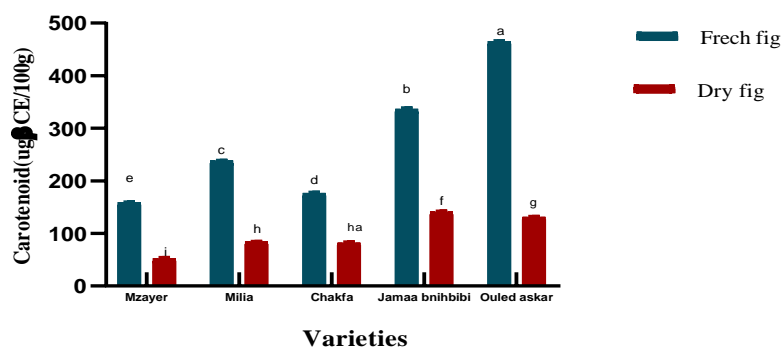
3.1.4. Anthocyanin contents

Anthocyanins also belong to the class of phenolic compounds, and are responsible for the orange, pink, red, purple and blue colors of several fruits and vegetables (Khoo *et al.*, 2017). The overall anthocyanin content is considered a differentiating mark for figs, which possess a diversity of colors, ranging from dark purple to green (Solomon *et al.*, 2006)

As indicated in the table 2 and figure 4, the anthocyanin contents of fresh and dried fig varieties investigated in the present study, which varied significantly (p < 0.05). Fresh fig varieties have a higher content of anthocyanins, with values varying from 2.3±0.09 (Ouled Askar variety) to 4.27±0.13 mg Q3GE/100 g (Milia variety) than dry figs which gave values from 0.69±0.03 (Mzayer variety) to 1.59±0.07 mg Q3GE/100 g (Djemaa Beni Hbibi variety).

Our results are lower than those reported by Chauhan *et al.*(2015) with contents of 4.78 4.67 mg Q3GE/100 g for dry fig. Kamiloglu and Capanoglu(2015) indicated a decrease in anthocyanins after drying, and they recorded levels of 4.6 mg Q3GE/100g and 0.1 mg Q3GE/100g for fresh and dried figs, respectively. It is reported in the literature that anthocyanins are very sensitive to high temperatures (Steyn, 2008). Thus, these pigments are rapidly destroyed during drying (Al-Farsi *et al.*, 2005; Mazza & Miniati, 2018).

In addition, several other factors such as light, storage and temperature are responsible for the degradation of anthocyanins in sun-dried fruits. The anthocyanin compounds can be degraded by enzymatic browning by glycosidase and polyphenol oxidase or non-enzymatic browning phenomenon(Al-Farsi *et al.*, 2005; Shahidi, 2004).This hypothesis may perhaps help to explain the anthocyanins' reduction after drying in the current investigation.

3.1.5. Carotenoid contents

Plants produce the fat-soluble pigments known as carotenoids. They are in charge of giving colors like yellow, orange, and red (Ferreiro-Vera *et al.*, 2011).

According to the results of total carotenoids amount analysis, fresh figs contained more carotenoids than dry figs (figure 5). Among the fresh figs, the highest concentration was recorded for Ouled Askar variety (464, 78 ug βCE/100g). For the dry figs, we noted that Djemaa Beni Hbibi variety has the highest content (140, 96 ugβCE/100g), while the lowest amount is recorded for Mzayer variety in both dry and fresh states (table 2).

Our findings are in line with numerous earlier investigations on various fruits and vegetables, including figs(Yemiş *et al.*, 2012;Fratianni *et al.*, 2013;Loizzo *et al.*, 2013;Liu *et al.*, 2014).It is apparent that these studies confirm that the total carotenoid content of fruits and vegetables mainly decreases after various drying treatments.

The degradation of carotenoids during drying has been attributed to their high sensitivity to oxidation. The oxidation reactions

are stimulated by light and heat, which activates enzymes. Thus, the oxidation reactions are most likely the primary reason of carotenoid loss. In this regard, compared to other transformation processes including heat treatment, drying leads to a greater degradation of carotenoids with the higher in porosity (Türkyılmaz *et al.,* 2014)**.**

3.2. Study of antioxidant activity

To assess in vitro antioxidant activity, three distinct techniques based on different mechanisms of action were used. The composition of extracts using a variety of assays act through a variety of mechanisms including the prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging (Li *et al.,* 2008)**.**

3.2.1. Free radical DPPH reducing

By calculating the percentage of inhibition for each variety of fresh and dried figs, we established the antiradical activity profiles presented in figure 6 and table 3. The results showed that this activity differed significantly between the five fig varieties in fresh and dried states ($p <0.05$). For the fresh and the dry fig, Chakfa variety was more efficient against DPPH (57.33%) and (62.41±0.83%) respectively.

Many papers have reported the antioxidant activity of fig extract using DPPH. In a study of Aljane(2018)working on several varieties of fresh Tunisian figs, the author reported inhibition percentages ranging from 11.36% to 64.737%. In another study conducted by Ayoub *et al*. (2019), it has been reported that the inhibition percentages varied between 11.31% and 87.03% for fig varieties in Morocco.
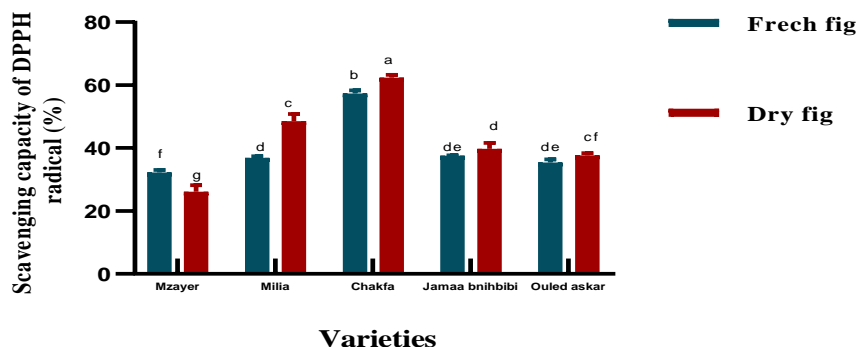
Figure 6. DPPH radical scavenging effect against the Extract of fresh and dry fig varieties
The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).

Table3. Antioxidant activities of the fresh and dry fig varieties

Varieties	DPPH (%)		FRAP (mg AAE/ 100g)		Hydrogen Peroxide Scavenging (%)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Mzayer	32.24±0.77	26.13±2.09	41.87±1.69	38.86±0.98	38.68±0.34	17.22±0.72
Milia	36.86±0.55	48.50±2.35	99.56±0.69	213.22±1.83	55.24±1.53	60.96±0.05
Chakfa	57.33±1.00	62.41±0.83	499.90±2.46	668.86±2.20	68.98±0.18	79.56±0.41
Jamaa Bni Hbibi	37.60±0.1	39.71±1.87	142.16±2.40	174.09±2.11	55.95±1.23	60.02±0.21

Ouled Askar	35.36±0.06	37.67±0.71	46.73±0.62	115.70±1.35	32.94±1.98	36.91±0.47

For dry fig, we noted that our results are superior to those of Bey and Louaileche (2015)who reported that the scavenging radical capacity of six varieties of light dried figs from Bejaia (Algeria) was ranging from 28.33% to 35.15%. Besides,Pourghayoumi *et al.*(2016) have also reported the DPPH scavenging activity of nine varieties of Irannian dry fig extracts (37.70% to 70.02%).

Overall, the statistical analysis showed a significant increase in the anti-radical activity by dried figs for the majority of the varieties studied with the exception of the Mzayer variety where a decrease in the percentage of inhibition was noted. Our results are consistent with the review paper on natural fig antioxidants by Arvaniti *et al.*(2019)that reported the consistent influence of sun-drying process on the antioxidant capacity of figs.

In some studies, the antioxidant capacity of dried figs has been reported to be higher than that of fresh figs(Chauhan *et al.,* 2015; Kamiloglu and Capanoglu, 2015; Konak *et al.,* 2015).This increase may be due to the quantity of phenolic compounds resulting from drying, which can be explained by a greater generation of these compounds and/or the release of

sequestration due to the rupture of the cell walls(Capanoglu, 2014).Also the Maillard reaction products can be a possible explanation for this increase which can occur as a result of heat treatment or lengthy storage and often exhibit significant antioxidant capacity (Nicoli *et al.,* 1999). By contrast, sun-drying can reduce the antioxidant capacity of figs by degradation or transformation of the active phenolic compounds of the fruits into a non-antioxidant form, leading to degradation of flavonoids, particularly anthocyanins despite the increase in the rate of total phenolic compounds(Bachir Bey *et al.,* 2017; Nakilcioğlu and Hışül, 2013). Indeed, the anthocyanin content is closely linked to the anti-radical activity (Steyn, 2008).

3.2.2. Iron-reducing power test

Iron ions reduction property of the tested samples is expressed in mg of ascorbic acid equivalents/100g.

As seen in figure 7 and table 3, in fresh figs, the reducing activity of the analyzed fig varieties varies between 41.87±1.69 mg AAE/100g (Mzayer) and 499.90±0.69 (Chakfa). For dried figs, the reducing power of iron varies between 38.86 ± 0.98 mg AAE/100g (Mzayer) and 668.86 ± 2.20 mg AAE/100g (Chakfa).

Figure7. Reducing power assay of fresh and dry fig varieties

The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).

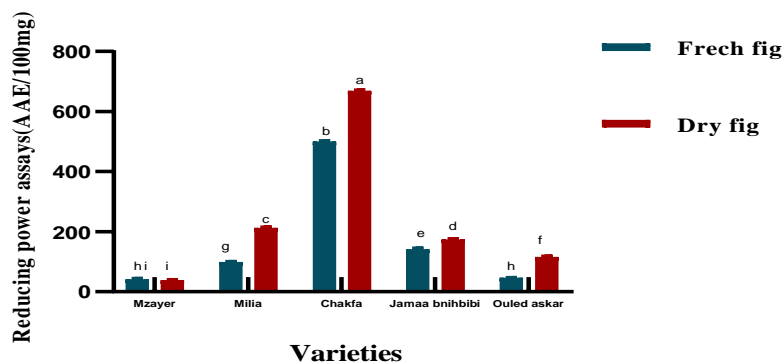**Figure8.** Hydrogen Peroxide scavenging inhibition of fresh and dry fig varieties
The results are expressed as mean values ± standard deviation (n=3). Bars labeled with different letters are significantly different (p < 0.05).
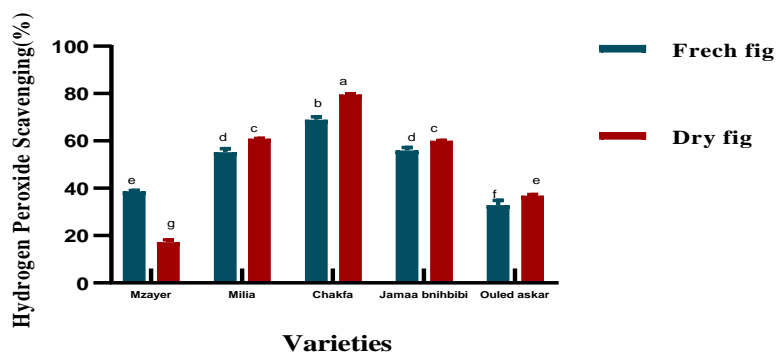
The mechanism of this activity is based on the ability of extracts especially to their polyphenols to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by electron-donating (Yıldırım *et al.,* 2001).

Previous studies on reducing power activity of dry fig extracts has been adopted in different works including that carried out by Nakilcioğlu and Hışül (2013), where they reported an average reducing activity of 222.71 mg $FeSO_4$/100 g (mg of iron sulphate / 100g MS)of Turkish fig variety.

In another study carried out by Capanoglu(2014) on the same Turkish dried fig varieties, iron reducing capacity was of 117 mg ET/100g. This value is lower than that obtained by Kamiloglu and Capanoglu (2015)(140 mg ET/100 g) or those reported by Reddy et al.(2010) on Indian fig varieties, where they recorded an inhibition with value of 3578.69 mg $FeSO_4$/100 g of fig.

Overall, the statistical analysis reveals a significant increase in iron-reducing activity after drying for the four varieties of Milia, Chakfa, Ouled Askar and Djemaa Beni Hbibi, inversely, we noted a slowly decrease in this activity in Mzayer variety. In this regard, the best drying method leads to the least alteration in phenolic content and enhances antioxidant activity of the sample. The high drying temperature gave a product with better polyphenol content with enhanced antioxidant activity(Madrau *et al.,* 2009). Similar effects of drying on antioxidant capacity of fruits and

vegetables including sage and Enicostemma littorale (Blume) have been reported (Hamrouni-Sellami *et al.,* 2013; Sathishkumar *et al.,* 2009).

3.2.3. Hydrogen Peroxide Scavenging

The determination of the hydrogen peroxide detoxification capacities of the different fig extracts is based on their ability to trap this radical, presented as the percentage value (figure 8, table 3).

The values of scavenging effect of tested extracts were ranged from 32.94 ± 1.98% to68.98 ± 1.18% in fresh fig; while dry fig extract gave values varying between 17.22±0.72% and 79.56±0.49%. According to these findings, we can note a significant increase in hydrogen peroxide scavenging activity after drying for the majority of the studied varieties, with the exception of the Mzayer variety, which showed a weaker capacity.

Our results agree with those of certain authors who have treated the role of sun-drying process on the antioxidant capacity of figs. In fact, these authors reported that hydrogen peroxide scavenging activity in dry fruit extracts was higher than those of fresh fruits(Qusti *et al.,* 2010; Slatnar *et al.,* 2011; Igual *et al.,* 2012). This increase can be explained as being the consequence of the formation of new active molecules induced during drying, especially Maillard reaction products known by their antioxidant activity (Qusti et *al.,* 2010;Igual *et al.,* 2012). In contrast, the decrease in activity recorded for the Mzayer

variety can be explained by the decrease in the abundance of flavonoids that are sensitive to the high temperature. Moreover, A good correlation was recorded between inhibitory activity and anthocyanins content in the study conducted byGorinstein *et al*.(2004).

4. Conclusion

Our study was carried out in order to evaluate the effect of sun-drying on polyphenol composition, as well as on the antioxidant activity of five varieties of figs from five Jijel regions (Chakfa, Milia, Mzayer, Djemaa Beni Hbibi and Ouled Askar). In fact, the phytochemical composition and particularly the content of total polyphenols, of flavonoids, andof pro-anthocyanidins (condensed tannins) showed a significant increase after sun-drying. On the other hand, a significant decrease (p<0.05) was noticed in the content of anthocyanins and of carotenoids of the five studied varieties of fig.

The *in vitro* evaluation of the antioxidant activity of the extracts showed that sun-drying had a positive influence on this activity (the scavenging of DPPH radical, the reducing power of iron and the scavenger effect of H_2O_2). This significant increase is due to their high content of bioactive substances. So, we can say that the dried fig is a good source of various antioxidants; it could be used in pharmaceutical field to prevent the lifestyle-related diseases in which free radicals are involved and to promote human health. Further researches should be carried out to complete this study by determination of other biological activities *in vitro* and *in vivo*, and by realizing of a comparative study by evaluating the impact of several drying processes (in the sun, in the oven and in the microwave) on the attributes of the quality of the figs, namely the physicochemical, nutritional, phytochemical quality and in particular the antioxidant activity.

5. References

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OBTAINING CRAFT GINGER BEER IN THE LABORATORY PHASE AND SENSORY, PHYSICO-CHEMICAL CHARACTERISTICS

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ABSTRACT
The purpose of this paper is the sensory and physico-chemical characterization of craft ginger beer obtained under laboratory conditions.
For this paper we prepared blonde beer (Indian Pale Ale - IPA) with ginger, the Ginger Beer assortment through the one-stage brewing method, in an amount of approximately 2 liters. The preparation resulted in a craft ginger beer with some insignificant defects (insufficient foaming, slightly high relative density, high acidity), defects explained by the too high temperature during primary fermentation and the continuation of secondary fermentation in bottles. On the other hand, the craft beer obtained has well-defined sensory qualities and is appreciated by the tasters (appearance, smell and taste), as well as a high alcoholic concentration, which qualitatively distinguishes it, compared to other commercial beers.
Once these deficiencies are identified and rectified, the craft beer recipe experimented in the laboratory can be successfully replicated in any micro-enterprise, which has as its profile the production of craft beer and opens the way for experimentation and innovation of other recipes.

1. Introduction

Craft beer is a beverage with a moderate percentage of alcohol, undistilled, which can be obtained by the fermentation process of malt juice boiled with hops (other aroma compounds may be used optionally) with the help of the brewer's yeast, in quantities restricted by independent producers.

Generally, craft beer drinkers prefer beers prepared from local varieties of malt and hops by brewery masters' products with a good knowledge of the local ingredients, as compared to the production obtained in large and medium-sized breweries (Baiano, 2021; Adams, 2006). This implies that breweries must be small in size and craft brewers must customise their brewing according to the tastes of consumers. According to a survey conducted by Alltech and The Journal of Brewers in 2016, the world number of breweries was 19000 and approximately 17700 (94%) of them were considered craft breweries (Zenith, 2017). Regarding the distribution of craft beer producers worldwide, the United States and Europe have a percentage share of 46% and 43%, respectively, followed at a distance by Canada (4.5%), South Africa (4.5%), Australia (3%), Japan (1.6%) and New Zealand (1%) (Callejo et al., 2019).

In the EU, the UK has the largest number of breweries (2430), followed by Germany (1492), France (1100) and Italy (868) (Baiano, 2021). Surprisingly, in countries with a tradition of producing beer in large breweries such as the Czech Republic, the number of craft breweries reached 480 in 2019 and have an encouraging market share of 2% (Brezinová, 2021).

For several years in Romania, the production of craft beer has started to grow, at the moment, with more than 40 microbreweries, no less than 17 opened in 2018.

As regards the ingredients used in the preparation of craft beers, the innovation of the recipe often refers to the use of new cereal mixtures or the rediscovery of old cereals, new varieties of hops, new yeast crops, fruits, vegetables and spices used to improve the sensory characteristics of the finished product (Donadini and Porretta, 2017; Li et al., 2017).

Craft beer can be classified according to its products and ingredients in several important types:
- Indian Pale Ale (IPA) tends to have a more bitter aroma, having more hops in its composition; this type of beer can be combined with ginger or other spices;
- Brown Ale, this beer has a high level of malt, which makes it softer and less bitter;
- Stout, it tastes similar to coffee because malt-free roasted barley portions are often used; it is a creamy beer rich in extract;
- Baltic Porter has a black texture, it is a dark beer with superior fermentation and has aromas of dried fruits (Hughes, 2020).

To meet consumer demands, craft beer producers have chosen to use distinct ingredients such as rice, honey, fruit, manioc, pumpkin, ginger, to induce positive sensory changes to the finished product (Tozetto et al., 2019; Pinto et al., 2015; Rio, 2013; Brunelli and Venturini Filfo, 2013; Brunelli et al., 2014; Matsubara et al., 2016).

The distinct aroma of ginger comes from the presence of volatile compounds such as camphene, borneol, geraniol, limonene, gingerol and other terpenes, while non-volatile molecules are responsible for the sharp, hot sensation in the mouth (Haniadka et al., 2013; Peter, 2006).

The production of craft beer is also an opportunity for local producers of raw materials to capitalize on their crops. Some studies have positively assessed the quality of the Romanian varieties of hops that are appropriate for the manufacture of craft beers (Mudura et al., 2010; Mudura et al., 2015; Mudura et al., 2016; Salanţă et al., 2012; Salanţă et al., 2015; Salanţă et al., 2016).

While craft brewers seek answers regarding possible new trends in beer consumption, many independent studies are directed towards assessing the quality of craft beer (Passaghe et al., 2015; Aquilani et al., 2015; Giovenzana et al., 2014).

In this context, our study aims to characterize from a sensory and physico-chemical point of view the craft beer with ginger obtained under laboratory conditions and to identify any defects that can be remedied in a future experiment.

2. Materials and methods
2.1. Materials

For the production of craft beer we used the following equipments: electronic scale, thermometer, areometer/densimeter, stainless steel pot, glass jar (capacity 3 L), grinding and filtering utensils.

The ingredients needed to obtain 2 litres of craft ginger beer are: malt - 800 g, water - 3.2 L, hops - 4.5 g, ginger - 11.2 g, yeast - 1.5 g

The ingredients were purchased from the S.C. Brico Ideea SRL Bucharest - Romania (Malt Weyermann Vienna 7 EBC, Hops Pellet Mandarina Bavaria and Yeast Fermentis Safbrew S-33).

2.2. Samples

The assessment of the sensory quality of beer was made using the 20-point assessment system and for the assessment of the different characteristics, scales from 0...5 points are applied for each organoleptic characteristic.

The determination of the density was carried out by the pycnometer method, the determination of the alcoholic concentration based on the relative density of the distillate, the determination of the acidity by titration with 0.1 N NaOH, and the determination of the colour by titration with 0.1 N iodine solution (Diaconescu and Theiss, 2004). The real extract was determined from correlation tables based on the relative density, and the primitive extract was calculated based on the real extract, the alcoholic concentration and some empirical coefficients.

Stages of making craft ginger beer in the laboratory
Weighing
The weighing of the ingredients (malt, ginger, hops, yeast) was done with electronic scales.
Mashing
We added the amount of water required (4 times the amount of malt), then we introduced the malt gradually for a good solubilization.
Obtaining mash sugar (brewing)
We have chosen the brewing by infusion in a single phase that involves keeping the temperature constant throughout the process. The ideal temperature for brewing is 65-68°C for 60-90 minutes. We stirred it permanently with a spoon for good sugar extraction.

Figure 1. Brewing, checking of starch saccharification

After 90 minutes of brewing, it was checked the sugar stage of the starch; for this purpose it was used a porcelain capsule where a few drops of must were placed, over which 2-3 drops of iodine solution were added and the color was monitored. If the colour of the composition changes to blue, the brewing must be continued and if the colour remains brown, there is no starch remaining, that did not turn into sugar, so and the next step can be taken.

Mash filtration
The filtration process is based on the separation of the soluble fraction of the mash from the insoluble part representing the malt pulp.

Wort boiling with hops and ginger
The process of wort boiling with hops takes 90-120 minutes. At the beginning of the boiling process, we added half the amount of hops in the standard Pellet form (90) to give the beer the bitter taste, and the other half in the same form we added it 5 minutes before the boiling was completed to give the beer flavor and savour. Also at that time we added freshly grated ginger. We added 2.5 times more ginger than hops to predominate the ginger flavor over the bitter taste.

Ginger contains the pungent compound gingerol, which is similar in structure to chilli's capsaicin and pepper's piperine. Heating converts gingerol to the less pungent zingerone, palatable.

Cooling
At the end of the boiling, I cooled the wort as quickly as possible to the temperature suitable for the adding of the yeast. We placed the pot in the cold-water bath until we cooled the wort to a temperature of 25°C.

Dry yeast inoculation
Before inoculating the yeast, we poured all the liquid into a 3-liter glass jar, then added the yeast culture. The yeast was diluted with lukewarm water at 30°C and we allowed it to acclimatize for 15 minutes.

Fermentation process
After mixing the wort with the yeast, we covered the container with gauze, creating anaerobic conditions of fermentation. We left the wort to ferment for a week at a temperature of 16-18°C (room's temperature). A control sample of wort prepared under the same conditions was fermented under ideal conditions of 7-9°C for a week. After 7 days of primary fermentation, the secondary fermentation phase followed, when the yeast settled. The completion of the primary fermentation was assessed by checking the density with the areometer.

Figure 2. Fermentation process, checking the degree of fermentation

Secondary fermentation

After primary fermentation, the beer was poured in 0.33 l bottles, which were stored in the refrigerator. The bottles were previously sterilised in the oven for 60 minutes at 180°C. Refrigerated storage for 2 weeks actually simulates conditions for secondary fermentation (2-4°C) (Diaconescu and Popescu-Mitroi, 2006; Diaconescu and Popescu-Mitroi, 2011)

3. Results and discussions
Sensory analysis

The sensory analysis of craft ginger beer was carried out in accordance with the approval of the Research Ethics Committee of "Aurel Vlaicu" University of Arad. 8 tasters over the age of 18, selected by prior screening that indicated consumption of blonde beer at least once a week, and who had no aversion to ginger, participated in the experiment. The attributes assessed were appearance, color, smell, taste, impregnation with carbon dioxide, and foam using a 5-point hedonic scale for each sensory feature (Bologa and Burda, 2006).
Summing up the scores

Depending on the importance of each characteristic in the formation of the quality of the beer, weighting factors are used:

Table 1. Sensory characteristic and weighting factor

Sensory characteristic	Weighting factor
Appearance	0.6
Color	0.8
Smell	0.2
Taste	1.4
Impregnation with CO_2	0.6
Foam	0.4

The result is calculated as an average, using the formula: $P_{mp}=P_{mnp}*F_p$

P_{mp} - weighted average score for each characteristic;

P_{mnp} - the unweighted average score of each characteristic, as the arithmetic average of the individual scores;

F_p - the weighting factor for the characteristic to be assessed.

Table 2. The centralization sheet of the results obtained in the sensory analysis of craft ginger beer

Current number	Taster code	Individual scoring					
		Appearance	**Color**	**Smell**	**Taste**	**Impregnation with CO_2**	**Foam**
1.	01	5	3	5	5	4	0
2.	02	5	4	5	5	3	1
3.	03	5	3	5	5	3	1
4.	04	5	3	5	5	3	1
5.	05	5	4	5	5	4	1
6.	06	5	4	5	5	4	0
7.	07	5	4	5	5	4	1
8.	08	5	4	5	5	3	1
Unweighted average score		5	3.6	5	5	3.5	0.75
Weighted average score		3	2.88	1	7	2.1	0.3
Total average score		16.28					

The score obtained in sensory analysis is 16.28 points, receiving the rating "Good", the product having specific positive qualities quite well outlined, the defects being insignificant. The appearance of the liquid is clear, without sediment or impurities, yellow-brown color, pleasant smell and taste specific to beer, without foreign smell and taste, harmonious combination between the taste of hops, malt and ginger. It is worth mentioning that for sensory characteristics, appearance, smell and taste, the analyzed product obtained maximum scores unanimously from the tasters.

However, following the evaluation carried out by 8 different tasters, the same sensory defect was identified: insufficient foaming, which is the consequence of poor impregnation with carbon dioxide during fermentation.

Table 3.Results of physico-chemical measurements for craft ginger beer

Physico-chemical measurements for craft ginger beer	Medium values	Commercial ginger beer „Crabbie's"	Limits according to SR 4230:2004 for superior blond beer
Density (g*cm^{-3})	1.019±0.01	1.018	1.018
Real extract (%)	4.83±0.01	2.16	3-5
Primitive extract (%)	12±0.01	10	10-12
Alcoholic concentration (%)	5.85±0.03	4.0	Minimum 4.3
Color (mL iodine solution)	1.10±0.10	1.4	Maximum 1.2
Total acidity (g*L^{-1})	5.70±0.08	3.78	2.8
pH	3.6±0.10	4	3.9-4.6

Physico-chemical measurements

For craft ginger beer, the following physico-chemical parameters were determined: density, real extract, primitive extract, alcoholic concentration, color, pH and total acidity. All physico-chemical measurements were performed 3 times and mean values were taken into account.

The results showed a higher alcoholic content of craft beer compared to commercial beer, a fact confirmed by other studies (Zhao et al., 2010). The high concentration of alcohol in craft ginger beer seems to be a parameter with a positive impact on the preferences of consumers. In addition, the ginger flavor induces positive sensory changes in the finished product, being highly appreciated by consumers.

The high alcohol content is correlated with the increase in total acidity, the same trend being reported in the other studies (Mascia et al., 2014).

Usually, unfiltered and unpasteurized craft beers tend to ferment again in bottles, in this way the increase in total acidity can also be explained (Marongiu et al., 2015).

Comparing craft ginger beer to commercial ginger beer (assortment „Crabbie's") it can be seen that the parameters alcohol concentration, color, real extract are clearly superior for craft ginger beer.

In the absence of a standard for craft ginger beer, the results were compared with the standard for superior blonde beer SR 4230 (2004).

4. Conclusions

The main advantages that personalize the product obtained are its appearance, smell, taste, but also the high alcoholic concentration.

The main defect identified in the sensory analysis (insufficient foaming) can be explained by the fact that the primary fermentation was carried out at a much too high temperature (16-18°C), the ideal temperature being 7-9°C. This defect can be remedied by driving the primary fermentation at lower temperatures.

The deficiencies identified by the physical-chemical examination are: higher density than normal, due to the presence of the suspended yeast - the cause being insufficient filtration and the total acidity higher than normal, but without the occurrence of the souring defect - the cause being the continuation of the secondary fermentation in bottles.

The results of the experiment carried out by us in the laboratory show that the preparation of craft beer by the method of single-phase brewing is not such an easy thing, resulting in some unpleasant surprises, materialized in small defects. Once these defects have been identified and remedied by "laboratory microprobes", it opens the way for experimentation and innovation of other recipes, initially at the laboratory phase, then at the pilot station and finally at the industrial phase (craft beer micro-enterprise).

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QUALITY CHARACTERISTICS AND STORAGE STABILITY OF GLUTEN-FREE CUPCAKES MADE OF BUCKWHEAT AND RICE FLOUR

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ABSTRACT
This study looked into the physicochemical and rheological properties of buckwheat (BW) and rice flour (RF) as the base ingredients for gluten-free cupcake formulations and how those properties related to the quality of the baked products. Different BW and RF ratios—10:90, 20:80, and 30:70—were used to create gluten-free cupcake recipes. Compared to wheat flour (WF), gluten-free BW, and rice-based composite flours showed substantial changes in proximate composition and declining number range, according to physicochemical and rheological analyses. The particle size of RF was finer than BW and wheat flour. When BW flour, the darkest flour, was blended with RF, the lightest flour, the resulting cupcakes were noticeably lighter than those made with wheat flour. With increasing amounts of BW flour, cupcakes received higher sensory scores overall. That increases the amount of BW flour in a product, which indicates more consumer appeal. Following the addition of more BW flour, the proximate composition and physical characteristics of cupcakes significantly changed. All of the cupcake samples showed a noticeable reduction in moisture content after 8 days of storage, but there was no discernible alteration in the amounts of fat or protein. Except for softness and stickiness, which showed just a slight difference in sensory scores after storage, all of the samples' sensory scores were significantly lower after 8 days. A non-significant (p<0.05) declining tendency was seen when cupcake height was compared to weight during storage.

1. Introduction

Gluten is the primary protein in flour that gives objects their forms. Additionally, it imparts elastic qualities to dough and influences the appearance and crumb shape of many baked items (Gallagher *et al.*, 2004). New approaches are being developed to change the structure and subsequently, the functioning of this unique protein component to give a variety of functional features at a lower cost than competitors like milk and soy protein. The sensitivity known as celiac disease (CD) (avenin) has been linked to the prolamin of rye (secarin), barley (hordein),

and possibly oats as well as the gliadin part of wheat. Celiac disease, often known as gluten intolerance, causes immunologically driven inflammatory damage to the small intestine mucosa (Arslan *et al.*, 2019). All across the world, celiac disease is becoming more common. Because so little is known about this disease, which is primarily immune-based, the prevalence has increased. It has been a long time since the cause of this sharp increase trend was discovered, but it might be connected to environmental factors that can lead to a lack of tolerance to dietary gluten

(Lebwohl and Rubio-Tapia, 2020). A gluten-free diet is a component of celiac disease treatment, but it is also extensively practiced outside of patient care. It becomes obvious that the majority of those who avoid gluten do not have undiagnosed celiac disease. In recent years, gluten-free products have become very popular since they cater to both consumers who follow a gluten-free diet and those who have medical demands (Pellegrini and Agostoni, 2015). RF is one of the grains that work well for the creation of gluten-free dishes due to its pleasant taste, hypoallergenic characteristics, lack of color, low sodium content, and readily digestible carbs (Gujral *et al.*, 2003; Gujral and Rosell, 2004; Lopez *et al.*, 2004).

Rice proteins are unable to generate the network needed to hold the gas produced during the fermentation process and baking. Rice only contains 2.5–3.5% prolamin, hence adding water to RF does not produce a viscoelastic dough. The resulting product has a low specific volume and doesn't resemble wheat bread since it can't hold the gases produced during baking and proving (McCarthy *et al.*, 2005). A pseudocereal is something that includes BW, quinoa, and amaranth. BW is a beneficial ingredient for enhancing food industry processing and marketing prospects due to its nutritious qualities (Mariotti *et al.*, 2008). Up to 50% of BW protein is made up of globulin, and 25% is low molecular weight chain polypeptide (Choi *et al.*, 2006). BW flour is an important dietary source of protein because of its high protein content. BW protein contains one of the highest concentrations of amino acids of any plant-based protein.

Prolamin and glutelin levels in BW flour are low, but albumin and globulin levels are high (Ikeda, 2002). Maximum levels of flavonoids and polyphenols can be found in BW. BW includes phenolic substances such as 3-flavanol, rutin, phenolic acids, and their derivatives as well as tocopherols, which may act as antioxidants (Sakac *et al.*, 2011). The goal of this research was to prepare gluten-free flour by combining rice and BW flour, analyze the physical, chemical, and rheological properties of the composite flour made from rice and BW, make gluten-free cupcakes, and assess the quality and storage stability of the cupcakes.

2. Material and methods
2.1. Collection of samples and consumables
The Pakistan Agriculture Research Center, Sakardu, supplied the common BW (*Fagopyrum esculentum*). The rice (*Oryza sativa*) variety (Green super rice) was gathered at the Food Science Research Institute, NARC-Islamabad. The wheat (*Triticum aestivum*) variety (AZRC) was collected from the Crop Sciences Institute, NARC-Islamabad. Sodium hydrogen carbonate ($NaHCO_3$), vegetable fat, sugar, eggs, and milk powder were purchased from the local market of Rawalpindi.

2.2. Milling of grains
Each grain was meticulously cleansed. 14 ml/100 g of moisture was used to condition wheat grains for one day (El-Porai *et al.* 2013). Quadrumat Senior Mill (Brabebder GmbH & Co.) was used to mill the grains of wheat and BW. Rice grains were ground using a Perten laboratory mill 3100 from Perten Instruments in Hagersten, Sweden.

2.3. Treatments plan
As a control sample, WF was used. RF and BW flour were combined in various proportions, such as 10% BW flour: 90% RF (B_1), 20% BW flour: 80% RF (B_2), and 30% BW flour: 70% RF (B_3).

2.4. Physicochemical and rheological properties
The test weight and thousand kernels weight were determined by following the previously stated procedure (Fred, 2008). Balance (SEEDBURO, Model 8800A) was used to weigh the grains. The particle size distribution of flours was evaluated by using sieves of particular pore size (>250 μm to <55μm), moisture by hot air oven (MEMMERT), ash by muffle furnace (CARBOLITE AAF 1100), wet gluten by glutomatic, dry gluten by glutork-2020, gluten index by centrifuge-2015, falling number by falling number instrument (Perten, Type

1500F), protein by behrotest[R] S5 distillation unit, fat by BUCHI Extraction System B-811 and farinographic parameters by Brabender Farinograph (Version: 3.2.6) according to reported procedures (Fred, 2008).

2.5. Cupcake preparation

The reported recipe was modified to make cupcakes (Carullo *et al*., 2020). Cupcakes were made with substances like sugar, eggs, vegetable fat, milk powder, and sodium hydrogen carbonate (NaHCO3). A mixer was used to combine the liquid entire egg for 10 minutes. Once a transparent consistency was achieved, 100 g of sugar and 100 g of vegetable oil were added. 4g of sodium hydrogen carbonate (NaHCO3) and 100g of the flour were combined before being added to the dough. 100g of the milk powder was added towards the end of the mixing process. A cake pan was filled with 100g of the dough after it was weighed. For 30 minutes, samples were baked at 180 °C in a baking oven. Baked samples were allowed to cool at room temperature.

2.6. Storage of cupcakes

A stated procedure was used after making certain adjustments to store cupcakes (Villanueva and Trindade, 2010). At room temperature, cupcakes were kept for around 0 days, 2 days, 4 days, and 8 days. Each cupcake with a paper mold as the base was individually wrapped in an aluminum foil multilayer film and kept in plastic zip bags for storage.

2.7. Physical characteristics, sensory attributes, and proximate properties of cupcakes

Vernier calipers were used to measure width and length. Above mentioned digital weighing balance was used to weigh cupcakes (Mancebo *et al*., 2015). After an hour of baking, cupcakes were tested for color, flavor, texture, softness, stickiness, and chew ability. Cake samples were rated on a 9-point Hedonic Scale (1 being the most disagreeable and 9 being the most agreeable). 14 judges—7 men and 7 women ranging in age from 30 to 47—scored each

sample. Scorecards and polyethylene bags containing cupcakes were given out. A trained panel of 14 judges, consisting of 7 men and 7 women, aged 30 to 47, evaluated each sample for the sensory qualities of the cupcake. Each judge evaluated two cupcakes, and the average scores were determined (Yousaf *et al*., 2020). The moisture, protein, and fat content of cupcakes were analyzed by following the reported procedures (Fred, 2008).

2.8. Statistical analysis

Following three repetitions of each test, the standard deviations for the average findings were noted. Version 16 of MINITAB software was used to conduct the statistical analysis. By creating Pearson correlation coefficients between cupcake quality measures and fine flour qualities, it was possible to assess how BW and RF quality attributes affected cupcake quality. (ANOVA) was used to determine how the samples differed. The least significant difference (LSD), a measure of the difference between mean values, was employed at a 5% level of significance.

3. Results and discussion
3.1. Test weight and thousand kernel weight

The test weight with the maximum value was found in WF, followed by BW and RF, respectively (Table 1). According to a study, Sham-3 had the lowest test weight (83.10 Kg/Hl) and Douma-29019 had the highest value (85.90 Kg/Hl) (Sakac *et al*., 2011). A study was done to assess the qualities of different rice types. Forme Chena had the highest test weight value (769.01 4.0 kg/m^3), whereas Barkat Chena had the lowest value (502.00 62.48 kg/m^3) (Rather *et al*., 2016).

As a result of the larger size of wheat grains compared to BW and rice grains, wheat had the highest value of a thousand kernel weight while BW and rice had the lowest (Table 1). Previous research comparing the physical and chemical characteristics of two BW varieties (commercial BW and the Guneş variety) revealed that the thousand kernel weight of commercial BW was 19.98±1.21g and that of

the Guneş variety was 21.74±0.81g (Unal *et al*., 2017).

3.2. Moisture and ash content

The addition of BW to other flour led to an increase in moisture content, which was seen over time. BW had the highest moisture content, which was followed by WF and RF (Table 1). The examination of the physical and chemical properties of BW, and WF used in gluten-free cookies revealed increased values of moisture % by the inclusion of BW flour (Ulfat *et al*., 2015). Compared to RF flour, which had the lowest percentages of ash, BW flour had the highest amount of ash. By increasing the ratios of BW flour, the ash content of blended flours was raised (Table 1). A study showed the impact of process variables on gluten-free rice-BW pasta made by a single-screw extrusion cooker, higher ash content values were found in BW flour (Bouasla and Wojtowicz, 2019). Research findings revealed an increasing trend in ash levels while examining the mineral content, antioxidant activity, and sensory evaluation of gluten-free rice and BW biscuits (Sakac *et al*., 2015).

3.3. Gluten and falling number

WF had a noticeable amount of gluten, whereas BW flour and RF had no gluten content. WF was found to have a sizable gluten strength (gluten index) (Table 1). The maximum amount of dry gluten (9.060.30%) was found in wheat flour, however, there was no gluten found when the nutritional characteristics of common BW from four separate Gilgit-Baltistan communities were studied (Kaushik *et al*., 2015). A declining tendency to falling numbers was noted in the RF and BW flour blends by increasing the BW percentage. The analysis of the highest falling number was done in RF, followed by B_1 and B_2, and the lowest value was discovered in BW (Table 1). The ultimate values of falling numbers may be affected by the RF pasting attribute. While researching the preparation of gluten-free biscuits and the impact of gluten-free flour on its physical qualities, a higher falling number was evaluated in rice (Mancebo *et al*., 2015). Similar results were obtained in a study on wheat gluten regeneration characteristics (Kaushik *et al*., 2015).

3.4. Protein and fat content

The highest protein content values were found in BW and the lowest in RF. In formulation, the BW percentage was raised to boost the protein content (Table 1). The inclusion of BW flour in the test recipe for gluten-free bread was found to significantly improve the proteins (KrUpa-KozaK *et al*., 2011). BW had the highest value of fat content, while WF had the lowest (Table 1). The addition of BW steadily raised the fat content of blended flours. After adding BW flour to gluten-free tarhana, the values of the fat contents were examined. According to the approximate composition of the flours, BW flour contained 1.81±0.03% fat, and refined WF included 1.78±0.08% fat (Bilgicli, 2009[a]).

3.5. Farinographic properties

Results showed that blend B_3 required more time for dough development, whereas blend WF required less time (Table 1). By adding BW to other flour, a rising tendency in dough development time was observed. Regarding the impact of using BW flour instead of WF on the production of pasta and cookies, our results values were in agreement with the earlier study. The trend of increase in dough development time was recorded after substituting wheat flour with millet and sorghum flour from 10% to 30% during a study on rheology and quality of composite flours (Torbica *et al*., 2012). Maximum water absorption was reflected in WF and minimum in B_3 (Table 1). There was a trend toward less water absorption during the addition of BW. When examining several quality indicators of bread and dough made with WF but substituted with BW flour and millet flour, a similar trend of declining water absorption was observed. In comparison to WF which contained 30% BW flour and reflected a value of 52.6%, WF had a greater water absorption rate (54.8%) (Izydorczyk *et al*., 2013).

Table 1. Physicochemical and rheological properties of flour

Sample	FC	PC	MC	Ash	FN	WG	DG	TW (Kg/Hl)	TGM (g)	WA (%)	DDT (min)	DST (min)	MTI (BU)
WF	1.99a ±0.10	10.71b ±0.22	13.74d ±0.17	0.43b ±0.02	368c ±1.50	25.48a ±0.58	9.06a ±0.30	82.00c ±1.20	53.05c ±1.08	63.10 e±0.18	5.00a ±0.17	6.10a ±0.22	63.00a ±1.70
BW	3.57a ±0.14	11.21b ±0.13	15.88e ±0.15	1.70e ±0.03	226a ±1.60	0	0	78.40b ±1.18	29.00b ±1.03	61.3f ±0.25	19.5c ±0.08	18.1f ±0.13	390.0b±2.30
RF	2.13a ±0.24	8.64a ±0.17	11.82a ±0.20	0.31a ±0.01	611f ±1.50	0	0	57.00a ±1.24	19.00a ±1.06	53.2b ±0.31	19.9d ±0.15	13.2b ±0.20	507.0d ±1.50
B₁	2.15a ±0.19	10.76b ±0.15	12.77b ±0.14	0.38b ±0.04	509e ±1.80	0	0	N/A	N/A	57.6d ±0.16	16.5b ±0.25	14.0c ±0.17	507.0d±3.00
B₂	2.41a ±0.9	10.80b ±0.23	13.02bc ±0.19	0.50c ±0.03	432d ±1.90	0	0	N/A	N/A	54.1c ±0.22	19.5c ±0.14	15.5d ±0.10	488.0c±2.70
B₃	2.79a ±0.15	10.84b ±0.14	13.24c ±0.23	0.68d ±0.05	359b ±1.70	0	0	N/A	N/A	52.1a ±0.29	20.0d ±0.09	16.1e ±0.19	505.0d ±1.90

The mean value of replications (n=4) in a column differs significantly p<0.05, *FC* Fat content, *PC* Protein content, *MC* Moisture content, *FN* Falling number, *WG* Wet gluten, *DG* Dry gluten, *TW* Test weight, *TGM* Thousand Kernel weight, *WA* water absorption, *DDT* dough development time, *DST* dough stability time, *MTI* mixing tolerance index, *WF* Wheat flour, *BW* Buckwheat flour, *RF* Rice flour, *B₁* 10% BW flour: 90% RF, *B₂* 20% BW flour: 80% RF, *B₃* 30% BW flour: 70% RF

Table 2. Sensory characteristics and physical properties of cupcakes

Sample	Color	Taste	Flavor	Texture	Softness	Stickiness	Chew ability	Weight (g)	Height (cm)	Volume (ml)
WF	7.50bc ±1.08	8.00b ±1.24	7.90b ±1.05	5.50b ±1.21	3.00a ±1.01	2.00a ±1.30	9.00a ±1.43	90.00c ±0.47	4.90a ±0.29	281.0f ±0.43
BW	5.00a ±0.97	6.30ab ±0.97	6.20ab ±1.20	4.80ab ±1.04	2.50a ±1.31	3.00a ±1.08	8.50a ±1.71	90.40d ±0.29	4.80a ±0.43	266.1e ±0.19
RF	8.00c ±0.88	5.00a ±1.16	5.10a ±0.85	3.20a ±1.09	2.00a ±0.99	4.00a ±1.99	7.20a ±1.09	88.00a ±0.51	4.70a ±0.37	231.3a± 0.26
B₁	6.00ab ±1.04	5.90ab ±1.31	5.70a ±1.04	4.00ab ±1.11	2.20a ±1.25	3.40a ±1.57	8.00a ±1.68	89.00b ±0.73	4.73a ±0.18	238.3b ±0.28
B₂	6.80abc ±1.15	6.60ab ±0.89	6.40ab ±1.22	4.40ab ±0.99	2.30a ±1.09	3.30a ±0.86	8.30a ±1.19	89.40bc ±0.44	4.76a ±0.32	246.8c ±0.35
B₃	7.00bc ±0.99	6.50ab ±1.05	6.60ab ±0.91	4.60ab ±1.14	2.40a ±1.51	3.20a ±1.25	8.60a ±1.03	89.70bc ±0.31	4.78a ±0.41	250.5d ±0.21

The mean value of replications (n=4) in a column differs significantly p<0.05, *WF* wheat flour, *BW* buckwheat flour, *RF* rice flour, *B₁* 10% BW flour: 90% RF, *B₂* 20% BW flour: 80% RF, *B₃* 30% BW floor: 70% RF

When compared to wheat flour, BW flour demonstrated greater dough stability over a longer period (Table 1). BW was added to RF, and an increase in dough stability time was observed. A research work reflected that the dough stability was significantly higher, particularly for dough with a buckwheat flour percentage of 30 g/100 g, which was 4.6 min compared to dough with only wheat flour at 0.3 min (Nada *et al.*, 2011). The minimum value of the mixing tolerance index was visible in the dough made from wheat flour. In the RF and BW, the highest values of the mixing tolerance index were found respectively (Table 1). According to a study, water absorption values and the mixing tolerance index for resistant

starch-wheat flour blends increased significantly as the quantity of resistant starch increased from 0 to 20% (Lei *et al.*, 2008).

3.6. Physical properties of cupcakes

With the addition of BW flour to RF, a rising weight trend was observed. The height and volume of the cupcake were reduced as compared to the control sample (Table 2). These findings are consistent with previous research on the textural and physical properties of millet-based muffins. The height of control muffins (no barnyard millet flour) was 33.88 mm. When refined wheat flour was replaced with barnyard millet flour, the height was reduced from 33.22 mm to 28.20 mm (Goswami *et al.*, 2015). The decrease in height of the bread containing BWF may be attributed to the poor air bubble retention capacity and limited CO_2-holding ability of the buckwheat dough during baking (Mariotti *et al.*, 2013).

3.7. Sensory characteristics of cupcakes

The results of the sensory attributes of cupcakes are shown in Table 2. WF cupcakes received the highest scores for taste, flavor, texture, softness, and chew ability, whereas RF cupcakes received the lowest scores for taste. Research on the physical qualities and quality traits of gluten-free biscuits produced similar findings. WF biscuits received the highest taste rating, 18.80 out of 20, followed by blended flour made from rice, sorghum, and corn (1:1:1), which received an 18.00 out of 20 (Mancebo *et al.*, 2015). The same flavor ratings were obtained when examining the quality attributes of cookies made with BW and RF. In this investigation, sensory qualities were graded on a hedonic scale of 1 to 5 (1 being much disliked, and 5 being very liked) (Torbica *et al.*, 2012). A study on the quality traits and physical characteristics of gluten-free biscuits investigated textural differences. Biscuits were made with rice, sorghum, and corn flour, with WF serving as a standard (Mancebo *et al.*, 2015). Similar results were found in earlier studies of the quality of steamed cakes supplemented with ordinary and tartary BW. The sensory qualities of steamed cakes were evaluated using a 7-point Hedonic scale

(Cho *et al.*. Earlier studies looking at the qualitative features of cookies made with BW and RFs revealed equal chew ability results. In this study, sensory qualities were graded on a hedonic scale with 1 being much disliked and 5 being much liked. Cookies made with rice and BW flour (80:20) received the highest chew ability rating of 4.22 (Torbica *et al.*, 2012).

Cupcakes made by RF received the highest grade for stickiness, and cupcakes made by WF received the lowest score. The results of the present experiment are consistent with previous studies on the impact of BW flour on the sensory attributes and quality of eriste, Turkish noodles. A five-point scale (1-extremely detest, 3-acceptable, and 5-extremely like) was used to grade the cooked eriste that was produced (Bilgicli, 2009[b]). Cupcakes made by RF had the highest color score while BW received the lowest color score. The impact of whole BW flour and debittered lupin flour on the nutritional and sensory quality of the gluten-free cake had a significant finding that was closely related to previous research. On a scale of 1 to 5, the sensory qualities of cake samples were rated: 1 equaled a strong dislike, 3 an acceptable rating, and 5 a strong liking (Levent and Bilgicli, 2011).

3.8. Storage stability of cupcakes
3.8.1. Proximate composition

Table 3 shows the close composition and physical characteristics of cupcakes when they are being stored. The cupcakes made by BW had the highest moisture content on the first day, whereas B_1 had the lowest. During storage, cupcakes' moisture content progressively drained out. On the eighth day of storage, the range of moisture loss was highest in cupcakes made by WF and lowest in cupcakes made by RF. All of the other samples showed the same pattern. A similar pattern for moisture content after storage was seen in research on the storage study of pan bread. The moisture content was decreased in the crumb of pan bread during storage at 15ºC (Besbes *et al.*, 2014). Protein content marginally but not dramatically decreased after storage. According to a study on the

impact of replacing xylitol in cookies, protein content barely changed over 60 days of storage. According to a previous study, the fat content of cupcakes cooked by BW had the greatest value on the first day, measuring 23.10±0.22%, while those made by RF had the lowest value, 20.04±0.21% (Mushtaq *et al.*, 2010). The fat content of cupcakes made by WF marginally altered throughout the storage; it reduced on the eighth day.

Table 3. Proximate composition and physical properties of cupcakes at storage

Samples	D	MC	PC	FC	Weight(g)	Height (cm)	Volume (ml)
WF	0	15.73a±0.17	6.71a±0.22	21.30cc±0.13	90.00cd±0.47	4.90a±0.29	281.00f±0.43
	2	15.45a±0.18	6.72ab±0.23	21.31a±0.12	90.00cd±0.21	4.89c±0.73	280.98f±0.15
	4	15.00a±0.14	6.70b±0.21	21.26ac±0.20	89.91ad±0.61	4.79a±0.37	280.81f±0.30
	6	14.50ab±0.15	6.67a±0.17	21.21ab±0.21	89.60ac±0.66	4.67b±0.65	280.52e±0.41
	8	13.69a±0.12	6.60a±0.15	21.16a±0.19	89.20c±0.38	4.44a±0.34	280.29e±0.42
BW	0	17.87b±0.15	7.21c±0.13	23.10a±0.22	90.40d±0.29	4.80a±0.43	266.07e±0.19
	2	17.58b±0.11	7.20c±0.18	23.10c±0.13	90.38e±0.38	4.71ad±0.74	265.86ab±0.21
	4	17.00ab±0.14	7.20a±0.15	23.07d±0.17	90.25e±0.49	4.65d±0.55	265.65cd±0.22
	6	16.58bc±0.11	7.19d±0.10	23.06f±0.12	89.99ae±0.56	4.55c±0.63	265.37ef±0.43
	8	16.02a±0.12	7.16ad±0.20	23.02f±0.14	89.75f±0.62	4.43a±0.38	265.11ac±0.41
RF	0	13.79a±0.20	4.64abc±0.17	20.04e±0.21	88.00a±0.51	4.70a±0.37	231.3a±0.26
	2	13.49ab±0.17	4.63a±0.13	20.04a±0.15	87.95ab±0.67	4.65a±0.62	231.07f±0.32
	4	13.03ac±0.16	4.64bc±0.20	20.02f±0.18	87.78b±0.46	4.50b±0.72	230.96cf±0.23
	6	12.49a±0.18	4.62bc±0.19	20.00a±0.12	87.56b±0.53	4.47c±0.30	230.77e±0.34
	8	12.05b±0.14	4.60a±0.17	19.99b±0.16	87.35b±0.71	4.23c±0.52	230.59f±0.31
B_1	0	13.78ab±0.14	5.76ab±0.15	20.10ab±0.19	89.00b±0.73	4.73a±0.18	238.30b±0.28
	2	13.34b±0.15	5.78ac±0.10	20.10c±0.22	88.97c±0.25	4.65ab±0.61	238.04ab±0.42
	4	13.01b±0.11	5.75a±0.16	20.08a±0.21	88.79c±0.39	4.62abc±0.77	237.85c±0.26
	6	12.47ab±0.12	5.71c±0.18	20.04b±0.22	88.57ac±0.46	4.43ac±0.53	237.68e±0.15
	8	12.00c±0.17	5.66d±0.11	20.00ab±0.18	88.48e±0.52	4.21a±0.48	237.41f±0.19
B_2	0	14.03ab±0.19	5.80b±0.23	20.30cd±0.17	89.40bc±0.44	4.76a±0.32	246.75c±0.35
	2	13.71ac±0.16	5.81abc±0.17	20.29cd±0.18	89.31c±0.61	4.61c±0.65	246.56e±0.29
	4	13.25bc±0.09	5.79c±0.22	20.25e±0.14	89.19d±0.59	4.52a±0.76	246.22f±0.28
	6	12.77b±0.18	5.75e±0.21	20.25e±0.11	89.02d±0.27	4.45ab±0.38	246.10c±0.35
	8	12.26a±0.14	5.70b±0.18	20.21a±0.21	88.99d±0.67	4.23abd±0.29	245.83d±0.31
B_3	0	14.25ab±0.23	5.84e±0.14	20.41ab±0.15	89.70bcd±0.31	4.78ac±0.41	250.50d±0.21
	2	14.01ab±0.22	5.85ac±0.21	20.41a±0.21	89.59abc±0.77	4.63c±0.72	250.28a±0.34
	4	13.61b±0.20	5.81e±0.22	20.37c±0.14	89.44ac±0.48	4.54b±0.64	250.03ad±0.41
	6	13.11a±0.18	5.80d±0.18	20.31ab±0.17	89.28bc±0.38	4.39b±0.45	249.81d±0.26
	8	12.66a±0.14	5.78f±0.11	20.31bc±0.11	89.06b±0.45	4.21b±0.39	249.66e±0.23

The mean value of replications (n=4) in a column differs significantly p<0.05, *MC* moisture content, *FC* fat content, *PC* protein content, *WF* wheat flour, *BW* buckwheat flour, *RF* rice flour, B_1 10% BW flour: 90% RF, B_2 20% BW flour: 80% RF, B_3 30% BW flour: 70% RF, *D* days.

Weight loss was less in cupcakes based on RF as compared to BW flour and WF during storage (Table 3). Similar results were noted in a study on the quality of gluten-free bread. Bread made of rice flour and corn had 47.42% and 45.27% moisture content during the storage period of 01 to 03 days (UCok and Hayta, 2015). The same findings were reported in research on muffin coating with starch-based bio-composite films, the edible muffin line performed best with 2% potato starch-based films integrating 5% CNF and 30% glycerol

(w/w starch) (Shih and Zhao, 2021). Volume values for all of the samples dropped considerably after storage. Similar findings were made when examining how long cupcakes were stored frozen and after par-baking. After storage, the specific volumes for cakes par-baked for 15 and 20 minutes decreased from 2.5±0.12a to 2.32±0.02b (cm^{3}g^{-1}) and 2.51±0.03a to 2.33 ± 0.02b (cm^{3}g^{-1}) respectively (Karaoglu *et al.*, 2008).

3.8.2. Sensory characteristics of cupcakes in storage

Table 4 provides the average sensory ratings given to cupcakes following storage. After 8 days of storage, scores for color were lower for all cupcake samples. The cupcakes made with RF received the highest rating, followed by those made with WF and BW flour. For every other cupcake, the same scoring trend was seen.

Table 4. Sensory properties of cupcakes at storage

Samples	D	Color	Taste	Flavor	Texture	Softness	Stickiness	Chewability
WF	0	7.50bc±1.08	8.00b±1.24	7.90b±1.05	5.50b±1.21	3.00a±1.01	2.00a±1.30	9.00a±1.43
	2	7.77a±1.02	8.00a±1.28	7.75bc±1.29	5.29ac±1.29	3.02ba±1.11	2.01b±1.27	8.90b±1.77
	4	7.30ab±0.99	7.55a±1.08	7.46bc±1.09	5.00bc±0.87	3.05±1.09	1.99cf±0.97	8.69b±0.72
	6	7.00a±1.01	7.11b±1.04	7.21d±1.03	4.75b±1.29	2.99a±0.91	1.95c±1.44	8.54b±1.56
	8	6.79a±1.05	6.57bc±0.93	7.00ad±0.99	4.53b±1.22	2.80cd±0.82	1.90c±1.32	8.17b±1.67
BW	0	5.00a±0.97	6.30ab±0.97	6.20ab±1.20	4.80ab±1.04	2.50a±1.31	3.00a±1.08	8.50a±1.71
	2	5.08a±0.91	6.00e±0.91	6.05ab±1.08	4.70a±1.33	2.52a±1.23	2.99e±1.02	8.40a±1.76
	4	5.00b±0.94	5.59e±1.12	5.77ac±1.28	4.39a±1.20	2.47ab±0.89	2.95f±1.26	8.00a±1.42
	6	4.91b±1.03	5.13a±1.21	5.39c±1.31	4.00a±1.19	2.25c±0.99	2.80c±0.96	7.82c±0.69
	8	4.66b±1.05	4.68a±1.15	5.00a±1.34	3.74ab±1.04	2.05c±1.20	2.65c±1.29	7.69d±1.61
RF	0	8.00c±0.88	5.00a±1.16	5.10a±0.85	3.20a±1.09	2.00a±0.99	4.00a±1.99	7.20a±1.09
	2	8.05a±0.94	4.79d±0.89	5.02b±0.81	3.12c±0.90	2.01bc±0.82	3.99ac±1.37	7.07b±1.88
	4	8.00ac±1.02	4.33ad±1.27	4.77b±0.86	3.00a±0.83	1.98bc±0.97	3.85b±0.87	6.99ab±1.02
	6	7.89ab±1.09	4.00ac±1.19	4.48a±0.86	2.86a±1.33	1.92c±1.23	3.81b±1.36	6.77c±0.91
	8	7.60a±0.98	3.67c±1.14	4.15a±1.09	2.59a±1.26	1.78c±1.08	3.72bc±1.55	6.55e±1.48
B$_1$	0	6.00ab±1.04	5.90ab±1.31	5.70a±1.04	4.00ab±1.11	2.20a±1.25	3.40a±1.57	8.00a±1.68
	2	6.07ac±1.11	5.92c±1.18	5.69ab±1.21	3.99bc±0.91	2.12a±1.09	3.32bc±1.31	7.95ae±1.88
	4	5.95b±1.09	5.55c±1.32	5.31ab±1.08	3.65bc±1.29	2.01a±1.02	3.18bc±0.78	7.66f±1.92
	6	5.69b±0.99	5.11b±0.97	5.11c±1.35	3.37cd±1.27	1.95a±0.91	3.04a±1.01	7.37a±1.72
	8	5.50e±0.87	4.77b±1.06	4.00bd±1.26	3.00d±1.09	1.81a±0.90	2.91a±0.78	7.13cd±0.90
B$_2$	0	6.80bc±1.15	6.60ab±0.89	6.40ab±1.22	4.40ab±0.99	2.30a±1.09	3.30a±0.86	8.30a±1.19
	2	6.80e±1.11	6.40bc±0.82	6.12e±1.31	4.36cd±1.00	2.21a±1.02	3.21c±1.66	8.15b±1.27
	4	6.65e±1.17	6.00a±1.26	6.00f±1.26	4.16ad±1.29	2.11cd±1.21	3.01c±1.29	8.02cd±1.36
	6	6.46a±0.95	5.76c±1.18	5.70e±0.88	4.00ac±0.91	1.98d±1.19	2.93e±1.37	7.82c±0.77
	8	6.20a±1.05	5.39c±1.10	5.33c±0.82	3.88b±1.22	1.86d±1.28	2.81e±1.63	7.39c±1.28
B$_3$	0	7.00bc±0.99	6.50ab±1.05	6.60ab±0.91	4.60ab±1.14	2.40a±1.51	3.20a±1.25	8.60a±1.03
	2	6.99c±0.98	6.44d±1.03	6.50c±0.92	4.44a±0.91	2.25e±1.20	3.04ab±0.94	8.48b±1.28
	4	6.86e±0.87	6.01d±0.93	6.19c±0.94	4.21a±1.28	2.11ae±0.85	2.92b±1.67	8.24a±1.23
	6	6.59a±1.17	5.68d±1.02	6.02d±1.22	4.00a±1.33	2.02c±1.42	2.81a±1.22	8.00a±0.99
	8	6.38d±1.09	5.32d±1.09	5.76a±1.28	3.78c±1.21	1.92a±1.40	2.67ae±1.87	7.79b±1.59

The mean value of replications (n=4) in a column differs significantly p<0.05, *WF* wheat flour, *BW* buckwheat flour, *RF* rice flour, *B$_1$* 10% BW flour: 90% RF, *B$_2$* 20% BW flour: 80% RF, *B$_3$* 30% BW flour: 70% RF), *D* days

On the first and second days of storage, scores for taste and flavor of cupcakes made by WF were maintained, and then they started to decline. Cupcakes made with WF received the highest flavor rating on the eighth day of storage, followed by those made with BW flour and RF. After storage, cupcakes made with WF received the greatest scores for softness, stickiness, and chew ability, followed by those made with BW flour and RF. The cupcakes made by WF and B3 received the highest ratings during the organoleptic examination.

Similar outcomes were determined in a prior investigation employing acceptance tests to examine the sensory characteristics of chocolate and carrot cupcakes. After 180 days of storage, scores for texture and flavor were much worse. After 180 days of storage, the mean scores for texture and flavor decreased from 7.4 to 4.2 and 7.1 to 4.9, respectively (Villanueva and Trindade, 2010). A previous study on the sensory aspect of biscuits reflected panelists' assessment of general acceptability as well as the attributes for which substantially different scores were assigned to each formulation. The evaluation of odor, aroma, and mouth-feel qualities yielded mean scores ranging from 5.27-5.61, 4.75-5.63, and 5.05-5.69, respectively (Oksuz *et al.*, 2016). The impact of flaxseed on bread production was documented in a study.

The sensory scores for the softness of linseed rolls and linseed cinnamon rolls (bakery products) decreased from 7.3 to 5.3 and 6.3 to 2.9, respectively, after 6 days of storage (Pohjanheimo *et al.*, 2006).

4. Conclusions

A significant difference (p≤0.05) was recorded in the physicochemical, rheological, and sensory properties of BW flour, RF, flour blend, and wheat flour. Cupcakes prepared with BW flour resulted in maximum moisture, fat, and protein content compared to cupcakes prepared with RF and WF. Overall sensory properties of cupcakes prepared by BW flour and RF increased the quality, sensory scores, and acceptability. RF contained more BW flour resulting in maximum weight, height, and volume of cupcakes. Sensory scores for cupcakes prepared by BWF and RF blends were significantly decreased at 8 days of storage whereas, softness and stickiness decreased non-significantly. A non-significant decreasing trend was noticed in the height of cupcakes as compared to weight during storage. RF and BW flour blends are applicable substitutes for gluten-containing flours. Blend B3 was evaluated as most suitable for gluten-free cupcakes based on quality parameters and sensory attributes. Research findings can be utilized for other products to explore functional properties.

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Acknowledgment

MODELLING OF THE ECOLOGICAL DRYING PROCESS OF TOMATOES BY THE NON-CONVENTIONAL DESIGN OF EXPERIMENTS METHOD

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ABSTRACT
The preservation of perishable foods through their transformation is always a major challenge for producers. Several methods are applied for this purpose, and progresses are recorded by improving these processes or by finding new means. In our work, the method of dehydration and natural drying of tomatoes using a natural dryer with indirect solar energy with air circulation by extractor is used to describe and then predict the process of obtaining the finished product in the form of tiny dried fragments or powder. This is the novelty of this research, which uses the unconventional design of experiments. This consists of finding a mathematical model based on the interaction of 3 parameters that interact with each other, namely the trays temperature, the drying speed and the relative humidity in the solar dryer. The interference of the values of one of these 3 parameters on the 2 other parameters acts directly on the result of the drying process. The matrix treatment of the data of several carried out experiments in the resolution of 13 equations gives us the required responses and permits us to draw the graphs, the contours and the responses surfaces that access us to a more detailed analysis.

1. Introduction

The description of the process of conservation by drying of tomatoes with a mathematical model according to the temperature of the trays on which the tomatoes are deposited, the relative humidity inside the dryer and the drying speed which describes the mass loss of the product during the experimental duration, has never been treated so to date by experimental design. This method does not take into account the heat and mass balances or other parameters often cited in any researches (Badaoui et al., 2019; Eichenlaub et al., 2015; Gary, 2002; Lopez-Quiroga et al., 2019; Azeez and al., 2019; Seyfi, 2014) but considers the

parameters that affect the drying process (Akshay, S., Sumit, S.P., Rama, C. P. (2021). It is all the more important as it is considered as a predictive method, since it can predict results in the experimental domain with the values not considered during the manipulations. This is realised by modelling the dehydration and drying process by a quadratic mathematical expression in the form of a polynomial that establishes a linear relationship between a variable called explained variable, and several other variables called explanatory variable (Goupy, 2005; Goupy, 2013; Louvet, 2005).

Therefore, this is different from other modelling done before and based on other

criteria than the ones mentioned in this paper. The drying and dehydration process is explained differently (Çelen et al., 2013; Sivakumar et al., 2013; Haddad et al., 2008; Manashi et al., 2013; Nazmi et al., 2018; Petro-Turza, 2009; Pushpendra et al., 2018; Doris et al., 2010; Nagwekara et al., 2020; Chauhan et al., 2021). For this purpose, a series of experiments is carried out in a solar radiation dryer without additional heating by electrical energy, but with forced ventilation of air from the container inside to the outside, thus avoiding the combined process (mixed-method) (Alkanan et al., 2021).

This is deliberately done to lower the price of the completed product. To compensate the low airflow from the bottom to the top, a vacuum cleaner is added at the air outlet of the dryer; it starts whenever the average trays temperature exceeds 56°C, thus lowering the maximum level of tomatoes damage. This means is preferred in the region because the quantity of tomato production is becoming more and more impressive and the conditions for its application exist. The number of sunshine hours reaches 3488.5 hours per year; the temperature in the sun mainly in the months from May to August is 48 °C with a maximum humidity rate of 80%.

The predictive result given by the modelling method using non-conventional experimental designs allows knowing previously the hours number of dehydration and drying of the food under changing conditions of the 3 mentioned parameters without making additional experiments. This method, in addition to being based on experiments: a- It has the ability to be descriptive and predictive at the same time, b- It does not require a high experiments number, c- It is polynomial form. b- It does not include any difficult functions, d- It has an interactive correlation between the factors. The finished product, obtained in the form of powder is preserved for a long time, it is ecological since its density is reduced, does not require great means of packaging and finally nutritional in

vitamins that are found in the initial fruit with an accessible price.

2. Materials and methods
2.1. Elaboration of the experimental test rig for the used method

In order to elaborate the experiments of tomatoes dehydration and drying, a test bench constructed by us in our laboratory is used as experimental material. This dryer with indirect solar conversion, composed of 2 distinct compartments, is elaborated with wood. Aluminium sheets envelop the first chamber; it is comported trays. This material is used to reinforce the thermal conduction, while the second chamber with the absorbing plaque is painted with a black colour to avoid the excessive reflection of the solar rays outside.

The first compartment, with a rectangular section, called the drying chamber is designed to contain the grilled trays, while the second, communicating with the first, is of parallel-piped form and is equipped with a glass inclined of 30°, called solar radiations collector; these rays end up at the absorbing tray.

They are destined to be transformed in calorific energy inside the chamber. This ergonomics is studied so that the solar rays do not strike directly on the food; this adaptation allows the heat to dry it, avoiding the deterioration and the putrefaction of the tomatoes (figure 1).

Nine positions for different sensors are chosen on the dryer.

Three positions for sensors at the grilled trays level inside the drying chamber, one at the level of the air inlet, a fourth at the top of the chamber, another at the air outlet, a seventh at the heat-absorbing tray, an eighth at the glazing window level. The last one is installed at the outside to measure the parameters of the ambient air. The used main devices to measure the parameters during the drying process are:

Figure 1. The experimental test rig of drying

Table 1. Experimental and coded values of the 3 parameters

Hours number of	Temperature trays °C	Coded values of temperature	Drying speed g/Hm²	Coded values of drying	Relative humidity %	Coded values of Relative
1	28.3	-1	11.53	1	66	1
2	36.6	-0.385	5.24	-0.0987	63	0.897
3	41.2	-0.0444	3.15	-0.464	59	0.759
4	45	0.237	2.12	-0.644	52	0.517
5	47.8	0.444	1.51	-0.75	43.4	0.221
6	49.4	0.563	1.11	-0.82	37	0
7	50.3	0.63	0.81	-0.872	43.7	0.231
8	51.5	0.719	0.61	-0.907	36.4	-0.0207
9	53.5	0.867	0.45	-0.935	33	-0.138
10	53.7	0.881	0.32	-0.958	27.9	-0.314
11	54.1	0.911	0.22	-0.976	23	-0.483
12	55.2	0.993	0.14	-0.99	16	-0.724
13	55.3	1	0.08	-1	8	-1

1. Digital hot wire anemometer, wind speed meter, airflow, 0 to 30 m/s, with USB Interface and thin sensor, GM8903.
2. Development board for Arduino, humidity sensor with hygrometer LM393.
3. Temperature sensor probe 0-400 °C, M6 thermocouple thread Type k1/2/3/4/5m, temperature controller.
4. Digital hygrometer with probe, LCD display, integrated temperature and humidity.

2.2. Experimental measures

Thirteen values of each of the 3 parameters influencing the dehydration and drying process were recorded along 13 hours of sun exposure. They are displayed in Table 1 above.

The values of the 3 parameters (Trays temperature, drying speed and relative humidity) are used obligatorily in the polynomial mathematical model in coded form. The sum of the 10 terms (Equation 1) is only possible if they have the same dimensions. However, the parameters have different units and values at different scales. One eliminates the units of the 9 monomials and one gives their values between -1 and +1 (Table 1).

$$y = a_0 + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_{11} x_1^2 + a_{22} x_2^2 + a_{33} x_3^2 + a_{12} x_1 x_2 + a_{13} x_1 x_3 + a_{23} x_2 x_3$$

(1)

Since 13 experimental measurements have been carried out, one then forms 13 equations of type 1 by replacing each time in the polynomial the values of x_i by forming a regression from Y into X. They are represented in matrix form, which facilitates their resolution and allows us to obtain the coefficients a_i and a_{ij} of the researched model (1) by the least squares method in equation (2). The sine qua non condition is the necessity to have a number of distinct experiments (here 13) superior or equal to the number of coefficients (here 10). (Goupy, 2005; Goupy, 2013; Louvet, 2005).

Table 2. Descriptive table of the elements composing the mathematical model.

Name and		Symb	Value
Constant	Cst	a_0	5.08907
Temperature of trays	Tpl	a_1	3.06431
Drying speed	Dry	a_2	-1.98757
Relative humidity	Hum	a_3	-0.731186
Temperature of trays *Temperature of trays	Tpl*Tpl	a_{11}	1.05761
Drying speed*Drying speed	Dry*Dry	a_{22}	-0.27053
Relative humidity *Relative humidity	Hum*Hum	a_{33}	-0.281137
Temperature of trays *Drying speed	Tpl*Dry	a_{12}	-0.141348
Temperature of trays *Relative humidity	Tpl*Hum	a_{13}	-1.39046
Drying speed*Relative humidity	Dry*Hum	a_{23}	0.24204

$$a_{ij} = \left(X^t X \right)^{-1} \left(X^t \right) (Y)$$

(2)

Where:

X: Model matrix with 10 columns and 13 lines,
X^t: Matrix transpose (Reverse of X),
$(X^t X)^{-1}$: Information matrix (idem),

(Y): is response matrix with 1 column and 13 lines. The informations and values of the a_{ij} coefficients are shown in table 2.

Then equation (1) takes the final form of the mathematical behavioural model of the dehydration and drying process (Equation 3). (Goupy, 2005; Goupy, 2013; Louvet, 2005).

$$y = 5.08907 + 3.06431x_1 - 1.98757x_2 - 0.731186x_3 + 1.05761x_1^2 - 0.27053x_2^2$$

$$- 0.281137x_3^2 - 0.141348x_1 x_2 - 1.39046x_1 x_3 + 0.24204x_2 x_3$$

(3)

3. Results and discussions

This part is composed of 2 distinct analyses, the first one being statistical and consists in evaluating the obtained model by coefficients and tests in order to know its descriptive and predictive quality, and then applying it on the elaborated experiments.
The second is a detailed analysis of the action of the 3 parameters and their interactions on the drying process of tomatoes.

3.1. Quality of the mathematical model

The statistical calculation carried out by the Modde 6 software allows us to obtain the values of two quality coefficients.

The statistical calculation carried out by the Modde 6 software allows us to obtain the values of two quality coefficients.

3.1.1. Descriptive and predictive coefficients

The first one being the coefficient of the descriptive quality of the model R^2.

It is the percent of the variation of the response explained by the model ($0 \leq R^2 \leq +1$). It is equal at 0.995 and is very close to +1.

The second one being the coefficient of the predictive quality of the model Q^2 ($-\infty < Q^2 \leq +1$).

It is the percent of the variation of the response predicted by the model. His value is 0.988 and it is very close at +1.

These 2 very satisfactory values show that the expression (3) describes and predicts the studied process. (Goupy, 2005; Goupy, 2013; Louvet, 2005).

3.1.2. Fisher test

The F-Fisher test is a statistical hypothesis test that allows comparing 2 variances by doing their ratio which must not exceed a certain theoretical value that we extract from the Fisher-Snedecor table.

One generally compares the observed Fisher number F_{obs} from the experiments, and the critical Fisher number F_{crit} from the established tables.

The first one must be higher than the second one, then the regression is globally significant, the model is acceptable with a risk of 5% that it is not realised (Choice initially taken at the beginning of the study). (Goupy, 2005; Goupy, 2013; Louvet, 2005). In our case $F_{obs}=336.19>F_{crit}= 8.81$.

3.1.3. Student test
The Student "t-test" is a statistical test allowing to compare the means of 2 groups of values having a link (here the values of the observed and predicted responses of "y") in order to know if they are significantly similar or different. It is therefore used to test the significance of the coefficients that compose the mathematical model. The method is based on the following variance (Equation 4), and the t_{crit}-Student coefficient. (Goupy, 2005; Goupy, 2013; Louvet, 2005):

$$S^2 = \frac{1}{n-p} \sum_{i=1}^{30} e_i^2 \qquad (4)$$

The student test shows that 4 coefficients on 10 of model (3), namely a_{22}, a_{33}, a_{12} and a_{23} have a lesser effect on the response and the 6 other are preponderant. This does not affect the quality of this modelling.

3.2. Parameters actions of the model on the response

As the model (3) sh0ows, firstly, each parameter acts separately, and each combination of parameters 2 by 2, acts secondly on it. The sum of these influences gives deviations between the experimental and the theoretical expression.

3.2.1. Deviations analysis

The 10th measure is the one with the lowest deviation evaluated at 0.22%. The drying behaviour of tomatoes at a temperature of 53.7 °C, with a dehydration speed of 0.32 g/Hm^2 and reaching a relative humidity of 27.9% shows that these values are theoretically attained after 9 hours and 59 minutes instead of 10 hours (Figure 2).

The biggest deviation is observed in experiment 7; it is evaluated at 6.48%. At this level, the measured average temperature on the trays is 50.3 °C, the tomatoes speed of dehydration and drying is 0.81 g/Hm^2 when the relative humidity inside the dryer is 43.7%.

These 3 parameters are reached after 6 hours and 32 minutes instead of 7 hours. On the graph in Figure 2, the points with the least deviations are the points of experiments 1, 10, and 13.

Contrary, the most deviated points are located in experiments 6 and 7. The average of the 13 deviations in absolute values is 0.22 hours, while taking into account the positive and negative signs is $7.76 \cdot 10^{-7}$ hours. This is considered as very encouraging results.

Figure 3 illustrates the initial state of the tomatoes on the 4 trays before the drying process is started. The temperature is equal to the ambient temperature at 18 °C in the morning, the relative humidity is around 80%, and the drying speed has not yet started.
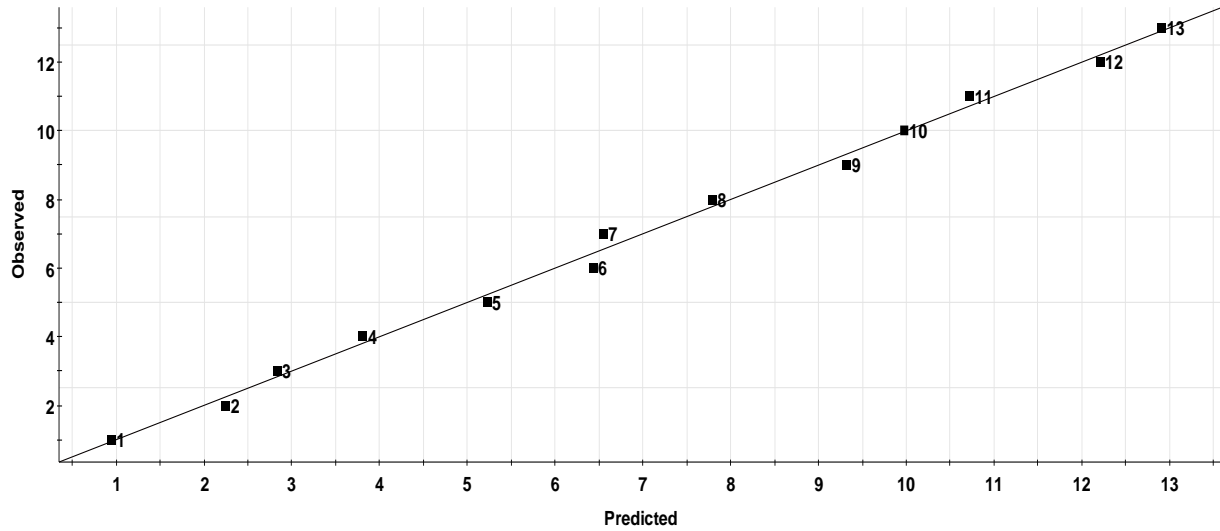
**Figure 2.** Illustrative figure of the deviations between model and experiments

Figure 3. General view of the 4 dryer trays before drying processes.

3.2.2. Analysis of the drying process under the effect of the parameters interaction

Through these 6 graphs, one can visualize the behavior of the dehydration and drying process along the 13 experiments. As many graphs can be represented as one wishes. Three cases are sufficient to understand the phenomenon, when the relative humidity has its higher value of 66% at 1 hour of drying, when it has its lower value of 8% after 13 hours of drying, then finally for an average intermediate value of 37% at exactly 7 hours of the dryer exposure to sunlight (Figure 4).

HR= 8% **(a)**   HR=37% **(b)**   HR=66% **(c)**
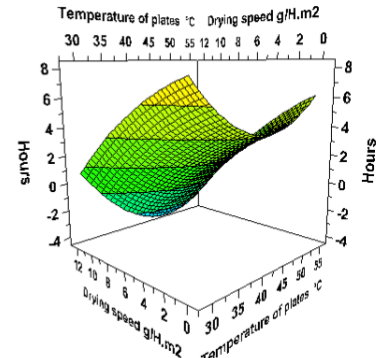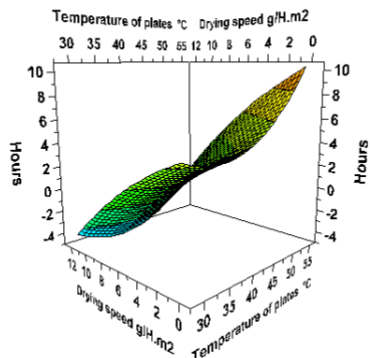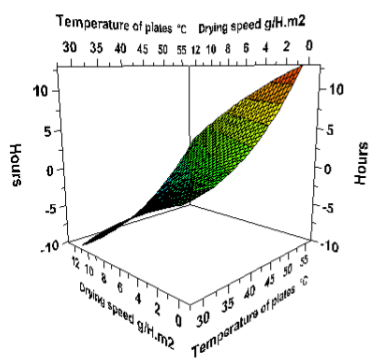
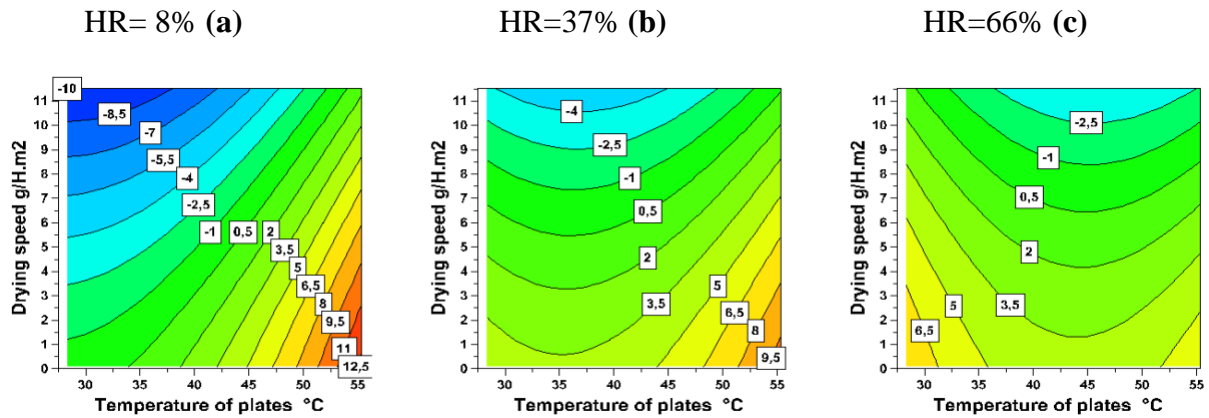**Figure 4.** Illustration of the drying process by response surfaces and contours at relative humidity of 8% and 66%

Firstly, the negative hourly values represented on the contours of the 3 cases are only theoretical values, they are given by the model (3) and are therefore rejected. Only the positive dehydration and drying hours values are taken into account and analysed. Secondly, the theoretically negative number of drying hours of tomatoes becomes less by going from 8% relative humidity (-8.5 hours) to 66% (-2.5 hours).

Table 3. Values for drying speed and trays temperature at 8% relative humidity

Relative humidity (%)	Dryin speed g/Hm^2	Trays temp (°C)	Time Hours
8%	6.5	47.5	1.37
	5	50	4.55
	3	51.3	7.36
	1	52.5	9.79

For a relative humidity of 8%, the number of drying hours increases with the increase of the temperature of the trays and the decrease of the dehydration speed, reaching 12.5 hours of drying at a temperature of 55°C and a speed lower than 1 g/Hm^2. The table 3 below mentioning some values confirms what has been said before.

For a relative humidity of 37%, the increase in the number of drying hours is done in 2 steps. For all contours the drying speed decreases to a minimum value and then increases to the maximum value of drying hours. There, the simultaneous increasing of the dehydration speed and the temperature of the trays increases the number of hours of exposure to the sunlight. Table 4 shows the minimum points of each contour. Before this point, it is the decrease of the drying speed and the increase of the temperature of the trays that keeps the drying time constant, but after this point, it is the simultaneous increasing of the 2 mentioned parameters that keeps the dehydration time in constant value.

Table 4. Minimum points of the 3 contours changing the drying behaviour for HR=37%

Relative humidity %	Dryin speed g/Hm^2	Trays temperature °C	Time Hours
37%	5.5	36	0.5
	3.25	35.7	2
	0.5	35.5	3.5

For the case of 66% relative humidity, the domain of definition of the drying process is larger since the negative theoretical number of hours is less.

One notices the same behavior as before with minimal contours points is around 45°. Below, are some values of parameters for a relative humidity of 66% (Table 5).

After 13 hours of the dryer exposure to the sunlight, the tomato dries by shrinking and losing its mass. Its water content, which is at the 1st hour of 11.53 g/kg of dry matter, becomes 1.09 g/kg of dry matter, i.e. a loss of 90.54 % of its initial mass.

These pieces of dried tomatoes are then transformed into tomatoes powder (Figure 5-b) by a mixer with a sharp blade turning at very high speed. (Castoldi et al., 2015).

After 13 hours of the dryer exposure to the sunlight, the tomato dries by shrinking and losing its mass. Its water content, which is at the 1st hour of 11.53 g/kg of dry matter, becomes 1.09 g/kg of dry matter, i.e. a loss of 90.54 % of its initial mass.

Table 5. Values for drying speed and trays temperature at 66% relative humidity

Relative humidity %	Dryin speed /Hm^2	Trays temperature °C	Time Hours
66%	10	32	0.5
	6	36.5	2
	4.5	47.5	2
	8	53.8	0.5

These pieces of dried tomatoes are then transformed into tomatoes powder (Figure 5-b) by a mixer with a sharp blade turning at very high speed. (Castoldi et al., 2015).

What remains in slices or in powder is without diseases and pure raw substance devoid of water, which is a nutritional source of vitamins A, C and E, of minerals, of dietary fiber and of antioxidants with beta-carotene (Figure 5). (Arslan et al., 2011, Clarke et al., 1979, Podsędek et al., 2003, Galhardo et al., 2009).

The water content of the tomato is very high at its harvest, reaching up to 90% of its mass, which favours its degradation in its fresh state and its physicochemical and microbiological deterioration. Its dehydration and its drying are more than necessary (Rajkumar, 2007).

The ascending hot air in the dryer can lead to diseases and degrade lycopene, carotene and ascorbic acid of the tomato; the control of the drying paramete99rs is thus primordial (Manzo et al., 2019, Demiray et al., 2013; Zanonia and al., 1998)
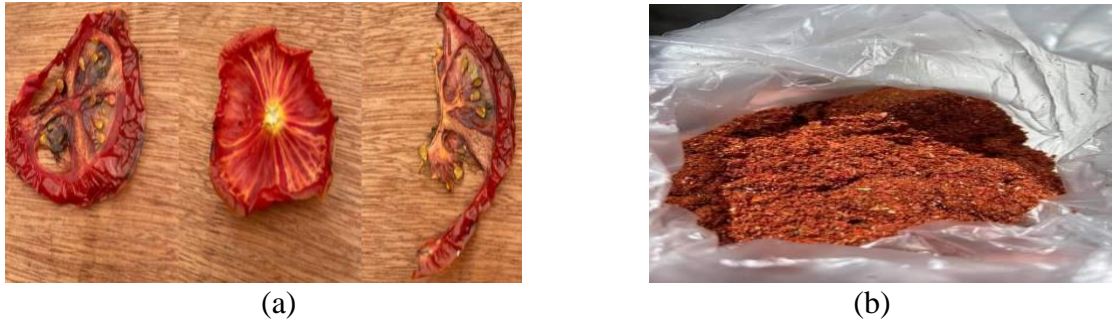
(a)   (b)

Figure 5. Tomatoes status (slices and powder) after 13 hours of drying

3.2.3. Variation of the drying time according to the experimental values of the 3 parameters

In the following analysis, one shows the variation of the dehydration and drying time as a function of each parameter acting on the process when the 2 other are invariant and relative to experiments 1, 7 and 13 (Table 1). In the 9 following equations of curves, the parameters x_i are expressed in coded values

(Table 1), while the response y (drying time) is expressed in real values, i.e. in hours.

When T_{pl} =28.3 °C, D_{speed}=11.53 g/Hm², HR=66% (Experience 1): One remarks that the domain of definition of y=f(T_{pl}) gives a negative number of theoretical hours of tomatoes drying, which is practically impossible to realise, therefore one rejects this case (Figure 6-a).
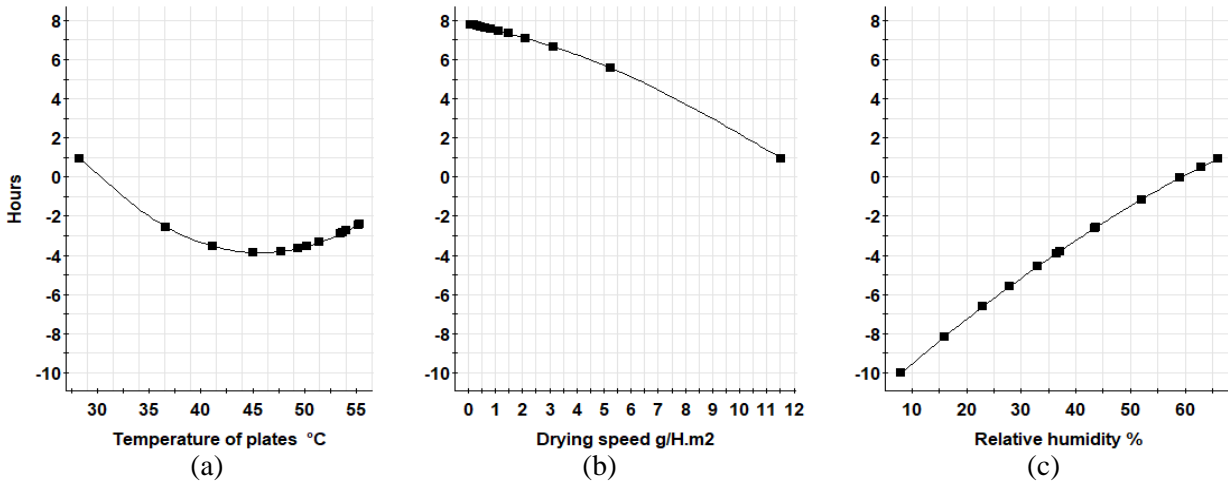

(a)   (b)   (c)

Figure 6. Drying time variation with experiment number 1

On the graph of Figure 6-b, the curve designed by y=f(S_{drying}) is decreasing. It shows that with the increasing of the drying speed, the hours number of the tomatoes dehydration process decreases from 7.78 hours at the process beginning to 0.933 hours (at 11.5 g/Hm² of drying speed) and this, under the influence of the 2 other parameters, i.e. temperature of plates and relative humidity. The decline of close to 12% of the tomatoes drying hours in the dryer is important with the increase of the dehydration

speed. On the 6-c ascending curve y= f(HR), for the values of the 3 mentioned parameters, the ascending variation of the hours number of tomato drying between the beginning of the process and 1 hour is explicable only for relative humidity rates varying between 58.8% and 66%. All the rest of the graph being theoretical with a negative hours number, in other words, an area to be rejected. When T_{pl} =50.3 °C, D_{speed}=0.810 g/Hm², HR=43.7% (Experience 7): In Figure 7-a, the number of drying hours decreases from

5.08 hours at 28.2 °C to 3.81 hours at 37.7 °C, and then increases again to 8.98 hours when the average tray temperature reaches 55.2 °C. Roughly speaking, under the influence of the drying rate of 0.81 g/Hm^2 and the relative humidity of 43.7%, the drying hours number

decreases from 5.08 hours at 28.2 °C to 3.81 hours at 37.7 °C, and then increases again to 8.98 hours when the average tray temperature reaches 55.2 °C. Roughly speaking, under the influence of the drying rate of 0.81 g/Hm^2 and the relative humidity of 43.7%.
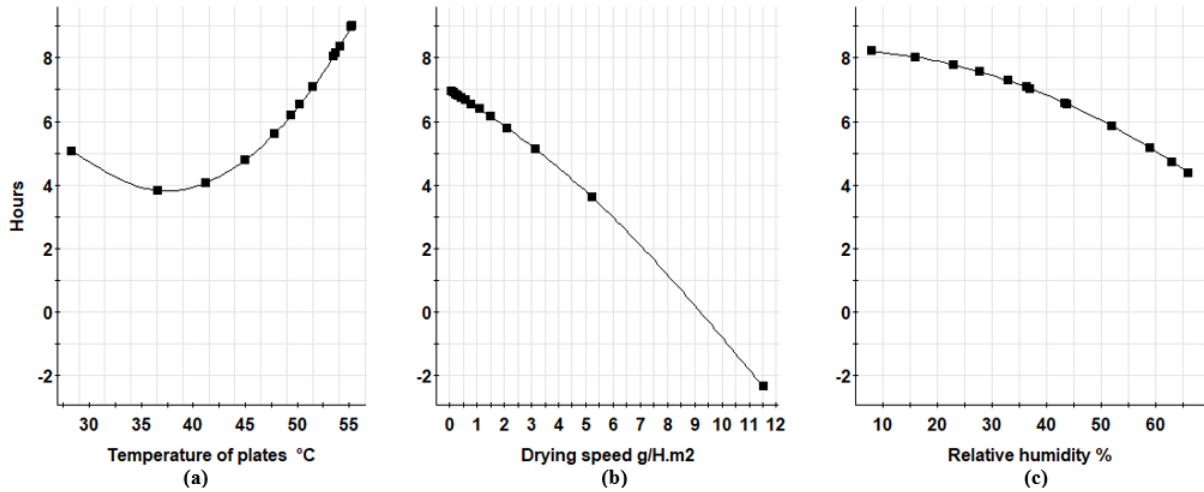

Figure 7. Drying time variation with experiment number 7

Paradoxically, the increase of the trays temperature increases the hours number of tomato drying for the reason of the interactions of the 2 other parameters acting on the mathematical model.

Figures 7-b and 7-c, show both, the decreasing number of drying hours with the

increase of the dehydration speed on one hand and the relative humidity on the other hand (table 6), always under the interactions of the 2 other remaining parameters as showing the mathematical model (3).

Table 6. Illustrative values of figures 7-b and 7-c

Figure 7-b		Figure 7-c	
Dryin speed g/Hm^2	Dryin g time hours	Relative humidity %	Drying time hours
0.03	6.93	7.89	8.2
9.13	0	65.7	4.36

When T_{pl} =55.3 °C, D_{speed}=0.08 g/Hm^2, HR=8% (Experience 13): In the analysis of this 3rd case, concerning specifically the experiment 13, where the average temperature of the trays takes its maximum value, the drying speed and the relative humidity take their minimum value (Figure 8). One notices that the 3 curves occupy

totally a real and large physical definition domain because the dehydration and drying hours are completely positive.

Again, the variation of drying hours under the effect of one of the 3 parameters (Figure 8-a-b-c) is dependent of 2 others, here of the drying speed of the tomatoes which is equal at 0.08

g/Hm2, and of the relative humidity equal at 8% for drying temperature parameter (for example). The interaction of the 3 parameters between them on the drying hours number of the tomatoes is omnipresent, it always responds to the mathematical model (3). Thus, increasing

the trays temperature increases the number of drying hours (Figure 8-a), and increasing the drying speed and relative humidity decreases it. (Figure 8-b and 8-c). Below, there are some illustrative values of this behaviour (Table 7).
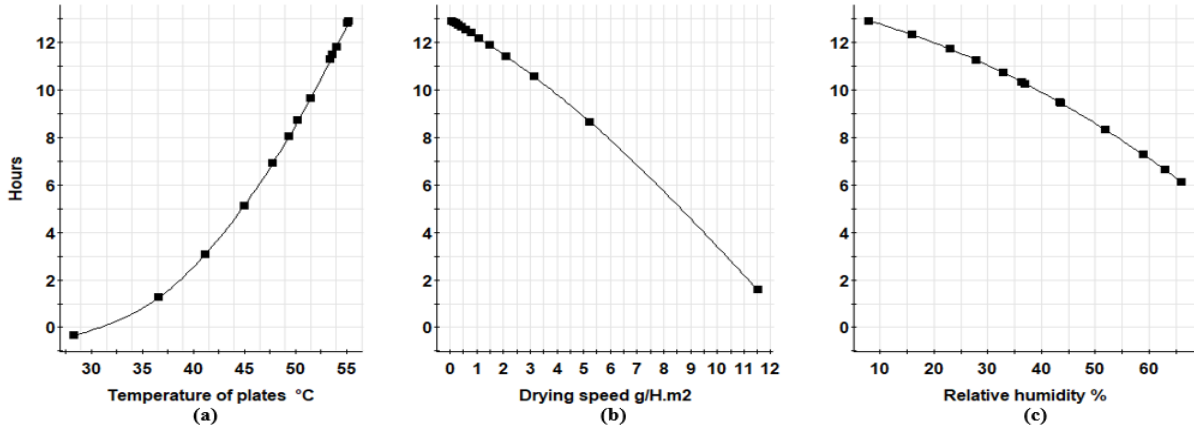

Figure 8. Drying time variation with experiment number 13

Table 7. Some values of the third case with the experiment parameters 13

$Time_{dry}= f (T_{trays})$		$Time_{dry}= f (Dspeed)$		$Time_{dry}= f (HR)$	
T°_{trays} °C	**Time** Hours	D_{speed} g/Hm^2	**Time** Hours	**HR** %	**Time** Hours
36.5	1.25	0.04	12.9	7.72	12.6
44.9	5.12	2.07	11.4	23.1	11.7
49.3	8.03	5.20	8.67	43.5	9.47
53.3	11.3	7.96	5.68	58.8	7.27
55.2	12.8	11.5	1.57	65.9	6.16

By comparing the 3 series of graphs, one notices that the aspect of the curves remains practically the same. The values change of the parameters in the order from "Low-High-High" (experiment 1) to "High-Low-Low" (experiment 13) while passing by "Medium-Medium-Medium" (experiment 7), shows that one tends towards physically real values and towards a practical experimental predictive behaviour of tomato drying.

3.3. Densitometry Study of a dried Tomato slice

A tomato slice is not dried uniformly in its volume; some parts are subjected to the heat

conduction of the dryer, while others contain a remaining quantity of humidity. The following study analyses the areas of the slice using the digital image processing software Scion Image (Software Scion, 2021; Software user guide, 2002.). It shows grey levels scaled from 0 to 255 on a similar of a photonegative. The 0 indicates a grey that tends to absolute black, while the value 255 corresponds to a perfect white. The interpretation of the real grey levels of the areas is therefore done by deducting the value shown on the profile from the value 255. Figures 9-a-b-c show the tomato slice before drying, after drying and finally the digitalised photo by Scion Image.
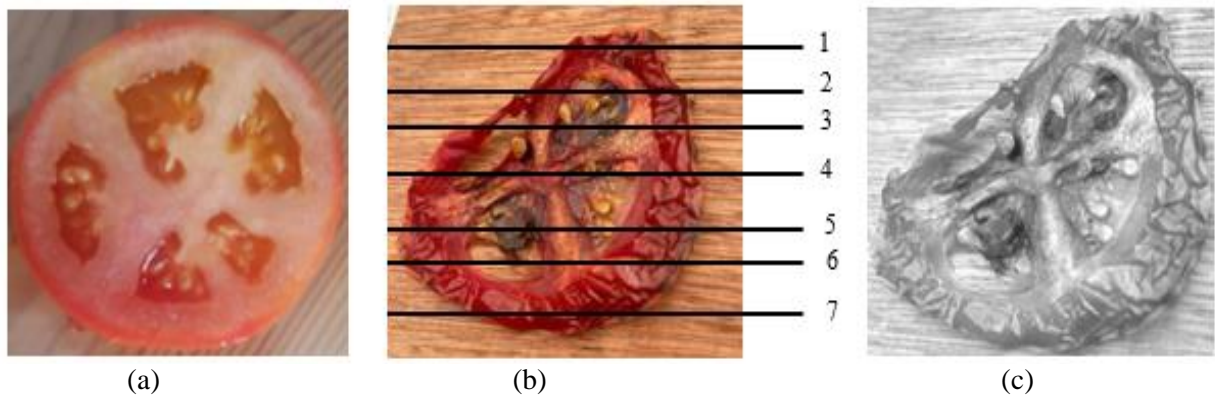
(a)   (b)   (c)

Figure 9. States of the tomato slice during the drying process.

If one looks at these figures, one can see that the radial and external peripheral nervures remain white and are therefore the least affected by heat conduction, these are the hard parts of the tomato that are difficult to dry, whereas the lobes (central portions) are blackened, and therefore strongly dehydrated. Sections 3 and 5 in Figure 9 are very close and have similar profiles because they are located on either side of the central circular nervure.

(a)   (b)
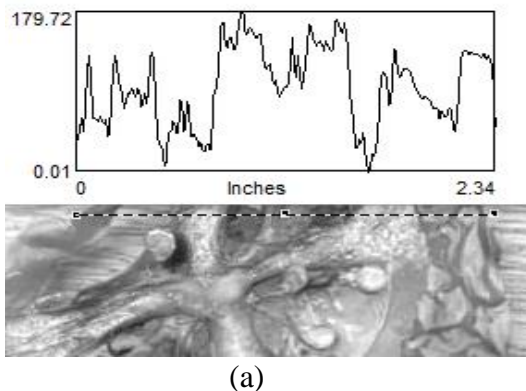
Figure 10. Densitometry of sections number 3 and 5 of the tomato slice

The first is measured on a width of 59.4 mm and the second on a width of 80.7 mm and reach a high grey of 75.28 (Figure 10-a) and 39.0 (Figure 10-b) (close to 0 value of absolute black) on a scale of 255.

In both figures, there are many peaks synonymous with parts affected by the thermal conduction; the central area between sections 3 and 5 of the tomato slice is suitably dried. However, a minor part of both sections remains white with values reached up to 255; this shows that these small areas have not sufficiently dried and that the 13 hours exposure to the sun is insufficient.
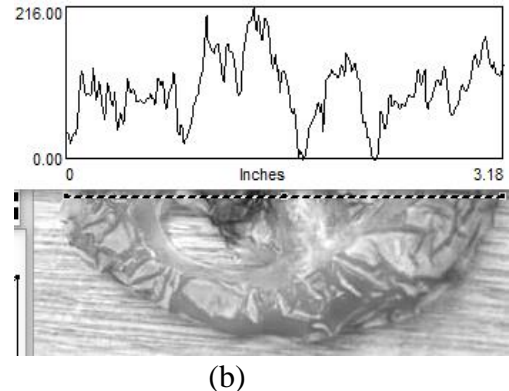
The effect of thermal conduction on the zones 2 and 6 is different. In Figure 11-a, on a width of 41.6 mm, located inside the dryer, a very dried central area reaches a grey scale of 50.24 on a scale of 255, which corresponds to a good drying.

(a)   (b)
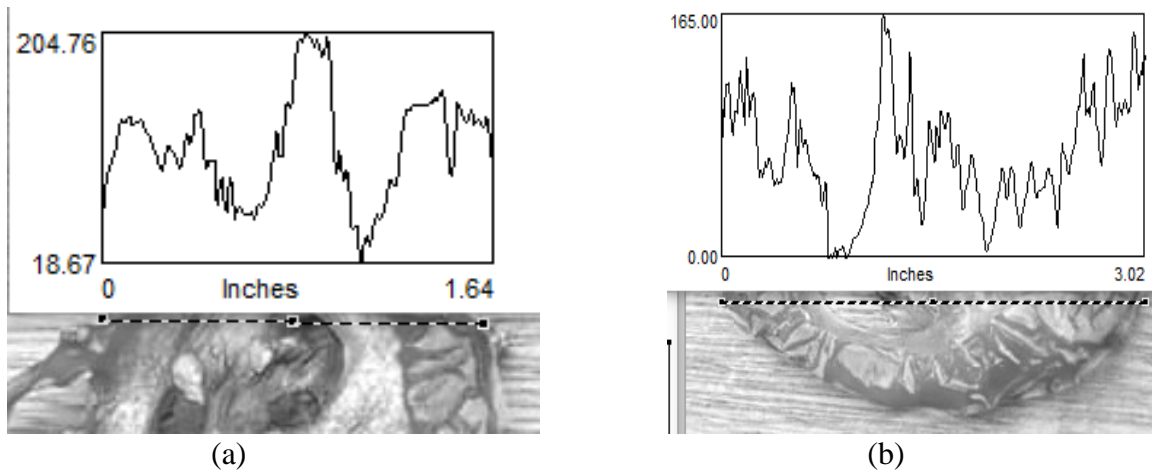
Figure 11. Densitometry of sections number 2 and 6 of the tomato slice.

Just to its right, a badly dehydrated area reaches a value of 246.33 very close to 255, which corresponds to a zone containing still water, therefore a poorly dehydrated area.

On the contrary, in Figure 11-b, in the area towards the dryer window, the obtained profile with a width of 76.7 mm has several valleys whose values tend towards 255, which corresponds to a nuance of grey tending towards white, in other words towards an absence of drying.

In these areas, drying is done more correctly inside the dryer than on the window side.

(a)   (b)
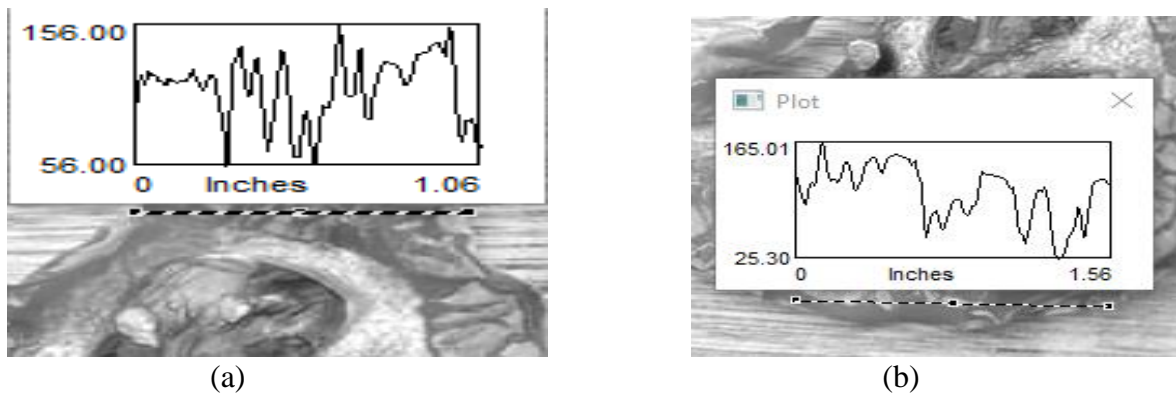
Figure 12. Densitometry of sections number 1 and 7 of the tomato slice

The areas 1 and 7 (Figure 12) represent the external circular nervure of the tomato slice; this is an area subject to direct unobstructed thermal conduction reaching a nuance of 89.99 of grey showing that this external zone is relatively more dried than the previously mentioned radial nervures. The average drying around the perimeter of the tomato slice have a nuance of 100.57 on a scale of 255, which corresponds to a relatively acceptable dehydration.
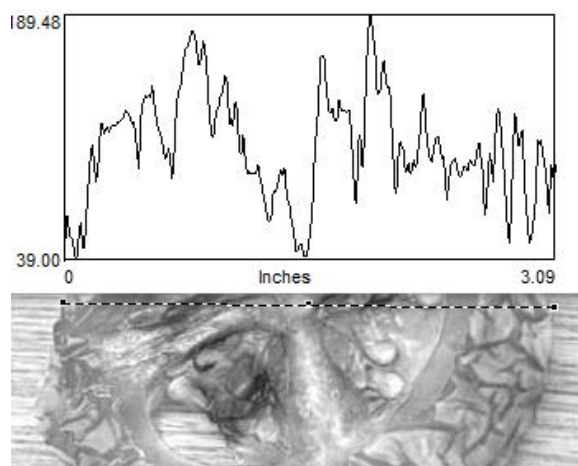
Figure 13. Densitometry of sections number 4 of the tomato slice

Finally, the profile of zone 4 (Figure 13) shows a correctly dried surface, it is characterized by the central nervure whose value reaches a nuance of grey of 216, very close to perfect white. This shows that this nervure remains significantly under-dried.

4. Conclusion

Through the analyses and comments carried out during this study by using the modelling by the non-conventional designs of experiments, one notices that the responses and the solutions are diverse and diversified. This is due to the interaction of the parameters that act separately and then in a combined form on the obtained results. One can see through the proposed graphs, contours and response surfaces without limit in treating an infinity of cases. The established design of experiments is thus a series of rigorously organized tests, in order to determine with a minimum of experiments and a maximum of precision, the respective influence of the 3 various mentioned parameters in the study in order to optimize the performances and the cost. The modelling by design of experiments is thus the only method, which analyses a process, here, the drying hours, by taking into account the effect of each of them, and the effect of each combination of our 3 parameters. These results permit us to describe the experimental dehydration and drying process, to separate it into theoretical values to be rejected and practical values to be applied, and to predict even unknown cases, not executed during the experiments. Since the different areas of the tomatoes, slices are differently affected by thermal conduction, and in order to attenuate this phenomenon, the installation of rotating trays is proposed. This will expose all areas to the same thermal effects.

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Conflict of Interest

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INVESTIGATION OF THE ANTIOXIDANT EFFECT OF TWO THIOLS, γ-GLUTAMYL CYSTEINE AND GLUTATHIONE, IN SUNFLOWER OIL UNDER ACCELERATED STORAGE

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ABSTRACT

Sunflower oil is an oil that is prone to oxidation due to its chemical structure and prevention of such oxidation is widely studied. This study assessed the the effectiveness of γ-glutamyl cystein (γGC, GC) and glutathione (GSH), in preventing oxidation of sunflower oil stored at 50°C for a period of 15 days. TBHQ was used as a positive control while no additive oil selected as a negatif control. Oxidation level indicators such as peroxide (*PV*), free fatty acidity (*FFA*), p-anisidine (*p-AV*) but also total oxidation (*Totox*), colour (*L*, a*, b**) and fatty acid profile were determined. At the end of storage, oxidation in sunflower oil was substantially reduced by 40 mg/L of GC. Analysis with 2,2-diphenyl-1-picrylhydrazyl (DPPH) resulted in the following order of IC_{50}; T (0.08±0.01), BHA (0.13±0.03), GC (0.3±0.01), GSH (0.41±0.00), BHT (0.42±0.02). The samples resistance to the generation of primary and secondary oxidation products was T>GCT>GC>GSHT>GSH>C for up to 15 days under storage conditions. The fatty acid profile analysed by GC/MS further demonstrated that these thiols outperformed the control group in terms of performance. Findings demonstrated that GC, precursor of GSH, has stronger antioxidant activity than GSH. As a result, it is recommended to be explored as a potential source of antioxidants in applications for the food industry to prevent lipid oxidation.

1.Introduction

Based on its high content of unsaturated fatty acids, especially essential 9-cis and 12-cis-octadecadienoic acid, sunflower oil is considered as one of the highest quality vegetable oils for human nutrition (Mariamenatu & Abdu, 2021). Beside this, it is also one of the most prone to oxidation upon storage (Lu et al., 2022). Compounds formed by lipid oxidation such as hydroxyperoxides, aldehydes, carbonyl compounds, hydrocarbons (alkane, alkene) formed during oil oxidation have adverse affects on human health but also alter the chemical and sensory properties of oils. Oxygen is mainly responsible of the initiation and acceleration of oils oxidation (Echegaray et al., 2022) although, temperature, light, degree of unsaturation and the presence of metal ions such as copper and iron also accelerate oxidation (Bastos, 2010). Therefore, it is challenging to avoid oil oxidation without adding any antioxidant substances.

Antioxidants are compounds that prevent or delay oxidative reactions based by a combination of scavenging free radicals, chelating prooxidative metals, quenching singlet

oxygen among other (Choe & Min, 2009). Based of their lower cost and greater efficiency, antioxidants obtained by chemical synthesis, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary hydroquinone (TBHQ) are commonly utilized in the food industry (Abdelazim et al., 2013). However, they are also associated with issue in safety to use (Honold et al., 2016). Consumers avoid the use of these products due to the fact that these chemically synthesized antioxidants have a harmful affect on human health. Therefore, the demand for natural antioxidants is constantly increasing.

In cells, different thiol compounds has antioxidants fuction prevent damage caused by free radicals and provide detoxification. Glutathione (GSH), cysteine (CYS), homocysteine (HCYS), N-acetylcysteine (NAC), captopril (CAP) and γ-glutamylcysteine (γGC,GC) are the best known biological thiols (Ulrich & Jacob, 2019). GC, a dipeptide composed of cysteine and glutamate is the cellular precursor of glutathione. GC and GSH are thought to protect cells against oxygen toxicity by destroying peroxides, disulfides, and other oxygen-generated species (Kritzinger et al., 2013). In particular, GSH is used in the cosmetic, medical and food industries as an active ingredient of these products to alleviate harmful oxidative processes and prevent the formation of toxic compounds such as radicals (superoxide, hydroxyl, peroxyl, alkoxyl) and non-radicals (hydrogen peroxide hydroperoxide etc.). It is also used to strengthen and repair skin whitening due to its anti-aging affect (Hamad et al., 2018).

The oxidative stability of oils can be determined during storage and packaging under normal ambient conditions. However, oxidation takes a long time to occur, which makes it impractical for routine analysis. Therefore, accelerated oxidation test have been developped, notably by increasing storage temperature to 50 °C upon 15 days (Bandyopadhyay, 2008).

The objectives of this study were to evaluate the ability of GSH and its precursor GC to prevent or at least reduce oxidation of sunflower oil and to compare thier antioxidant capacity between them and other used industrial antioxidants. For this purpose, sunflower oil was subjected to accelerated oxidation under laboratory conditions and the affect of temperature, sun light and oxidation was monitored during storage period.

2.Material and methods
2.1. Materials and reagents

The refined organic sunflower oil used in this study was purchased from Beyorganic company located in Istanbul, Turkey. Glutathione (GSH), γ-glutamylcystein (GC), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA).

2.2. Oxidation of Sunflower oil in the presence of glutathione, γ-glutamyl cysteine and TBHQ

Accelerated oxidation test were performed according to the method of Papadopoulou and Roussis (Papadopoulou et al., 2008). Briefly, 50 ml of sunflower oil were stored in 100 ml flasks in the presence of glutathione (GSH; 40 mg/L), γ-glutamylcystein (GC; 40 mg/L), TBHQ (T; 200 mg/L), γ-glutamylcystein+TBHQ (GCT; 40 mg/L+40 mg/L) and glutathione+TBHQ (GSHT; 40 mg/L+40 mg/L). Oil without additives was selected as a control (C; no additive). The experiment carried out with 3 repetition for each sample. Prior incubation, antioxidants were dispersed in the oil phase in an ultrasonic water bath (Bandelin, RK 1028H, Berlin) for 10 minutes. All samples were incubated at 50°C and in sun light conditions for 15 days. Analyzes were performed in 2 replications on days 0, 3, 6, 9, 12 and 15.

2.3. Determination of free radical scavenging activity for glutathione, γ-glutamyl cysteine, BHA, TBHQ, BHT

The radical scavenging activity of GSH and GC and commercial antioxidants used in food, namely BHA, BHT, TBHQ were tested according to Blois et al (1958) with some modification (Blois, 1958). Briefly, solutions of

GSH, GC, BHA, TBHQ and BHT were prepared in ethanol at concentrations ranging from 0.04 to 1.5 mM. 390 µl of DPPH solution (25 mg/L ethanol) was mixed to 100 µl of the different antioxidant solutions. After, 30 minutes of incubation in the dark at room temperature (25°C). The results was measured at 517 nm absorbance by visible spectrophotometer (Perkin- Elmer, USA). For the reference sample, a mixture of ethanol and DPPH was used (Kamkar et al., 2010). The scavenger activity was calculated according to Eq. (1):

$$DPPH\ radical\ scavenging\ activity\ (\%) = [(Ab-As)\ /\ Ab] * 100 \tag{1}$$

Ab is the blank absorbance and As is the absorbance of the sample.
IC_{50} was calculated for each sample on inhibition %.

2.4. Determination of peroxide value, free fatty acids and p-Anisidine value

To determine the peroxyde value (*PV)*, 1 g of sunflower oil was dissovled by gentle mixing in 25 mL of chloroform-acetic acid solution (2:3 v/v) mixture. One ml of saturated potassium iodide solution was then added and the mixture was incubated in the dark for 5 minutes. It was then diluted by adding 75 mL of distilled water. One mL of 1% starch solution was added and the final solution was titrated with 0.01N sodium thiosulfate ($Na_2S_2O_3$). The *PV* values were calculated according to AOCS, 2003, cd 8-53 method (Matthäus 2006) using Eq. (2):

$$PV= (V \times N \times 1000\ meq\ g\ O_2/kg)\ /m \tag{2}$$

V: Spent sodium thiosulfate solution, mL
N: Normality of sodium thiosulfate solution
m: sample weight, g.

The amount of free fatty acids (*FFA*) in oil samples was calculated as oleic acid. To determine the *FFA*, 1g of oil sample was dissolved in ethanol-diethyl ether (1:1 v/v) solution. The resulting solution was then titrated by 0.1 N KOH in ethanol in the presence of phenolphthalein. Fatty acidity content was calculated according to the AOCS Ca 5a-40 method (Tyburczy et al., 2013) using Eq. (3):

$$\%\ FFA = V\ x\ N\ x\ Ma\ /m \tag{3}$$

V: 0.1 N spent ethanolic potassium hydroxide solution, mL
N: Normality of ethanolic potassium hydroxide solution,
Ma: Molecular weight of oleic acid,
m: Sample weight, g.

The p-anisidine value (*P-Av*) was determined according to the modified AOCS 1990 method. First 0.5 g of oil sample was dissolved in 10 ml of hexane was measured at a wavelength of 350 nm with Lambda 25 UV-VIS (Perkin- Elmer, USA) spectrophotometer. Then 2.5 ml of the solution was taken and 0.5 ml of p-anisidine solution (0.25 g/100 ml of acetic acid) was added, and absorbance was measured at 350 nm (Pocklington & Dieffenbacher, 1988). Finally, the *P-Av* was calculated according to Eq. (4):

$$P-Av= 10*(1.2\ x\ A2- A1)/m \tag{4}$$

A2: last reading,
A1: first reading,
m: sample quantity.

2.5. Measurement of Colour (L*, a*, b*) and calculation of total oxidation (Totox) value

Colour measurement in oil was performed by Chroma meter CR-400(Conica Minolta, Japan) colourimeter. In the CIELAB colour space *L*, a*,* and *b** values indicate lightness, green to red, and blue to yellow, respectively. The *Totox* value in oil was calculated on *PV* and *P-Av* according to theEq. (5):

$$Totox = (2*PV) + p-AV \tag{5}$$

2.6. GC-MS analysis of Fatty Acids

For determination of fatty acid composition, the methylesters were prepared by cold transmethylation with potassium hydroxide according to IUPAC 2.301-2.302 method (Paquot, 2013). Briefly, 10 mg oil was dissolved in 10 ml hexane solvent. Then 0.5 ml of 2N

KOH was added to the oil sample and left in the dark for 2 hours. Then, the upper phase was collected and filtered with a 0.45 µm filter before being analysed by gas chromatography-mass spectrometry (Agilent- 7890B GC -7010B MS) with an autosampler (Gerstel, Germany) equipped with the flame ionization detector (FID). A capillary DB-WAX column (Agilent J&W; 60m x 0.25 µm x 0.25µm) was used. The oven temperature was held at 50ºC for 1 min, raised to 200ºC at a rate of 25°C/min held for 10 min and then to 230ºC at a rate of 3 ºC/min held this temperature for 25 min. The injector (250 ºC) and detector temperatures (300ºC) were set. The sample size was 1µl and the the flow rate of helium carrier gas was 1 mL/min. The split used was 1:40. The identification of fatty acid was determined by checking with the retention times of known fatty acid standards and given as a percentage of the total.

2.7. Statistical analysis

The results of the analyzes were evaluated by analysis of variance (ANOVA), and Tukey tests. "Rstudio 2022.02.03 version "agricolae" package was used for statistical analysis. Principle component analysis was done by Xlstate 2023 software. Results were evaluated in biplot.

3.Results and Discussions
3.1 Antioxidant properties

Figure 1 presents the antioxidant properties using DPPH assay of the GC and GSH, along with the reference standards TBHQ, BHA, and BHT. DPPH is a stable free radical widely used for screening compounds with free radical scavenging abilities (Sethi et al., 2020).
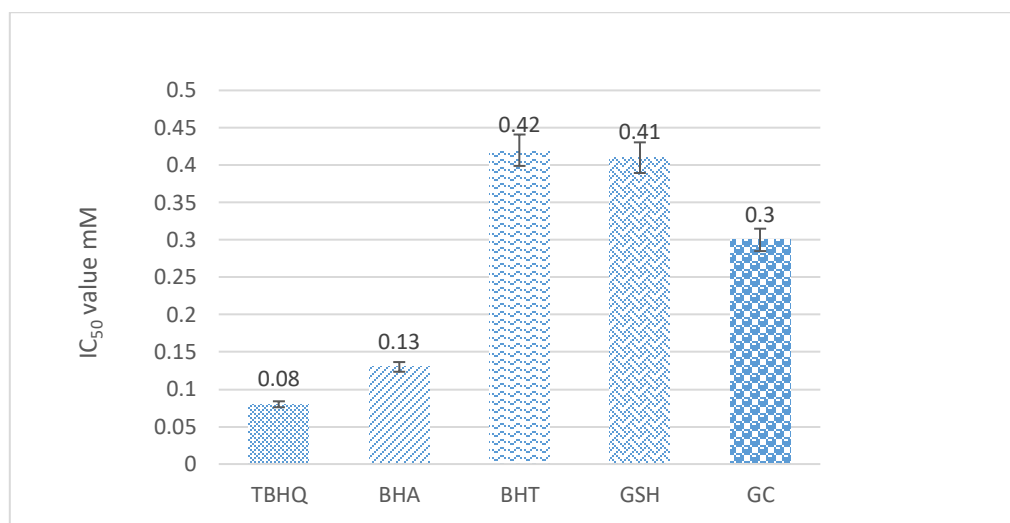
Figure 1. DPPH radical-scavenging activity of GC and GSH compared with BHA, BHT, and TBHQ at different concentrations. Mean ± SD, *n*=3.

In the present study, IC_{50} values were found in the range of 0.08 to 0.42 mM. It was observed that the DPPH radical-scavenging activity of the GC and GSH were found to be higher than the TBHQ and BHA, but lower than the BHT (Figure 1). Their scavenging activity of DPPH radicals decreased in the following line TBHQ>BHA>GC>GSH>BHT. Superoxide anion and hydroxyl groups are two of the most important free radicals. Superoxide anions are produced by adding an electron to molecular oxygen and are harmful reactive oxygen species because they damage cellular components in biological systems (Yu et al.,2016). It was noted that the presence of -SH groups in the medium significantly eliminated the superoxide anion and hydroxyl groups. Since GC and GSHS contain -SH groups, it has a powerful scavenging affect on free radicals (Jie et al.,2016).

Many studies regarding DPPH radical-scavenging activity in the literature are on plant extracts and oils comparing with synthetic antioxidants. Although there are not many studies on DPPH scavenging activity of GC and GSH in oil, Fileger et al (2020) compared hydroalcoholic extracts from *Aegopodium podagraria* L. with ascorbic acid and glutathione. It was stated that the inhibition concentration of glutathione was around 65% for 0.7 mM. In present study, it was found 64.2 % for 0.75mM glutathione. In another study, the synergistic effect of glutathione (50-200 μM) with various flavones was studied using DPPH. It was noted that inhibition % increased when GSH amount raised (Pereira et al., 2013).

TBHQ IC_{50} value was determined as 6.87 μg/ml and 29.81 μg/ml in two studies (Zheng et al., 2011;Gharib et al., 2013). In the present study, the IC_{50} value corresponds to 13μg/ml (0.08 mM) for TBHQ. Chen et al (2014) compared rosemery extract with commercial antioxidants in their study, the IC_{50} value was ordered as TBHQ>BHA>BHT. Our finding was also in agreement with the results reported by previous studies (Laulloo et al., 2015; Prevc et al., 2013). The maximum limit of commercial antioxidants usage is determined as 200 mg/L by the Food Drug Administration (FDA). Although the determined IC_{50} values of these antioxidants are low, the limit used is high considering their negative effects on human health (Sharma et al., 2019).

3.2. Determination of *PV* and *FFA* and *P-Av* in accelerated oxidation of sunflower oil

The degree of oxidation in oils is usually characterised by the peroxide value that relates to hydroperoxides, the primary oxidation products that are unstable and readily decompose to form mixtures of mainly volatile aldehyde compounds. It is known that oils are rapidly oxidized by heat, light and air, thus increasing the *PV* (Gharby et al., 2011). The affect of antioxidants on peroxide value in the sunflower oil samples is in shown in Figure 2. Results show that *PV* increases linearly for all samples with storage time. The increase in PV accelerated after 3rd day. As a result, the control

sample had the highest PV till the end of storage time 4.67– 168.33 meq O2/kg. When the samples are compared in terms of PV, the order was C>GSH>GC>GSHT>GCT>T. There are significant differences between groups, particularly at the end of oxidation (p<0.05). As mentioned above, GC, a dipeptide, is the precursor of GSH. According to the DPPH result, GC, which showed higher activity than GSH, showed a stronger antioxidant affect against GSH during accelerated oxidation. Crapiste et al (1999) measured the peroxide value of sunflower oil before and after storage. The values obtanied indicated that while the peroxide value was 3.36 meq/kg at the beginning, it reached 90 meq/kg in 30 days at 47°C. These results are in line with the peroxide value (168.33 meq/kg, at 50°C, 15 days) recorded in the present study corraborating the impact of temperature. In this study, we aimed to highlight any synergistic affects of GC and GSH with TBHQ, as antioxidant in sunflower oil that will allow subsequent utilisation of lower concentration of TBHQ. According to the results, values close to each other were GCT, 19.33 meq O2/kg and GSHT, 27.33 meq O2/kg (p<0.05), TBHQ was recorded when 200 mg was used (15 meq O2/kg (p<0.05)). This means that it showed a positive synergistic affect with the synthetic antioxidant. Glutathione was compared with synthetic antioxidant, and its antioxidant affect was investigated by measuring the peroxide value in butter. Glutathione affect showed results close to BHA at the end of storage (Papadopoulou et al., 2008). There are also studies close to our results to prevent the increase of PV in sunflower oil (Erol et al., 2022; Naserzadeh et al., 2018; Saeed et al., 2022). *FFA* refers to the free fatty acids and is expressed in mg of potassium hydroxide or sodium hydroxide required to neutralize 1 gram of fat. *FFA* is an important quality index for oil and is constantly used as a shelf-life monitoring parameter in oil. An increase in *FFA* means a decrease in stability to oxidation. This is one of the important indicators that the oil will start to become rancid (Frankel, 2014). The concentration of *FFA* calculated as % oleic acid are shown in Figure 2.
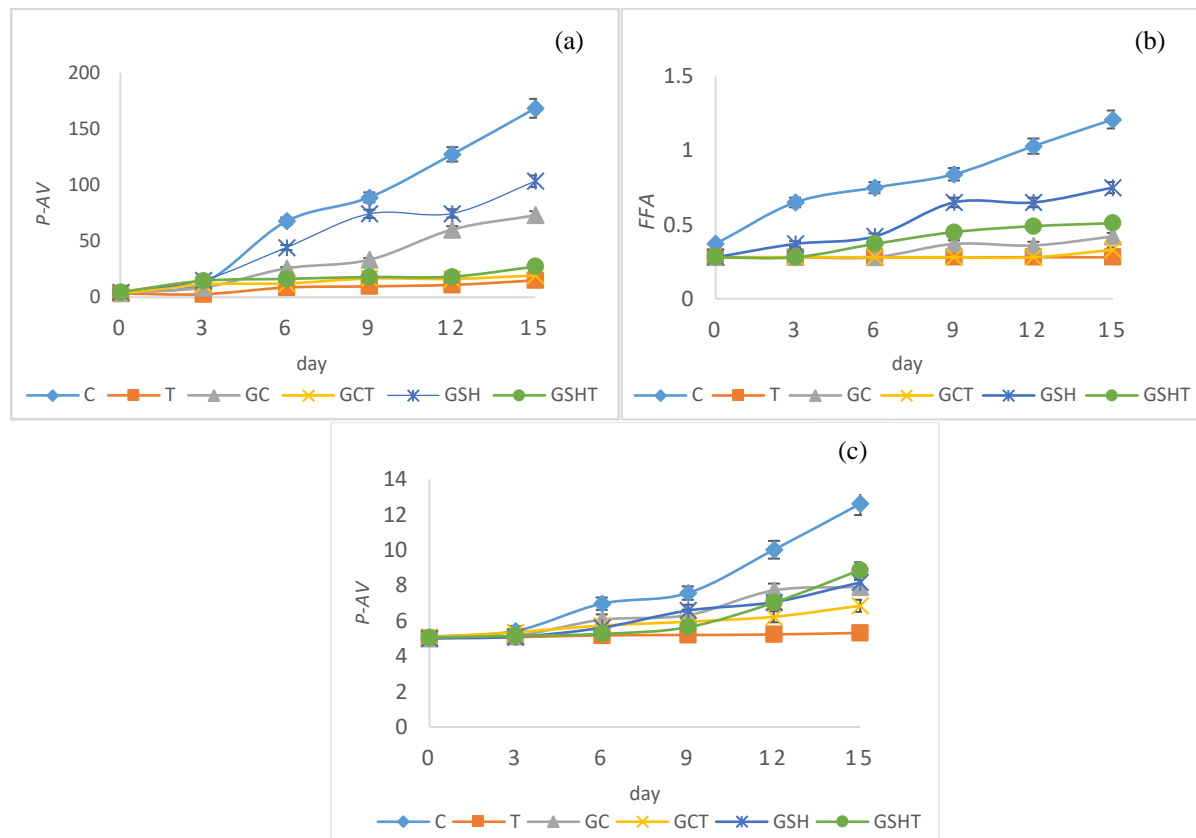
Figure 2. Affect of adding TBHQ, GC, and GSH on peroxide (a), FFA (b) and p-anisidine value (c) in sunflower oil under accelerated oxidation conditions. C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3.

According to the *FFA* data, the best result during the storage period belongs to the oil sample containing TBHQ. A significant difference was not observed between groups till 1the 6th day (p<0.05). At the end of storage time, *FFA* of sunflower oil sample C, TBHQ, GC, GCT, GSH, GSHT were 1.21±0.16, 0.28±0.00, 0.42±0.00, 0.33±0.08, 0.75±0.16, 0.51±0.08 (% oleic acid) respectively. According to Duncan's multiple comparison, there was no significant difference between T, GC, GCT, but there was a statistical difference between these groups with GSH and GSHT. This shows that GC has antioxidant affects close to TBHQ. In addition, when we examined the combination of GC and GSH with TBHQ, GCT showed the highest antioxidant result. Generally *FFA* result increased in parallel with *PV* results (Dhibi et al., 2022). *FFA* measurement by the accelerated oxidation method in sunflower oil

has been measured in many studies. While *FFA* TBHQ value (0.28 oleic acid %) in this study had a similar to the literature (Chen et al., 2013), GCT results showed higher antioxidant activity than the literature compared to plant-derived antioxidants. GCT results in this study showed higher antioxidant activity than literature (Chang et al., 2015; Iqbal et al., 2007; Mei et al., 2014).

The oxidative degradation of oils begins with the formation of primary compounds such as hydroperoxides. They react to undesirable secondary oxidation products, such as aldehydes, alkanes, esters, alcohols etc. Secondary oxidation products are determined by *P-Av* in oils and fats (Ramadan & Mörsel, 2004). *P-Av* results are shown in Figure 2. The *P-Av* results were parallel to the *PV* results and increased on the 3rd day, with the highest value at the end of storage.

The data presented in Figure 2 showed that sample C has the highest secondary oxidation products. A statistically significant difference was observed in *P-Av* between the GC and GSH at the end of storage. After 15 days the increase in *P-Av* value is in the order of C (7.95±0.68) > GC (6.37±0.65) > GSH (6.26±0.55) > GSHT (6.17±1.14) > GCT (5.87±0.66) when all days are averaged. As a result of *P-Av*, lower value was recorded in all the doped groups than the control group. Although GC and GSH values were close, there was a statistically significant difference between TBHQ and GSH groups, but no difference was observed between TBHQ and GC groups (p<0.05). There is no difference between GC and GCT. It is thought that if the GC concentration is increased in oil, it will slow down the increase of *P-Av* without TBHQ.

Two major methods seperate the oxidation level; the peroxide number measures the hydroperoxide level, while the anisidine value measures the secondary oxidation level. In a similar study the ability of glutathione and N-acetyl-cysteine for all conditions showed results close to the BHA sample in *P-Av* measurement. As a result N-acetyl-cysteine and GSH may be taken into account as antioxidants in corn oil during storage, cooking or frying (Papadopoulou et al., 2008).

When the two measurements are made together and applied to the formula, the *Totox* level is revealed and the oxidation level is determined. According to many studies, the maximum levels are 5 meq/kg for peroxide level, 20 for anisidine and 26 for totox (FAO, 2015). *Totox* value results shown in Figure 3.
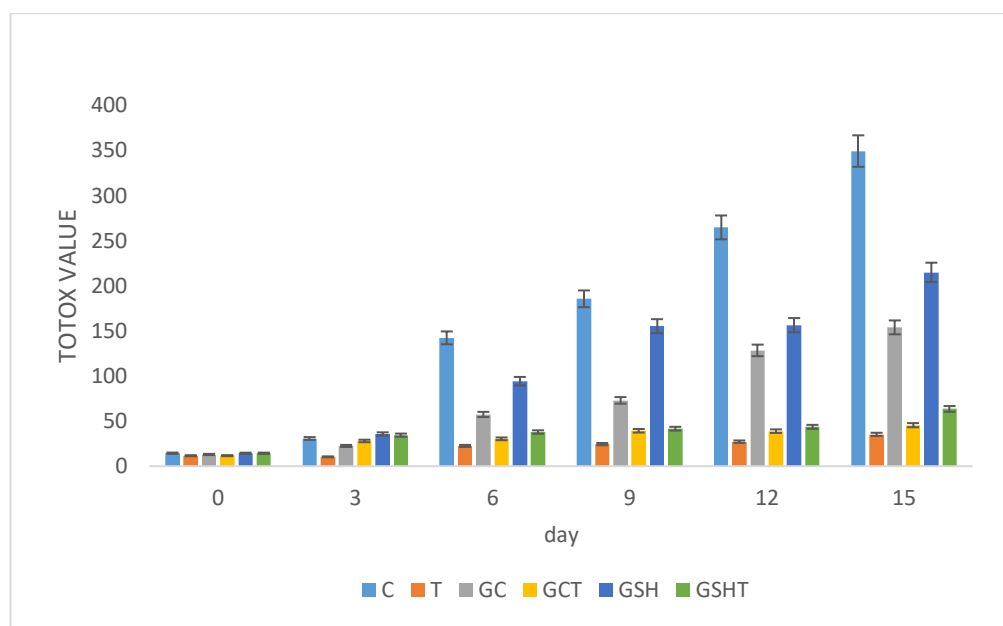
Figure 3. Comparison of p-Anisidine value of different treatments of sunflower oil during 15 days at 50°C. C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3

At the end of the storage period, the *totox* value of all groups was above 26 meq O_2/kg. However, when the antioxidant groups were compared with TBHQ protected oil, a rapid increase was observed in the total oxidation value at the end of the 3rd day in parallel with the *PV* and *P-Av*. In the end of storage time *totox* value of C, T, GC, GCT, GSH, GSHT determined (349.28±6.79) meq O_2/kg,

(35.32±1.22) meq O_2/kg, (153.92±4.71) meq O_2/kg, (45.52±3.99) meq O_2/kg, (214.84±2.34) meq O_2/kg, (63.54±0.56) meq O_2/kg respectively. In a study in which lemon peel was used as an antioxidant, the *totox* value was compared with the oil sample containing BHT and the *totox* value was found higher than in the study compared to data given by (Okhli et al., 2020). In another study researchers reported that

using of fennel seed extract at 100-800 ppm in soybean oil reduced totox values in oil samples. The extract at levels of 300 and 400 ppm showed higher antioxidant activity than BHT and BHA (Mazaheri et al., 2014).

3.3. Colour properties of the samples

The colour attributes of the oil are the main criteria affecting consumer acceptance. The colour data of the samples are displayed in Figure 4. As a measure of the colour (*L**, brightness/darkness and *b**, blueness/yellowness) of the oil. All treatments showed darkening over time (ie, decreasing *L**). *L** value was 54.1±0.14 at the beginning of the storage process for all samples. Then *L** decreased to 49.98, 53.70, 50.68, 51.71, 50.80 and 51.64 after 15 days under heating and light conditions for C, T, GC, GCT, GSH and GSHT, respectively. At the same time, the initial *b** value of 3.6 increased to 5.68, 4.13, 5.04, 4.92, 5.10 and 5.19 for C, T, GC, GCT, GSH and GSHT, respectively. The yellow colour of these oils is defined by increasing of *b** over time during storage, because of the natural carotenoids in the sunflower oil.
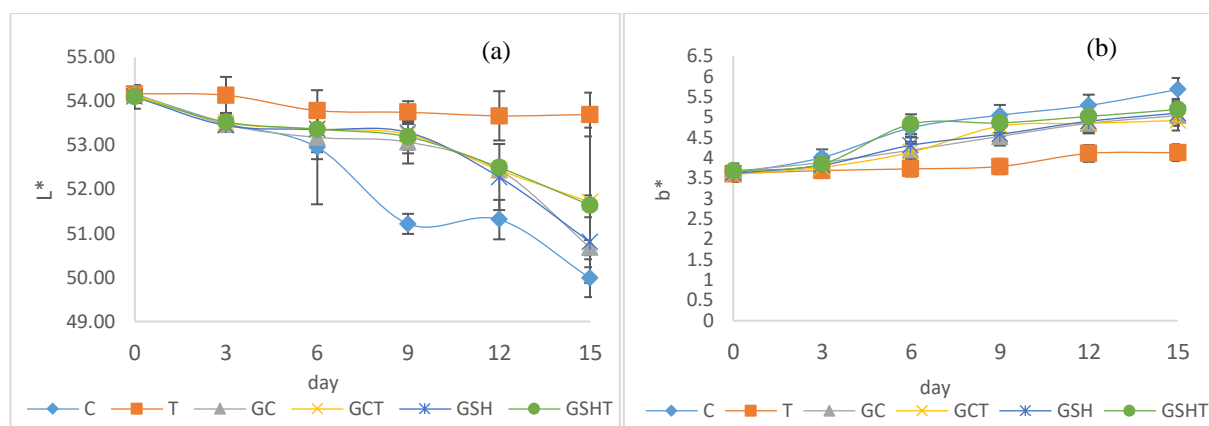
Figure 4. Changes in the *L** (a) and *b** (b) value in sunflower oil during 15 days at 50˚C. C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3

This study states that significant difference between the *b** value of GC and GSH samples. However there was no difference for *L** value (p>0.05). In the control sample, while the *L** value decreased dramatically, the *b** value increased. Colour value is an important criterion especially in frying oils (Erol et al., 2022). A colour change was observed in frying oil during oxidation in a study. In another study, it was observed that the *L** value decreased rapidly and the *b** value increased significantly (Urbančič et al., 2014).

Principal component analysis (PCA) was utilized to demonstrate a better explanation of the chemical composition of the sunflower oil with different additives. Figure 5. shows the correlation biplot for the composition of oil samples. The plot indicates that the first two components (F1 and F2) account for 92.09 percent of the required information regarding the differences between oils oxidation profile. Control sample replaced in the first region of the coordinate system, GSH alone fell in the same region as the C, GSHT found its place in the 2nd region with T. GCT and T added oil groups fell into separate regions on the graph, although they had the lowest value when looking at oxidation criteria. This indicates that they have close affects on oxidation, especially in maintaining the *L** value. Additionally there was positive correlation between *PV* and *FFA* (r=0,94), *b** and *P-Av* (r=0,92), *P-Av* and *FFA* (r=0,93) while *L** and *b** showed negative correlation (r=0,93).
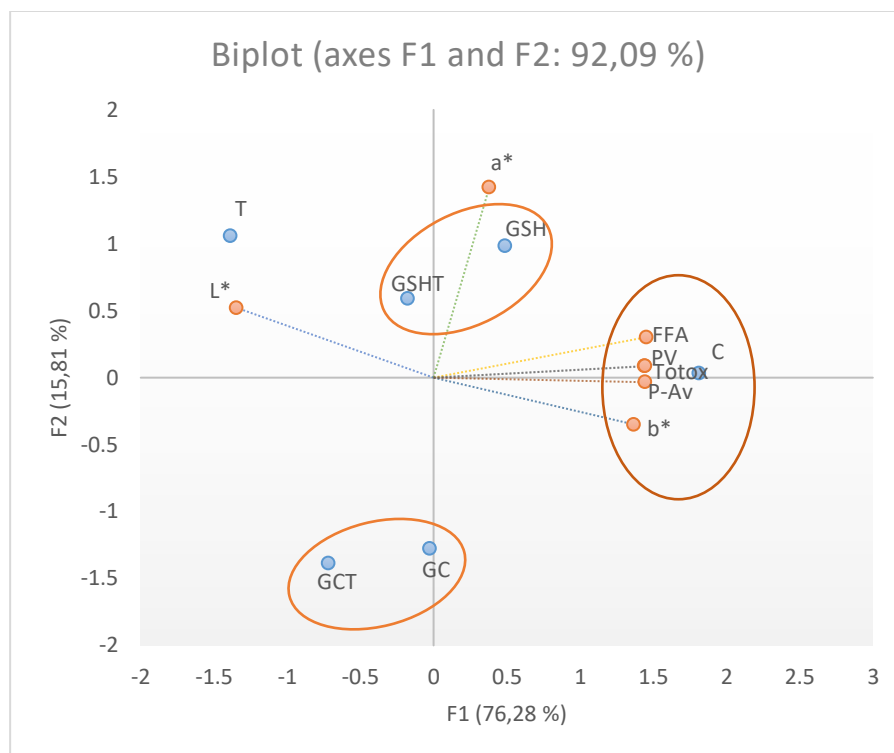
Figure 5. Principle component analysis according to the sunflower oil samples based composition of oils et the end of storage, C, GSH (40 mg/L), GC (40 mg/L) and TBHQ (200 mg/L), GCT (40 mg/L+40 mg/L) and GSHT (40 mg/L+40 mg/L)

Table 1. Fatty acid composition of sunflower oil by GC/MS

	C16:0 (Palmitic acid)				C18:0 (Stearic acid)				C18:1 (Oleic acid)				C18:2 (Linoleic asit)			
	IS		ES		IS		ES		IS		ES		IS		ES	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	6,72[a]	±0,97	7,61[ab]	±0,05	2,84[a]	±0,45	3,2[a]	±0,02	31,65[b]	±0,03	32,45[a]	±0,08	55,96[a]	±0,28	55,6[ab]	±0,37
T	7,48[a]	±0,06	7,46[c]	±0,03	3,16[a]	±0,03	3,17[a]	±0,05	32,01[a]	±0,1	31,93[b]	±0,11	56,39[a]	±0,25	56,39[a]	±0,35
GC	7,50[a]	±0,07	7,51[bc]	±0,09	3,16[a]	±0,02	3,20[a]	±0,02	32,05[a]	±0,09	32,18[ab]	±0,27	56,27[a]	±0,17	55,83[ab]	±0,43
GCT	7,48[a]	±0,03	7,49[bc]	±0,08	3,19[a]	±0,02	3,19[a]	±0,04	31,88[a]	±0,05	31,89[b]	±0,19	56,15[a]	±0,01	56,17[a]	±0,3
GSH	7,46[a]	±0,05	7,66[a]	±0,00	3,19[a]	±0,02	3,25[a]	±0,00	32,01[a]	±0,11	32,47[a]	±0,00	56,24[a]	±0,27	55,35[b]	±0,00
GSHT	7,44[a]	0,02	7,48[bc]	±0,02	3,17[a]	±0,02	3,18[a]	±0,01	31,87[a]	±0,00	32,08[a]	±0,09	56,18[a]	±0,02	56,36[a]	±0,06

IS: initial storage, ES: end of storage, C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3

3.4. Fatty acide profile

Refined sunflower oil contains approximately 15% saturated, 85% unsaturated fatty acids and forms of 14-43% oleic and 44-75% linoleic acids in unsaturated fatty acid content. Fatty acid composition of oil samples shown in Table 1.

Adding 200 mg TBHQ appears benificial in shielding the oil from oxidation, based on comparing the results before and after storage for all fatty acids. The level of oleic acid is the measure of oxidation. When GC was compared to GSH, it was shown that GC inhibited oleic acid growth (p>0.05). Palmitic acid and linoleic acid showed no appreciable change before and

after storage. The results show lower levels of stearic, palmitic and linoleic fatty acids compared to test conducted with sunflower oil and higher levels of oleic acid (Akkaya, 2018; Wang et al., 2018).

Loh et al (2006) investigated the effect of synthetic antioxidants (100-750 mg/L) on palm oil during a 5-week storage period. They determined the synthetic antioxidants effect on fatty acids to be in order of vitamin E<BHT<TBHQ<BHA<PG (Propyl gallate). Considering the IC_{50} values in our current study, we predict that increasing the amount of GC and GSH in future studies may decrease the fatty acid composition.

4.Conclusions

In this study, the antioxidant affect of two thiols, γ-glutamyl cysteine and glutathione, in sunflower oil under accelerated storage was studied. The findings of this study demonstrated that GSH and GC worked affectively as antioxidants in sunflower oil when it was stored. The antioxidant of GC, the precursor of GSH, was shown to be more important than GSH. When combined with the synthetic antioxidant, the use of GC and GSH produced a synergistic affect. This indicates that the synthetic antioxidant level in sunflower oil can be reduced by using GSH and especially GC as a curative strategy. It is estimated that these two compounds, which are affective even at low concentrations, will give affective results alone like TBHQ when the amount in the oil is increased.

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Author contributions

Huseyin Erten, methodology, analysis, data analysis, editing draft, Patrick Fickers editing draft, Serkan Selli methodology, editing draft. Mümine Guruk, investigation, methodology, validation, formal analysis, writing-original draft.

Statements and declarations

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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EXPLORING BIOACTIVE COMPOUNDS, NATURAL ANTIOXIDANTS, AND EXTRACTION TECHNIQUES FROM WATERMELON (*CITRULLUS LANATUS*) FOR HEALTH AND FOOD APPLICATIONS

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ABSTRACT
Watermelon (Citrullus lanatus) is a globally cherished fruit celebrated for its succulent sweetness. This article delves into the bioactive potential of watermelon, spotlighting its antioxidant-rich seeds, rind, and skin byproducts. Researchers are increasingly exploring the extraction of natural antioxidants from these byproducts due to their therapeutic potential. Watermelon's vibrant color and robust nutritional profile are attributed to compounds such as lycopene, carotenoids, phenolic compounds, and flavonoids, which act as crucial defenders against oxidative stress and its implications in various diseases. Various extraction methods are discussed, with ultrasound-assisted extraction (UAE) standing out for its efficient cavitation-driven mechanism. The pivotal role of phenolic compounds, particularly flavonoids, in plant antioxidant defense systems is underscored, exploring the distinct contributions of flavonoid and non-flavonoid phenolic compounds to plant health and coloration. Carotenoids like β-carotene and lycopene not only lend watermelon its vivid hues but also offer considerable health benefits. Techniques for evaluating antioxidant capacity, such as the DPPH assay, are explored, along with the application of bioactive natural compounds to enhance the stability of plant-based oils, addressing oxidation-related quality issues. The article also illuminates the potential anti-inflammatory and anti-diabetic properties of cucurbitacins, oxygenated steroidal triterpenes found in watermelon. Various extraction techniques, including maceration, infusion, percolation, and decoction, are briefly explored. In essence, this study highlights the significance of bioactive compounds in promoting human health and improving food quality, contributing to the harnessing of natural compounds from watermelon for health and food applications.

1. Introduction

Watermelon, scientifically known as Citrullus lanatus, belongs to the Cucurbitaceae family. Its name is derived from the Greek word "citrus," referring to the fruit, and the Latin term "lanatus," owing to the tiny hairs on its stems and leaves, giving it a wooly appearance (Mathew, *et al*., 2014).Where about 117 million tonnes of watermelon were produced last year (FAOSTAT, 2016). Due to its sweet and refreshingly juicy flavor, it is consumed all over the world including in Asian countries (Mushtag, *et al*., 2015).

Watermelon contains approximately 92% water and a significant number of byproducts, including seed, skin, and rind; these products may cause environmental hazards if not handled properly (Saeid, *et al*., 2016).These byproducts are rich in bioactive compounds. Watermelon seeds have powerful antioxidant and radical-scavenging properties and phenolic compounds which showed health benefits against various diseases including cancer, inflammation, viral infections, and skin flakes (Manivannan, *et al*., 2020; Yi, *et al*., 2013).

Other beneficial properties such as rancidity inhibition and lipid oxidation are also associated with these byproducts (Prochazkova, *et al.*, 2011). Due to the potential for therapeutic and other beneficent implementation of such natural antioxidants and bioactive compounds, such as the development of functional foods, the extraction, and use of these health-promoting compounds from watermelon byproducts are an important area of research. (Chua, *et al.*, 2019). The watermelon fruit is a source of multiple minerals, vitamins, and proteins that are present in the skin, pulp and seeds (Zayed, *et al.*,2021).Watermelon, with cucumbers, squashes, luffas, and melons, is a vital vegetable crop in the cucurbit family (Zhang, *et al.*,2020) *Citrullus lanatus* rind is an abundant source of natural antioxidants, lycopene, citrulline, and numerous polyphenols. Watermelon skin has a peppery and astringent flavor and calming impact. Watermelon skins along with seeds and rinds are also used as livestock feed (Dranca, *et al.*, 2016).

Watermelon fruit is divided into three major components including flesh, seed, and rind (Fig. 3). Flesh, also called pulp, rind, and seeds account for 68%, 30%, and 2% of the total biomass, respectively (Dietrich, *et al.*, 2016; Jawad, *et al.*, 2018).

Figure1. Watermelon
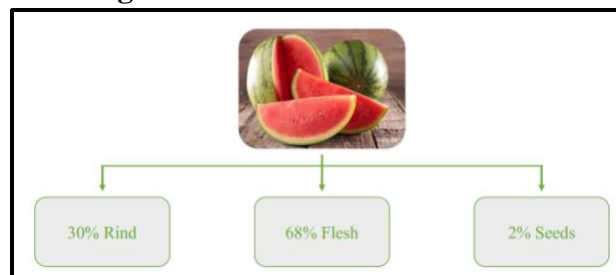

Figure 2. Total Calories in Watermelon

Figure 3. Breakdown of watermelon biomass

1.2. Bio refinery for watermelon

Almost all of the components of the watermelon can be used to make different valuable products, thus bio refinery of water melon would be feasible. Previous authors have documented various benefits associated with the techniques for watermelon biorefinery (Perkins-Veazie, *et al*., 2002). It is a fact that 1/3rd biomass of watermelon weight belongs to its rind, therefore, it can be as used for biomass refinery. This is the reason why rind gets the main focus. The foremost function of the bio refinery is the extraction of pectin which is a major constitute of the rind. Previously, (Montesano, *et al*., 2018), reported that this fruit is very useful for the extraction of pectin. However, the extraction of pectin from watermelon rind also gets much attention (Petkowicz, *et al*., 2017; Montesano, *et al*., 2018). Moreover, after the extraction of juice, the waste is also used for that purpose. To make the bio refinery concept more valuable in watermelon crops, considering value-added products for each of its components is essential. Numerous value-added products that can be derived from the harvest of watermelons are discussed previously (Montesano, *et al*., 2018). See in (Fig.4).
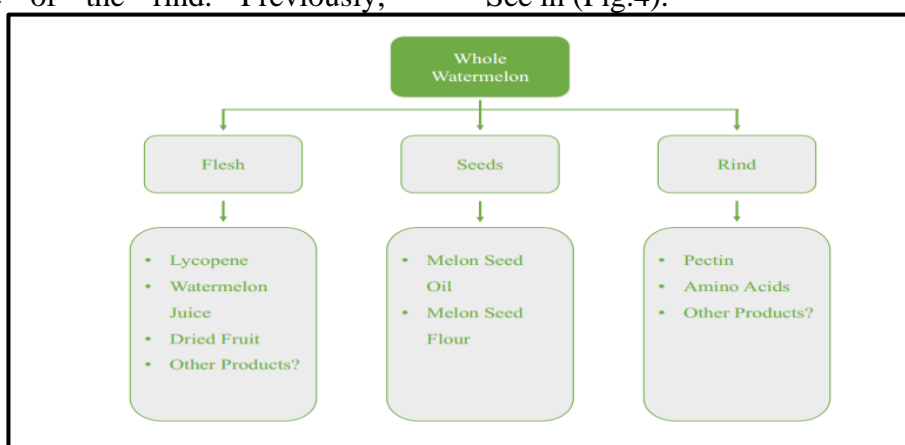
Figure 4. Overview of watermelon bio refinery concept
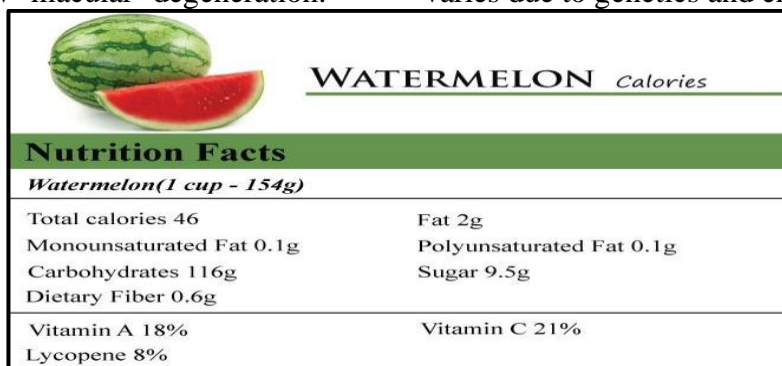
The flesh of watermelons can be produced to array of products with added value. Up to 60% of lycopene, also known as carotenoid with strong antioxidants, is present in watermelon flesh followed by typical tomato flesh (39%). Lycopene has numerous health benefits. It is commonly used as a nutraceuticals. (Montesano, *et al*., 2018). As mentioned above, the market for watermelon flesh is expanding; flesh juice would be a new addition in this respect. Numerous studies have evaluated the viability of pasteurizing and commercializing watermelon juice, without extracting seeds (Montesano, *et al*., 2018).

Even though watermelons with seeds continue to dominate the market. But, still, it is important to look into the watermelon's seed content. A number of studies on the viability of seed reported the manufacturing of flour for protein powders and oil production and stated that the biorefinery concept can be used to utilize the seed component for high return.

Proteins from watermelon seeds would be a beneficial addition to the majority of protein sources other than cereals (Mahindrakar, *et al*., 2020; Montesano, *et al*., 2018).Watermelon seeds are frequently used on a modest scale in many countries as a source of oil and protein. The flour of watermelon weeds contains an excellent amount of soluble protein it can be used in food products that are protein ideal. Moreover, it can also be used for the stabilization of the colloidal food system. Studies also stated that the extracted protein from watermelon can be used for cowpeas as a protein supplement as well. The protein assimilation of a blend of cowpea and watermelon proteins is about 80%, somehow lower than the lysine accessibility of watermelon flour which is 93%. Lycopene, a potent antioxidant found in tomatoes watermelon and other sources, has demonstrated efficacy against diseases like -

efficacy against diseases like cancer and cardiovascular issues. It's used as a food additive, supplement, and natural food coloring. However, it degrades due to factors like temperature and light during processing and storage. Carotene, an important antioxidant and precursor to vitamin A, also has benefits for skin health and may slow macular degeneration.

Challenges with its use in food include instability and poor solubility. Nanoencapsulation improves solubility. Xanthophylls like zeaxanthin and lutein aid ocular health. Red-fleshed watermelons are rich in lycopene, especially cis-isomers which are easily absorbed. Watermelon's lycopene content varies due to genetics and environment.

Figure 5. Total Calories in Watermelon
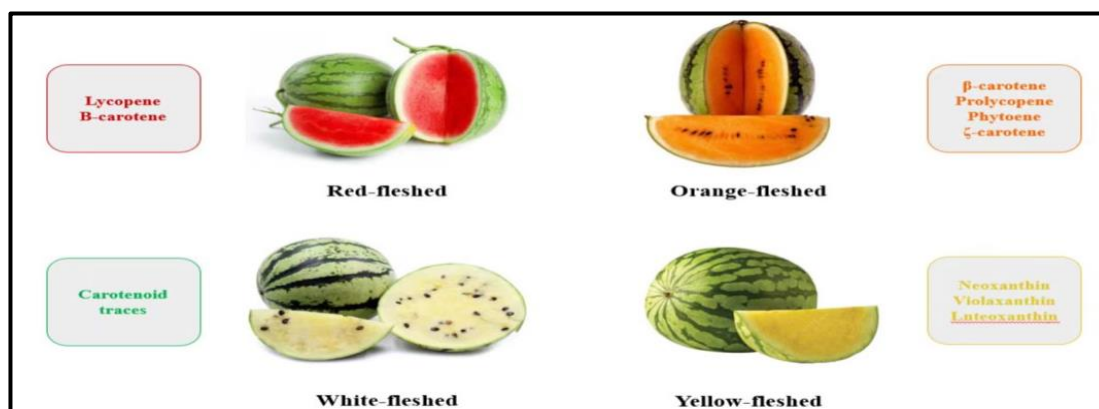
Figure 6. Main carotenoids in different color watermelons

Table 1. Studied carotenoid distribution in various watermelon cultivars, focusing on lycopene isomers and β-carotene

	Variety cultivar		Carotenoids	Amount mg/kg Fresh	References
	Xite (Hazera 6007	Fruit pulp	Lycopene (90% all *trans*- and 10% cis isomers)	98.84	Kim (2014)
	Hazera 5109	Fruit pulp	Cumulative lycopene	96.01	Naz (2013)
	Extazy (HZ6008)	Fruit pulp	Cumulative lycopene	91.92	Naz (2006)
	Crimson sweet	Pulp	Cumulative lycopene	45.50	Fiedor (2014)

	Variety cultivar		Carotenoids	Amount mg/kg Fresh	References
	Giza	Pulp	Cumulative lycopene	61.98	Fiedor (2014)
	Dumara	Pulp	Cumulative lycopene	45.90	Naz (2013)
	P403	Pulp	Cumulative lycopene	45.04	Fiedor (2014)
	P503	Pulp	Cumulative lycopene	63.95	Fiedor (2014)
	PWM25-4	Matured fruits	Cumulative lycopene	72.90	Naz (2013)
	Kiran	Matured fruits	Cumulative lycopene	75.95	Naz (2013)
	Kareena	Matured fruits	Cumulative lycopene	79.43	Nazet (2013)
	Zaohua (red-ripe)	Pulp	Cumulative lycopene	36.90	Fiedor (2014)
	var. Ole ´	Pulp	Provitamin A	9.40	Fiedor (2014)
	Minipool	Flesh	Provitamin A	8.90	Naz (2006)
	Mielhart (Hazera 5133)	Flesh	Provitamin A	9.98	Fiedor (2014)
	–	Juice	Provitamin A	0.25	Naz (2013)
	–	Rind	Provitamin A	0.65	Naz (2013)
Solanum lycopersicum		Consumable portion	Cumulative lycopene	103.97	Suwanaruang (2016)
		Raw fruit	Provitamin A	4.3	Kim (2014)
Papaya		Consumable portion	Total lycopene	46.02	Naz (2013)
Psidium guajava, Horana Red variety		Fruit	all *trans*-lycopene	42.99	Kim (2014)
Carrot	var. Florida F1	Consumable portion	Provitamin A	264.08	Kim (2014)
Carrot	var. Nevis F1	Consumable portion	Provitamin A	244.98	Kim (2014) ´

Table 2. Research on carotenoids from various Citrullus lanatus sources highlights their diverse health benefits, including antioxidant, antiproliferative, cardio-protective, and potential antihyperglycemic properties.

Source	Pigment	Role	Research Findings	Reference
Watermelon fruit	Carotenoid compound	Antioxidant, antiproliferative, cardio-protective, antihyperglycemic effects.	Watermelon: Abundant source of cis-isomeric lycopene	Naz (2013)
Watermelon lycopene extract	Carotenoid compound	Antioxidative and anti-inflammatory	Watermelon lycopene extracts counter free radicals, inhibiting iNOS and COX-2 mRNA expressions and proteins in a dose-dependent manner.	Kim(2014)
Watermelon juice and lycopene extract	Carotenoid compound	Antioxidant	Watermelon juice and lycopene extract exhibited antioxidant activity against free radicals.	Naz (2014)
Watermelon fruit	Lycopene, carotenes, and xanthophylls	Antiproliferative	Lycopene and other carotenoid-rich fruits and vegetables form the basis of a healthy diet. watermelon, resulted in protection against prostate cancer	Naz(2014)
Watermelon fruit	Lycopene	Antioxidant	Watermelon is rich in lycopene, exhibiting potent antioxidant properties in vitro. However, a three-week supplementation study on middle-aged adults found no impact on antioxidant or cholesterol levels.	Pinto(2011)
Watermelon juice	Lycopene	ND		Collins(2004)
Watermelon juice	Lycopene	Antioxidant, antidiabetic	Watermelon's lycopene-rich content showed strong antioxidant and antidiabetic properties through β-carotene bleaching and inhibition of α-amylase and lipase.	Naz (2014)

Numerous techniques are being used for watermelon rind to create products with added value. Although the rind alone can be used to extract juice, no known commercial uses have been found (Müller, *et al*., 2014; Medeiros, *et al*., 2019). Recently, USDA ARS started to create a patent that is used to obtain the rind citrulline, an amino acid that helps remove nitrogen from the blood and convert it into urine (Montesano *et al*., 2018). Other studies have also stated the use of rind as a component in cheese and pickles (Chen *et al*., 2015; Montesano *et al*., 2018).

Table 3. Mean values of Seeded, Seedless, Personal, Excursion, Fascination, Exclamation, and Captivation Varieties

Sample	Excursion	Fascination	Exclamation	Captivation	Seedless	Seeded	Personal
Melon	4.78a	4.00[a]	4.24[a]	4.85a	4.30[a]	4.16[a]	4.62[a]
Fresh	4.85a	4.35[a]	4.67[a]	5.00[a]	4.55[a]	4.43[a]	4.60[a]
Green*	3.52[b]	3.3[a,b]	3.3a,[b]	3.41a,[b]	2.82a,[b]	2.9[a],[b]	2.37[a]
Ripe	3.90[a]	4.26[a]	4.21[a]	3.95[a]	3.87[a]	3.76[a]	5.00[a]
Seedy	3.17a,[b]	3.67[b]	3.3a,[b]	2.9[a],[b]	2.94a,b	3.2[a],[b]	2.55[a]
Sweet***	4.76[b,c]	3.76[a]	4.26a,[b]	5.0[b],[c]	4.4a,[b,c]	4.[a,b,c]	5.28[c]
Sour***	2.72[c]	2.2[b,c]	2.14[b,c]	2.6[b],[c]	2.37[b],[c]	2.0[a],[b]	1.43[a]
Astringent	2.09[a]	2.10[a]	2.22[a]	2.1[a]	2.03[a]	2.05[a]	2.01[a]
Refreshing***	6.82[b,c]	5.44[a]	6.03a,[b]	7.21[c]	6.66[b,c]	6.0[a],[b]	6.2[a,b,c]
Wateriness	7.41[a]	6.75[a]	6.97[a]	7.5[a]	7.06[a]	7.02[a]	6.96[a]
Crispness***	5.82[c]	4.5a,[b]	5.06a,[b,c]	5.6[b],[c]	5.4a,[b,c]	4.47[a]	5.05[ab,c]
Mealiness*	3.75a,[b]	5.08[b]	4.41[a,b]	3.53[a]	3.62[a],[b]	4.6[a],[b]	4.36[a],[b]

Distinct letters in the same row indicate significant differences (*p< 0.05, **p< 0.01, ***p < 0.001).

1.3. The source & effects of free radicals, as well as benefits of antioxidants to human health

Free radicals, containing unpaired electrons, can harm cells. Reactive oxygen and nitrogen species (ROS and RNS) also contribute. Overproduction of radicals is linked to tissue damage, affecting lipids, DNA, and proteins. Environmental factors like toxins, radiation, and pollution generate free radicals, causing complications. ROS like oxygen radicals cause cell structure damage, nucleic acid alterations, and lipid peroxidation. Antioxidants counter oxidative damage by neutralizing radicals. Cells use endogenous and exogenous antioxidants for protection. Enzymatic (SOD, catalase) and non-enzymatic (vitamins) defenses exist. Free radical impact on health is illustrated in (Fig7). Watermelon, rich in vitamins and phytochemicals, particularly lycopene, acts as potent antioxidant that defends against oxidative stress.

Lycopene, the red carotenoid pigment, supports normal metabolism, guarding against cancer and degenerative diseases. With its versatility, watermelon can be enjoyed in various ways – from breakfast to snacks – while offering substantial vitamin C and β-carotene content that enhances its potential to combat cancer through antioxidant prowess (Caillet, *et al*., 2012).

1.4. Oxidation of Plant-Based Oils

Volatile oils (VOs) and fats are essential nutrients for humans, derived from plants or animals. Vegetable oil production is substantial at 207 million metric tonnes globally (2021/22).Volatile oils are obtained from oleaginous seeds and fruits through solvent extraction or mechanical expulsion. Quality is

determined by both sensory and compositional factors, influencing consumer preferences (Fadda, *et al.,* 2007). Off-flavors and odors in oils arise from triglyceride oxidation or hydrolysis, as depicted below.
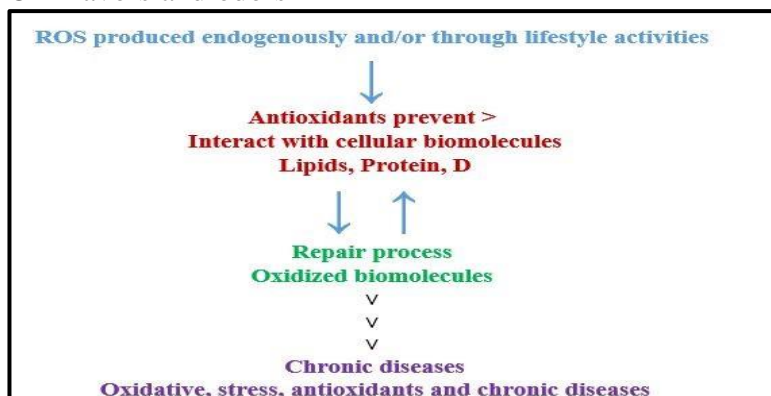
Figure 7. Oxidative Imbalance and its Link to Chronic Conditions
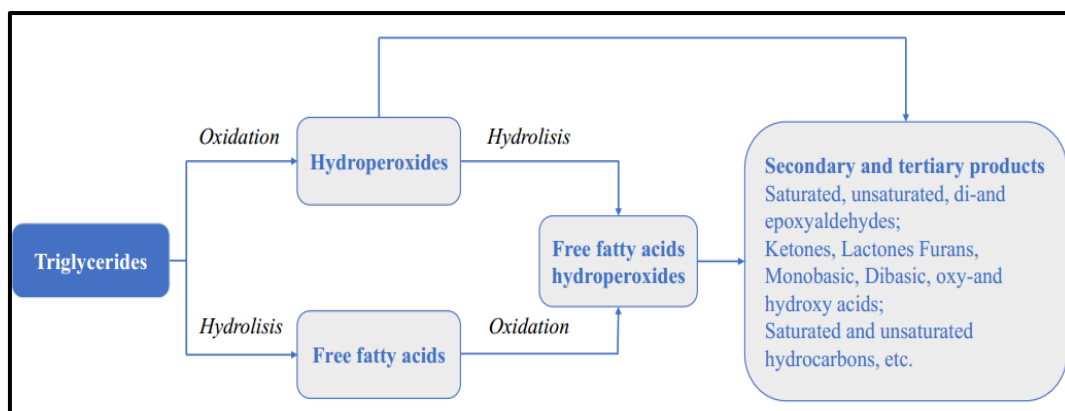
Figure 8. Oxidative and hydrolytic degradation reaction
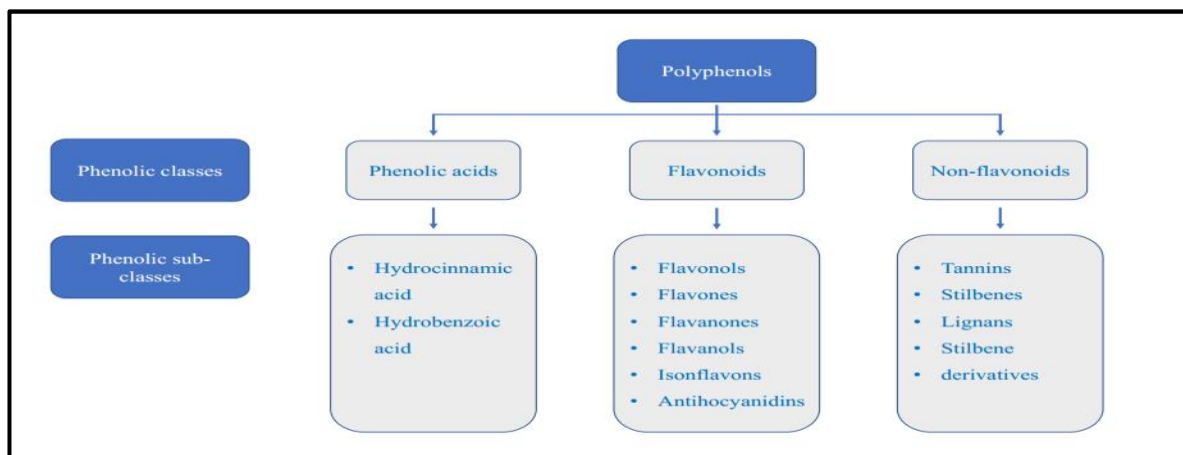
Figure 9. Phenolic classes and sub-classes

1.5 Phenolic Substances

Phenolic compounds are prominent in plants, found in various parts like roots, leaves, and fruits. Plants produce these secondary metabolites for protection and growth. Phenolic compounds are classified based on their subunits, leading to phenolic acids, flavonoids, and non-flavonoids. They serve as antioxidants, scavenging free- radicals and inhibiting their production. Phenolic acids, like hydroxybenzoic

and hydroxycinnamic acids, are vital subcategories. These compounds have bioactive potential and contribute significantly to plant health (Ghasemzadeh, *et al*., 2011; Azwanida, *et al*., 2015).

1.6 Flavonoid Substances

Flavonoids (pronounced "flavus" in Latin; it signifies "yellow") is derived from the Latin word "flavus," which means "yellow". It imparts color to the flower's shoots, petals, or fruits. They give red or blue color (Sandhar, *et al*., 2011; Vuolo, *et al*., 2019). They are physiologically active plant compounds with potential health benefits (Greenwell, *et al*., 2015). The antioxidant processes of flavonoids have been investigated through chelation abilities, straight forward neutralization of reactive Oxygen species and inhibition of oxygen species and inhibition of oxidative enzymes. (Karak, *et al*., 2019). Flavonoids are classified as flavonols, flavones, flavanones, Isoflavones, and anthocyanins based on species, environmental factors, plant tissues, growth circumstances, and level of maturity (Wang, *et al*., 2018)
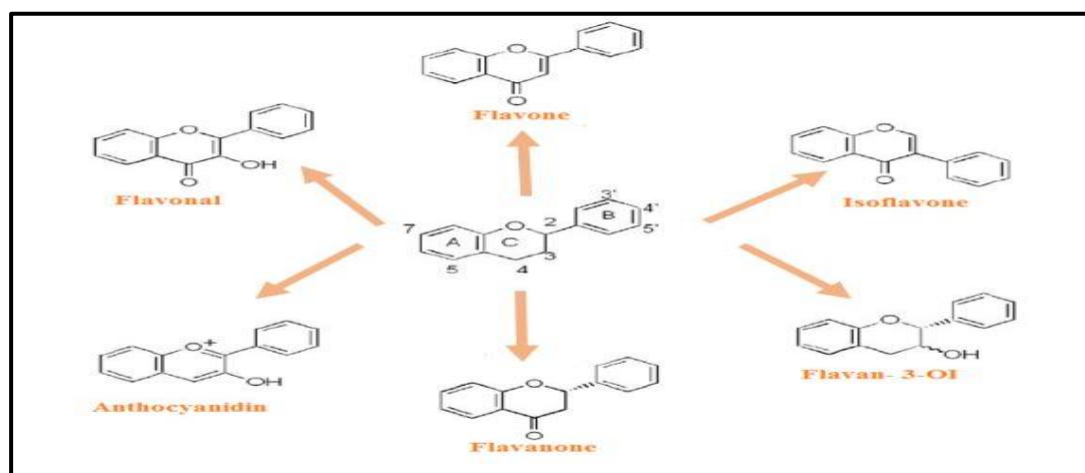
Figure 10. Flavonoid Substances

Table 4. Concentrations of phenolic compounds and flavonoids found in Modified Atmospheric Packaging

Vegetation	Substances	Levels	Reference
Licorice	Phenolics &terpenoids	4.94±0.42 g/100 g	Karak (2019)
Indian Snakeroot	Alkaloids	2.06 ± 0.13 g/100 g	Karak (2019)
Bloody Cranesbill	Catechins and proantho-cyanidines	2.02 mg/kg	Naz (2014)
Moldavian Balm	Rosmarinic acid	245.92 ± 24.67 mg/g	
Moldavian Balm	Chlorogenic acid	1.39 ± 2.68 mg/g	Naz (2014)
Moldavian Balm	Pigenin-7-O-glucoside	6.72 ± 2.30 mg/g	
Ficus microcarpa L. fil	Protocatechuic acid	6.80 ± 0.10 mg/g dry extract	

-	Catechol P-vinylguaiacol	10.91 ± 0.01mg/g dry extract 3.90 ± 0.06 mg/g dry extract	Karak (2019)
-	Vanillin	4.27 ± 0.02 mg/g extract	
-	Syringaldehyde	8.96 ± 0.29 mg/g extract	
Hibiscus cannabinus L.	Flavonoid content	82.11 mg/g extract	Patel (2010)
Trigonella arabica Delile.	Tannin content	2 ± 0.47 mg TA/g	Jaradat(2010)
Trigonella berythea Boiss. & Blanche		9 ± 0.47 mg TA/g	Jaradat (2016)
Origanum vulgare L.ssp. hirtum (Link)	Rosmarinic acid Carvacrol	116.7 grams per kilogram of dried extract 94.6 ± 21.16 grams per kilogram of dried extract	Karak (2019)
Origanum vulgare L.	Rosmarinic acid	12.88 mg/g plant	
	Chlorogenic acid Hyperoside	2.10 milligrams per gram of plant 1.05 milligrams per gram of dried extract	Karak (2019)
	Isoquercitrin	0.69 mg/g dry extract	
Satureja thymbra L.	Salvianolic acid A Cafeic acid	66.4 grams per kilogram of dried extract (± 1.7 g/kg) 2.69 grams per kilogram of dried extract (± 0.1 g/kg)	Karak (2019)
Thymus capitatus (L.) Hoffm.	Taxifolin Eriodictyo	4.28 grams per kilogram of dried extract (± 0.03 g/kg) 2.36 grams per kilogram of dried extract (± 0.12 g/kg).	Oreopoulou (2019)
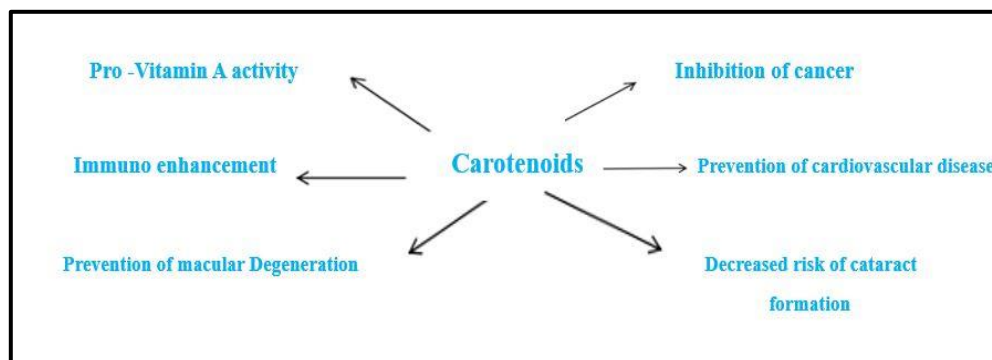
Figure 11. Non-Flavonoid Phenolic Substances

1.7 Non-Flavonoid Phenolic Substances

Tannins, also known as tannic acid found in numerous plants. They are non-phenolics. (Vuolo, *et al*., 2019). Tannins are frequently divided into two chemical categories: hydrolyzable tannins and condensed tannins (Vuolo, *et al*., 2019). Tannins have a more complex and homogenous structure than hydrolyzable tannins. Another type of phenolic chemical found in plants is stilbenes, lignans, and stilbene derivatives. All of these diverse compounds exhibit exceptional properties in plants like antioxidants or free radical scavenging (Oreopoulou, *et al*., 2019; Tabaraki, *et al*. 2011; Balboa, *et al*., 2014). Existing naturally in plants are numerous bioactive phenolic compounds with antioxidant properties they include phenolic substances, carotenoids, coenzyme Q, lycopene, and vitamins (A, C, and E) (Kücük, *et al*., 2017).

1.8 Carotenoids and their health benefit

Carotenes, present in both plants and animals, span yellow to red shades. These pigments, carotenoids, comprise lengthy aliphatic polyene chains with eight isoprene units. Found naturally in red, yellow, and orange hues, carotenoids abound in leafy greens and yellow-orange fruits. Notably concentrated in vibrant foods like plants, bird feathers, crustaceans, and marigold petals, their significant applications are shown in (Fig.12) (Eldahshan, *et al*., 2013; Taeymans *et al*., 2014).
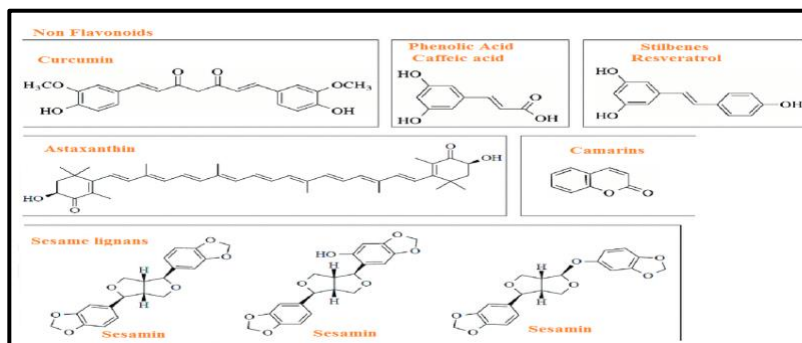
Figure 12. The Beneficial Roles of Carotenoids in Promoting Health

Fruits and Vegetables: Rich Sources of Vibrantly Colored Carotenoids in Human Diets Carotenoids are linked to health benefits like preventing cardiovascular diseases and cancer, and they provide essential vitamin A. Nature has over 600 carotenoids, but around 40 are typical in diets, with about 20 found in human tissues. Common carotenoids include β-carotene, α-carotene, lycopene, lutein, and cryptoxanthin. (Sethi, *et al*., 2020).These molecules share structural features with double bonds and symmetry. Isomerization to cis-trans isomers is possible due to double bonds, with Tran's isomers being more prevalent and stable. The complete implications of carotenoid

isomerization on human health remain uncertain. (Eldahshan, *et al*., 2013).

1.9 Evaluation of radical scavenging capacity (DPPH)

Antioxidant activity is assessed by measuring the ability to scavenge free radicals, turning them yellow. The extraction process is influenced by factors like temperature, solvent concentration, pH, and time. Extraction solvents depend on the compound's polarity; water for water-soluble antioxidants and organic solvents for fat-soluble ones. New methods combine ethyl acetate, hexane, and acetone, alongside traditional approaches like Soxhlet extraction and modern techniques such as ultrasound and microwaves. These newer methods require substantial energy input to enhance efficiency (Li, *et al*., 2018; Zhao, *et al*., 2013).

1.10 Usage of natural antioxidants to enhance oil property

Oil and fats are generally considered unstable because of oxidation but they do have many antioxidants such as tocopherols, carotenoids, tocotrienols, and sterols. When the oil is not oxidative stable it starts giving off many health problems that are serious. The quality of this type of oil becomes low and they do causes then cancers, mutagenicity, colds, CVD, and others (Farooq, *et al*., 2021). Many techniques have been introduced that

Are been used to stable the quality if oil and increase oxidative stability. The one mostly used technique is to use synthetic antioxidants like TBHQ, TBHA, PG, and BHT (Zamuz, *et al*., 2021; Samaram, *et al*., 2015).Synthetic compounds are causing health hazards. However, natural compounds are effective (Castelo-Branco, *et al*., 2016). Plants' volatile oil is found to be useful to enhance the quality of oil as well as flavor for oily products (Zheng, *et al*., 2019). The oils that have been rich in volatile oils and some other kinds of products are also been tried to get used as functional foods. As the fortified food product is enhanced with nutrients, the flavor can also be enhanced by the addition of aromatic compounds (Naknaen, *et al*., 2016). This strategy is rapidly spreading across the globe (Zheng, *et al*., 2019). A variety of flavored

oils containing various ingredients have been marketed (Van Belzen, *et al*., 2017; Oliveira, *et al*., 2018). Leaf, roots, kernels, or flowers can be considered as by-products for the plant which contain high concentrations of bioactive compounds. These bioactive compounds are used as natural antioxidants to stabile the oil against oxidation. These are phenolic acids, flavonols, and anthocyanidins (Galano, *et al*., 2015). Several studies on the ability of plant extracts to inhibit oxidative degradation have been conducted previously. Olive leaves (Zahran, *et al*., 2020), aromatic plants (Saoudi, *et al*., 2016), rosemary, Rambutan, and fruit skin (Phuong, *et al*., 2020), grape seed (Freitas, *et al*., 2017), cocoa bean shell (Patricia, *et al*., 2017), coffee husk (Ribeiro, *et al*., 2017), peanut skin (Franco, *et al*., 2018), and *Cressa cretica* (L.) leaves (Afshari, *et al*., 2018), extracts commonly used. Extract-enhanced soybean oil had a lower peroxide value as well they do contain a smaller amount of secondary product (Zahran , *et al*., 2020).The results come with fortified oil that contains a low amount of TBA. Thymus vulgaris extracts are normally used to delay the degradation process as reported previously (Kozowska, *et al*., 2018; Phuong, *et al*., 2020; Yang, *et al*., 2016). *Rosmarinus officinalis* (L.) and coffee husk also do works as antioxidants. In addition, they reduce free fatty acid production and peroxide value while boosting antioxidant activity (Kozowska and Isabel, *et al*., 2017). These studies indicated that these plant extracts could be used as a substitute for synthetic antioxidants.

1.11 Additional bioactive substances in watermelon.

There is a class of oxygenated steroidal triterpenes. This class is known as "cucurbitacins" as they do have a curcubitane skeleton. They possess antitumor, anti-inflammatory, and anti-diabetic properties (Kaushik, *et al*., 2018). Both the watermelon flesh and leaves are utilized for extracting Curcubitacin B, C, D, E, I, and curcubitacin L 2-O-b-glucoside. (Hassan, *et al*., 2011). It has been demonstrated that numerous anticancer activities inhibit cell proliferation, cell cycle arrest, and apoptosis (Chen, *et al*., 2011).Despite having

the same molecular structure, the cucurbitacins listed above can induce distinct cell cycle arrest stages. Cell cycle arrest induced by cucurbitacin was frequently associated with apoptosis (Chen, *et al*., 2011). The above-mentioned cucurbitacins inhibited cyclooxygenase (COX) 2 enzymes (Jayaprakasam, *et al*., 2003a). Inhibiting TNF-along with some other inhibitors like nitric oxide synthase-2, cyclooxygenase-2, and dihydrocucurbitacin B also showed their activity against inflammation (Escandell, *et al*., 2007; Jayaprakasha, *et al*., 2003). Cucurbitacins unquestionably contain the ability to initiate agonistic action. Cucurbitacin B exhibited hypoglycemic effects in differentiated enter endocrine L cells by initiating the AMPK and increasing GLP-1.This molecule improved hyperglycemia in diabetic mice with the help of activating intestinal AMPK and causing plasma GLP-1 (Li, *et al*., 2018). The translocation is considered an important step to make glucose entry into the body cells by acting at the AMPK pathway level. AMPK activation is crucial to numerous metabolic processes because it increases fatty acid oxidation, inhibits lipid synthesis, and enhances the action of insulin (Tan, *et al*., 2008; Ruderman, *et al*., 2005).One of the potential antioxidants to neutralize the free radicals is vitamin C which has the capability to convert an iron state that is high in oxidation to Fe^{+2}. Lanatus is considered to be a good source of vitamin C. Its flesh provides more than rind and seeds (Rahman, *et al*., 2013). The weight can be varied depends on the environmental conditions, genotype as well as pre-harvest and post-harvest conditions (Alka, *et al*., 2018; Ilahy, *et al*., 2019). Watermelon studies reported that the bioactives isolated from *C. lanatus* are an active source for the health of humans (Deshmukh, *et al*., 2015; Ekene, *et al*., 2014; Messaoudi, *et al*., 2014).

1.12 Maceration, infusion, percolation, and decoction

Plants either coarse or powdered are soaked in a container that has been solvent-sealed also called a menstruum. For at least three days at room temperature with continuous agitation until the soluble materials have dissolved. The mixture is then filtered, and the majority of the occluded solutions are extracted from the solid residue. The combined filtered and pressed liquid is then filtered to remove contaminants (Jovanovic, *et al.,* 2017). The liquid that has been filtered is evaporated and concentrated. Similar to maceration, infusion, and decoction are used to dip in hot water or cold water. Infusion, however, has a shorter maceration period. In general, maceration and infusion contain more fat-soluble compounds than decoction. The percolator is a one-of-a-kind piece of equipment used in the percolation extraction technique (Ribeiro, *et al.,* 2017).

1.13 Ultrasound-assisted extraction (UAE)

UAE technique is being used widely in the field of pharmaceutical and food industries during the last thirty years. They are considered an important part to increase the efficiency of extraction (Esclapez, *et al*., 2011). The mechanism is based on the cavitation phenomena. Ultrasound propagates, by a successive approach in the waves of compressional and rarefaction present in a system of liquid (Chemat, *et al*., 2012; Soria, *et al*., 2010). When a few cycles pass the diameters of those bubbles increase. It starts to expand until it does not reach the critical threshold. This point is the one at which the bubbles mix with each other and releases energy in a high amount. When the energy releases the temperature rises up to 5000k from the room temperature and pressure up to 1000 atm. During this technique plant cell walls can be damaged because of the presence of high temperature and high pressure. Due to this, from the plant cell wall bioactive compounds start coming out. (Rodsamran, *et al*., 2019; Muniz-Mˆarquez, *et al*., 2013). This is how the mass transfer rate improves. Temperature, pressure, and frequency are the factors that affect the frequency and yielding amount of extraction. For a thriving extraction, the type of solvent, solvent's volume, property of a sample like particle size, and their moisture content level (Talmaciu, *et al*., 2015). Ultrasonic extraction has a variety of benefits over conventional techniques in contexts of extraction efficiency and time. Methods of

operation concerning extraction yields and extraction durations (Virot, *et al*., 2010; Zhang, *et al*., 2020).

Table 5. Examples of extraction methods of natural antioxidants

Extraction Method	Plant	Main Compounds	Main Results (Extract)	Reference
Soxhlet extraction	Spearmint (*Mentha spicata* L.)	Flavonoids	Catechins = 0.144 mg/g	Bimakr (2011)
Maceration	Summer savory (*Satureja hortensis* L.)	Phenols Flavonoids Anthocyanins	TPC = 125.34 ± 0.13 mg GAE/g TFC = 16.27 ± 0.34 mg RU/g TAC = 115.21 ± 0.95 mg C3G/g	Maškovi (2017)
Micro-waves assisted extraction	*Pistacia* leaves (*Pistacia lentiscus* L.)	Polyphenols	TPC = 148.79 ± 8.22 mg GAE/g	Dahmoune (2014)
(UAE)	Rosemary leaves (*Rosmarinus officinalis* L.)	Polyphenols	TPC = 2030 ± 38 ppm GAE TPC = 35.0 mg GAE/g	Bellumori (2016)
Supercritical Fluid extraction	Rosemary (*Rosmarinus officinalis* L.)	Antioxidant compounds	EC_{50} (DPPH) = 0.22 mg/mL	Babovic (2010)
Pressurized liquid extraction	Spinach (*Spinacia oleracea* L.)	Vitamin E forms	α-T = 282 ± 12 µg/kg β-T = 8 ± 0.1 µg/kg γ-T = 82.9 ± 2 µg/kg	Viñas (2014)
High hydrostatic pressure extraction	Green tea (*Camellia sinensis* L.) leaves	Polyphenols	Yield of polyphenols at 4 min = 29.96 ± 0.6%	Shen (2009)
Pulsed electric field	Norway spruce (*Picea abies* L.)	Polyphenols	TPC = 9.20 g GAE/100 g	Bouras (2016)
Enzyme-assisted extraction	Stevia (*Stevia rebaudiana* (Bert.)	Bioflavonoids	Catechins = 86–103 g/100 g	Puri (2012)

GAE stands for gallic acid equivalent, EC denotes effective concentration, TPC represents total phenolic content, TFC includes total flavonoid content, TAC measures total antioxidant capacity, and DPPH is 2,2-diphenyl-picrylhydrazyl. Additionally, α-T, β-T, and γ-T stand for α-, β-, and γ-tocopherols.

Response surface methodology (RSM) is one of the most powerful and useful statistical procedures for optimizing multifaceted operations and evaluating the impact of process variables and their interactivity (Fiedor, *et al*., 2014).The past few years, this technique has

been extensively utilized to enhance the extraction of biologically active compounds from a variety of plant materials (Ghafoor, *et al*., 2009; Zhang, *et al*., 2016). However, only a few numbers of studies involving the optimization of UAE for the determination of total phenolic content (TPC) and antioxidant activity of watermelon skin (WMP) and seed (WMS) (Dranca, *et al*., 2016). Therefore, the present study was conducted to determine the influence of sonication temperature, sonication time, and ethanol concentration on the TPC and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of WMP and WMS extracts using RSM based on the Box-Behnken design (BBD)**.** In addition, phenolic acids in WMP and WMS extracts were quantified using gas chromatography-mass spectrometry under optimal conditions (GC-MS). In this work, the following hypothesis was tested: a designed experiment based on variable combinations of UAE process conditions may aid in establishing optimal conditions for maximum recovery of phenolic bioactive from watermelon byproducts. In this work, response surface methodology (RSM) was also used to simulate the impact of distinct variables (specifications) on phenolic compound extraction from different plants, as described previously (Mas'ud, *et al*., 2017; Rebollo-Hernanz, *et al*., 2021; Wang, *et al*., 2013). The results obtained were compared to percolation as a traditional extraction technique (Rebollo-Hernanz, *et al*., 2021)

1.14 Classification & preparation of hydrogels

In the literature, numerous hydrogel classifications and perspectives are presented. According to the ionic species on the bound units, hydrogels are classified as cationic, anionic, or neutral. According to the sources, there are two major types of hydrogels: those made of natural polymers and those made up of synthetic polymers. However, some authors have also used other terms such as physical, chemical, and biological hydrogels (Silva, *et al*., 2009). Changes in variables such as temperature, ionic concentration, and pH can cause physical hydrogels to transform from liquid to gel (Ahmad, *et al*., 2019). Chemical hydrogels, unlike other low-strength materials, rely on covalent bond formation to provide biomechanical stability and resistance to degradation. In biochemical hydrogels, biological agents, enzymes, and amino acids assist the gelation process. There are numerous other types of hydrogels, including crystalline, semi-crystalline, amorphous, and hydrocolloid clusters (Silva, *et al*., 2009; Ullah, *et al*., 2015).

Hydrogels are a three-dimensional polymer network that expands when exposed to water while retaining their mechanical integrity. Similar to extracellular matrices, hydrogels can hold vast quantities of water. The hydrophilic functional groups attached to the polymer membrane provide hydrogels with their water-retention capacity, while the cross-links between network chains give them the stability to resist degradation (Ullah, *et al*., 2015). Hydrogels are gaining popularity due to their ease of production, vast range of applications, and biocompatibility. Hydrogels, both natural and synthetic, are cross-linked hydrophilic polymeric materials in nature (Li, *et al*., 2006). Due to their high compatibility with human tissues, these polymers could be utilized in biomedical applications. In drug delivery, hydrogels offer numerous advantages including durability and sensitivity without side effects. Due to their well-established biocompatibility, hydrogels are frequently employed as drug-delivery hosts (Ullah, *et al*., 2015). Hydrogels are also used for spatial and temporal delivery of various medicinal substances, macromolecular pharmaceuticals, tiny drug molecules, and cells. Due to their controllable physical characteristics, variable degradation rate, and ability to preserve unstable compounds from deterioration, hydrogels serve as a foundation for a variety of physical and chemical interactions to regulate the release of the enclosed drugs (Wang, *et al*., 2019). Hydrogel beads are one of the hydrogel system's expansions. Beads are spherical objects that serve as a solid base for coating or encapsulating medicine within their core, in this way providing a controlled release. Moreover, beads are also known for their high bioavailability in

formulated medications. Gastro-retentive beads solve the problem of developing a gastro-retentive drug delivery system that maintains

drug release and prolongs the dosage form's stomach residence until the desired time of drug release (Amiri, *et al*., 2021).
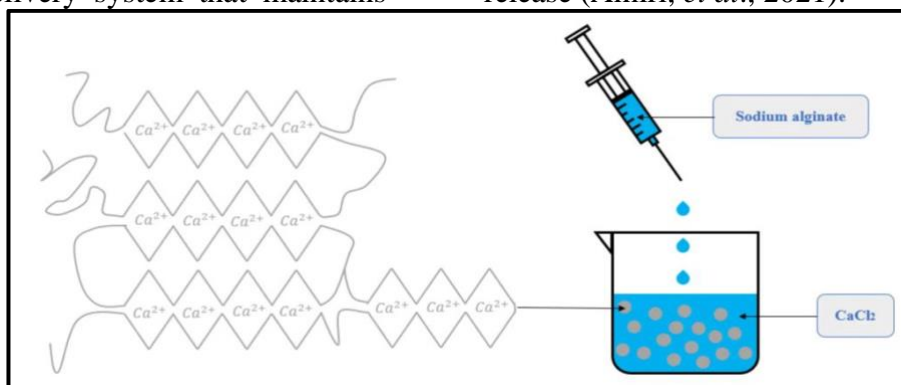
Figure 13. Preparation and cross-linking of hydrogel beads

1.15 Beads of oil

Oil beads contain liquid oil as their core material, created through dispersing vegetable oil into a wall material, resulting in a solution with consistent consistency. Ratios of oil-to-wall weight can reach up to 50% or more. Various proteins, carbohydrates, and gums are used as wall components, with A-Cyclodextrin being utilized due to its amphiphilic nature. Alginate and pectin are common choices for gel formation. Encapsulation efficiency varies with oil content and wall materials. Combining emulsifiers with ionic gelation can enhance oil loads and encapsulation efficiency. Formulation adjustments allow control over bead size, oil content, and mechanical characteristics (Lin, *et al*., 2020; Silva, *et al*., 2019).

1.16 Botanical Description of Watermelon (*Citrullus lanatus*)

Watermelons belong to the Cucurbita citrullus species, sharing a genus with pumpkin, squash, and bottle gourd. Its scientific name originates from Greek and Latin roots, "Citrullus" referencing the fruit and "lanatus" denoting "woolly." It's a summer crop with sprawling stems, pinnately lobed leaves, and shallow roots. The round to cylindrical fruits have edible endocarp and weigh between 8 to 35 lbs, with smaller Asian varieties weighing 2 to 8 lbs. Male and female flowers exist on the same plant. Watermelon seeds are obovate to elliptical, yellow to black, maturing with the

fruit's ripening. The seeds lack dormancy, germinating in 2 days to 2 weeks at temperatures above 60 °F.

1.17 Application of natural antioxidants

Natural antioxidants have been applied for years to preserve food, counteracting oxidation-related flavor, color, and texture changes in fat and oil-containing products. Oxidation generates free radicals during the process, leading to unwanted alterations in the product's quality and nutritional value. Unsaturated fatty acids are particularly vulnerable to oxidation, with double bonds accelerating the process. Efforts to enhance product stability have involved antioxidants, bioactive treatments, and controlled extraction methods. Synthetic antioxidants like BHT, BHA, TBHQ, and PG are used but raise health concerns. (Müller, *et al*., 2010). Oxidation can be categorized as physico-chemical autoxidation or enzymatically catalyzed lipoxidation. Synthetic antioxidants can harm health, while natural antioxidants from plants offer a sustainable solution to food preservation. Modern extraction methods like accelerated solvent extraction, microwave-assisted extraction, and ultrasound-assisted extraction are efficient for obtaining active components from plant organs. Ultrasound-assisted extraction (UAE) is particularly noteworthy due to its effectiveness and shorter time requirements, driven by acoustic wave-induced cavitation. UAE's benefits include

enhanced mass transfer and high extraction efficiency, although challenges like sample contamination and foam generation exist (Yao, *et al.,* 2020; Wang, *et al.,* 2013).

2.Conclusions

Watermelon, scientifically known as Citrullus lanatus, belongs to the Cucurbitaceae family and has a Greek-Latin name derived from "citrus" and "lanatus," meaning wooly. It is consumed worldwide for its sweet, juicy flavor. The fruit consists of flesh, seeds, and rind, with byproducts rich in bioactive compounds. Watermelon seeds have antioxidant properties and phenolic compounds, showing health benefits against various diseases. The potential therapeutic uses of these natural antioxidants have led to research on their extraction from watermelon byproducts. Watermelon's rind, seeds, and skin can be utilized in biorefinery processes. Lycopene, an antioxidant, is abundant in watermelon and is beneficial for various health aspects. Carotenoids like β-carotene and xanthophylls contribute to watermelon's nutritional value. Different varieties exhibit varying carotenoid profiles. Free radicals, formed during metabolic processes and due to environmental factors, can cause oxidative damage to cells and tissues. Antioxidants, found in watermelon and other sources, neutralize free radicals and protect against diseases. Watermelon's rich vitamin and antioxidant content, including lycopene and carotenoids, offer potential health benefits. Plant-based oils are essential nutrients obtained from seeds and fruits, with quality influenced by sensory and compositional factors. Oxidation and hydrolysis processes can cause off-flavors and odors in oils. Various aspects of phenolic compounds, flavonoids, non-flavonoid phenolic substances, carotenoids, antioxidants, and the usage of natural antioxidants in enhancing oil properties. It also highlights the presence of bioactive substances in watermelon and different extraction methods.

The importance of phenolic compounds in plants, including their classification into phenolic acids, flavonoids, and non-flavonoids, and their role as antioxidants. It explains the significance of flavonoids in plant pigmentation and physiological activity. The concentration of phenolic compounds and flavonoids in different plants, particularly in modified atmospheric packaging, is provided. The distinction between flavonoid and non-flavonoid phenolic compounds is outlined, mentioning tannins, stilbenes, and other antioxidants found in plants. Carotenoids, such as β-carotene and lycopene, are discussed for their role in providing color to fruits and vegetables, as well as their health benefits. The text also covers the evaluation of radical scavenging capacity using DPPH, the use of natural antioxidants to enhance oil stability, and the potential of plant extracts to substitute synthetic antioxidants. Oxygenated steroidal triterpenes, called cucurbitacins, found in watermelon, are explored for their anti-inflammatory and anti-diabetic properties. Maceration, infusion, percolation, and decoction extraction methods are explained briefly. Overall, the text emphasizes the bioactive compounds present in plants and their potential health benefits, as well as techniques for utilizing these compounds to enhance food quality and stability. Various techniques and methods used in the fields of extraction, encapsulation, and natural antioxidants. Ultrasound-assisted extraction (UAE) is highlighted as an efficient method for extracting bioactive compounds from plant materials using cavitation-induced energy release. The benefits of UAE include improved mass transfer and high extraction efficiency. Response Surface Methodology (RSM) is mentioned as a statistical approach for optimizing extraction processes. The passage also introduces the concept of hydrogels, three-dimensional polymer networks that can hold water while maintaining their structure. Different methods of oil encapsulation, including beads and capsules, are explained, with a focus on emulsion extrusion. The nutritional value and potential uses of watermelon seeds are discussed, highlighting their high protein content and essential amino acids. The passage concludes by providing botanical descriptions of watermelon and its growth characteristics. Overall, the passage covers a range of

techniques and applications related to natural compounds, extraction, encapsulation, and their potential benefits.

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LIPIDOMIC ANALYSIS, CAROTENOIDS CONTENT, AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF DIFFERENT PARTS OF *GANODERMA LUCIDUM* AND *GANODERMA ATRUM*

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ABSTRACT
This study determined fatty acid compositions of two cultivated *Ganoderma*. The canopy and stalk lipids of the *Ganoderma* were analyzed for fatty acid composition, β-carotene content and antioxidant activities. The results showed that *Ganoderma* samples contained 51 fatty acids. Their lipid extraction yields ranged between 0.45% and 1.09%. The β-carotene content of the red *Ganoderma* canopy extract was higher than its stalk extract. The canopy extract also had the highest DPPH hydroxyl radical scavenging activity. The data of the overall heat map showed that over 30 fatty acids were positively and highly correlated to the lipid in the black *Ganoderma* stalk. The main types of fatty acids in these *Ganoderma* samples were palmitic, oleic, and linoleic acids. The concentration of linoleic acid in the red *Ganoderma* canopy was as high as 164.02 mg/g lipid. These results suggested that these *Ganoderma* lipids are lipid-based antioxidants and potential sources of dietary supplements.

1. Introduction

Ganoderma (Ga) mushroom is a medicinal fungus that promotes good health. It has been used as traditional medicine to promote health in China for over 2000 years (Li *et al.*, 2019). The fungus is used as a dietary supplement in the United States. It is listed in the U.S. Pharmacopoeia of Dietary Supplements and Herbal Medicines, and its subsidiary entities are commonly used as primary medicinal parts (Xu and Yu, 2021). Some bioactive ingredients in Ga mushrooms that affect human physiology have been studied and developed as active ingredients in cosmetics and nutraceuticals (El Sheikha, 2022). The mushroom is a source of micronutrients and bioactive substances such as terpenoids and polysaccharides. Unsaturated

fatty acids (UFAs) are also the natural bioactive components of Ga mushrooms, but the studies on FAs and bioactivities of this mushroom are still lacking.

Ga lipid contains a variety of FAs, including linoleic and α-linolenic acids. These FAs cannot be synthesized in the human body and must be obtained from foods. The FAs in foods are mainly in the form of phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. They constitute major lipid components and cell membranes. Saturated fatty acids (SFAs) possess adverse health effects, whereas unsaturated FAs are thought to have protective effects. Trans FAs have been shown to possess detrimental effects on human health, but

conjugated FAs may be beneficial (Tvrzicka *et al.*, 2011).

Monounsaturated fatty acids (MUFAs) predominate in the Ga sample (Stojkovic *et al.*, 2014). Researchers have found large amounts of oleic and palmitic acids in the lipid extracts of *G. lucidum* (Salvatore *et al.*, 2020). Oleic acid is thought to have various physiological functions; a previous study has confirmed its cancer-preventive effect (Carrillo *et al.*, 2012). On the other hand, unsaturated FAs have the function of regulating immune response, which can reduce wound inflammation and enhance repair response (Cardoso *et al.*, 2011). Studies have also shown that the Ga spore oil has excellent antioxidant capacity, which can effectively scavenge free radicals and extend the life span of fruit flies (Zhang *et al.*, 2021). The unsaturated FAs in the fruiting bodies of Ga are effective antioxidants, but the study on the antioxidant activities of Ga lipids is limited.

In this study, we evaluated the fat extraction rates and β-carotene content in fat from different parts of four Ga species. We also determined the DPPH free radical scavenging ability and ferric-reducing power of the lipid extracts, quantitatively analyzed 51 kinds of FAs in the extract, and discussed the differences of different varieties and parts of Ga regarding the extraction rate, *in vitro* antioxidant activity and FA content. Reviewing the results of previous studies, we have not seen similar reports. The results obtained have the potential to use Ga lipid as a functional food for the prevention of age-related diseases and provide guidance to industrialists and manufacturers who are looking for the best excipients for their products.

2. Materials and methods
2.1. Materials
Cultivated red (*G. lucidum*) and black (*G. atrum*) Ga mushroom samples were obtained from the Guilin Chinese medicinal wholesales market in Guangxi. The red Ga was of Guilin-origin, whereas the black Ga was cultivated in the Yunnan Province of China. The fruiting bodies of all Ga samples were separated into canopy and stem parts, and their base-part was discarded. The canopy part of the cultivated red

Ga was named RCC, and its stem part was RCS. The canopy and stem of the black Ga were BCC and BCS.

The chemicals used in experiments are analytical grade unless otherwise specified. The n-hexane, ethanol, chloroform and sulfuric acid was purchased from Xilong Science Co., Ltd. (Sichuan, China); 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), β-carotene, potassium ferricyanide, trichloroacetic acid, ferric chloride and methanol from Yien Chemical Technology Co., Ltd. (Shanghai, China); ascorbic acid from Jingchun Biochemical Technology Co., Ltd. (Shanghai, China). The methyl salicylate was obtained from Sigma-Aldrich Shanghai Trading Co, Ltd. (Shanghai, China) and anhydrous sodium sulfate from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). Also, the 0.1 M phosphate buffer saline (PBS) of pH 7.4 were from Solarbio Biotechnology Co., Ltd. (Shanghai, China).

2.2. Lipid extraction
All Ga parts were washed, oven-dried, and pulverized to 50-mesh particles. The lipid in two different parts of the Ga fruiting body was extracted using hexane (Salvatore *et al.*, 2020). In brief, 2.0 g Ga powder was added into 50 mL of n-hexane and subjected to ultrasonic assisted treatment for 30 min. After putting to stand in a water bath at 60 °C for 6 h, the hexane was removed using a rotary evaporator. The Ga lipid was collected and stored at -20 °C before FA analysis.

2.3. FA analysis
Trace 1300 gas chromatography equipped with an ISQ LT mass selective detector (Thermo Fisher Scientific, USA) was used to analyze the FAs in Ga lipids. The column used was the Thermo TG-FAME capillary column (50 m × 0.25 mm, 0.20 μm). The injection volume was 1 μL, and the shunt ratio was 8:1. The lipid sample (1 mg/mL) was weighed and added with 1 mL of chloroform-methanol (2:1, v/v) solution. The lipid solution was homogenized at 60 Hz for 1 min and then ultrasonicated for 30 min at a room temperature (RT) of 25 °C. After centrifugation

at 10,000 rpm for 5 min at 4 °C, the supernatant was collected. The supernatant was added with 2 mL of 1% methanol sulfate solution, mixed thoroughly, and esterified in an 80 °C water bath for 30 min. After cooled to RT, the esterified FAs were extracted with n-hexane for 5 min and then added to the chilled distilled water (4 °C) for washing and centrifuged for 10 min before adding 500 ppm methyl salicylate as the internal standard (Hoving *et al.*, 2018). The esterified FAs were analyzed using the GC-MS. Fifty-one FA methyl ester mixtures (4000 μg/mL) were diluted with n-hexane at concentrations ranging between 1 and 2000 μg/mL for plotting standard calibration curves. The GC-MS data was analyzed using multivariate statistics, and the receiver operating characteristic (ROC) analysis was also performed.

2.4. Extraction and analysis of β-carotene

The β-carotene in the Ga lipid samples was extracted using dimethyl sulfoxide (DMSO) (Kozłowska *et al.*, 2016). In brief, a 0.1-g lipid sample was weighed and added with 2.5 mL of n-hexane and 2.5 mL of DMSO. The solution was homogenized and ultrasonicated for 20 min at RT and centrifuged at 4000 rpm for 5 min. The DMSO layer containing β-carotene was collected. The β-carotene content of the Ga lipid samples was determined based on a spectrophotometric method (Khoo *et al.*, 2009).

2.5. Determination of *in vitro* antioxidant activities

DPPH radical scavenging activities of the carotenoid extracts of Ga lipids were determined according to a previously reported method with slight modification (Farooq *et al.*, 2023). The DPPH reagent (0.02 mg/mL) was prepared by dissolving it in an anhydrous ethanolic solution. The DPPH reagent (2 mL) was mixed with 2.0 mL of the extract at concentrations of 0.25, 0.5, 1.0, 2.0, and 4 mg/mL. The mixture was stirred and left to stand at RT for 30 min in the dark, and the absorbance was measured at 517 nm. The scavenging activities of the extracts were then calculated, and the results were expressed at EC_{50} values. Ascorbic acid (Vc) was used for comparison.

The reducing power of the carotenoid extracts was evaluated according to a previous method (Mota *et al.*, 2022), also known as ferric-reducing antioxidant capacity (FRAP). The 0.5-mL extracts (0.25, 0.5, 1.0, 2.0, and 4 mg/mL) were added with 0.5 mL of 1% potassium ferricyanide solution and 0.5 mL phosphate buffer (0.2 M, pH 6.7) in test tubes. The test tubes containing the reagent mixture were incubated in a 50 °C water bath for 20 min. After cooling to RT, 0.5 mL of 10% trichloroacetic acid solution (TCA) was added. The supernatant was collected after centrifugation, mixed with 0.5 mL of 0.1% ferric chloride solution and 2.0 mL distilled water, and left to stand at RT for 10 min. The absorbance was measured at 700 nm. The standard curve was plotted by applying a ferrous sulfate solution of five different concentrations (0 to 6.58 μM). FRAP value was expressed as $\mu M\ Fe^{2+}/g$ extract. Vc was used for comparison.

2.6. Statistical analysis

All data were expressed as mean±standard error (SE). The results were statistically analyzed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance coupled with the Tukey range test was used to compare the mean differences between different groups, and $p<0.05$ was considered statistically significant. The FAs in the lipid samples were also analyzed based on the principal component and ROC analysis.

3. Results and discussions
3.1. Ga lipid yields

The lipids in different Ga species and parts of their fruiting bodies were extracted with hexane. As shown in Figure 1, the extraction yield of RCC was the highest (1.09%). The extraction yields of RCS, BCC, and BCS were 0.96%, 0.92%, and 0.84%, respectively. There was no significant difference in the extraction yields among RCS, BCC, and BCS ($p>0.05$). The total lipid content of the Ga samples was estimated based on their extraction yields. The results showed that the Ga canopies had total lipid content higher than that of their stalks.

The difference in Ga lipid content could be related to their growing environment. The high-temperature treatment during extraction might also cause a loss of volatile components. These volatile substances were trans anisinol, R-(−)-linalsol, S-(+)-carvone, and sesquiterpenol (Ziegenbein *et al.*, 2006). The lipid content of Ga could be varied among different species (Chen *et al.*, 2023).
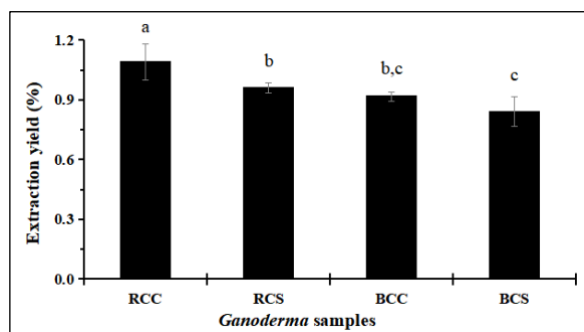
Figure 1. Lipid extraction yields of the Ga samples. *RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga.

3.2. FA composition of Ga lipids

The FA composition of Ga lipids was determined using a gas chromatographic system coupled with a mass spectrometer (GC-MS). The chromatographic parameters are shown in Table S1. The TICs of the samples and fatty acid

(FA) standards are depicted in Figure S1. As shown in the overall heat map (Figure 2), the types and levels of FAs in different Ga samples are varied. These FAs were divided into four categories. The results showed that RCC, RCS, BCC, and BCS had 0.28%, 0.1%, 0.24%, and 0.1% total FAs in the Ga lipids (Table 1). The black Ga samples had higher total SFA levels than the red Ga sample, whereas the total unsaturated FA levels were higher in the red Ga samples. Among the Ga samples, BCC had the highest total MUFA level, followed by RBC, BCS, and RCS; RCC had the highest total polyunsaturated FA (PUFA) level, whereas RCS had the lowest. The SFAs were highly distributed in the canopy of red Ga but not for the black Ga canopy. Moreover, the trans FA levels in the black Ga samples were higher than in the red Ga samples.

The result showed that 47 FAs were detected in the Ga samples (Table 1). They are 16 SFAs, 10 MUFAs, 11 PUFAs, and 12 trans FAs. Among the trans FAs, 7-trans nonadecenoate and 10-trans-nonadecenoate were not detected in all these Ga samples. The major FA was palmitic acid (C16:0), oleic acid (C18:1n9c), and linoleic acid (C18:2n6). They were the main SFA, MUFA, and PUFA, respectively, detected in the Ga lipids.

Table 1. Quantitative analysis of fatty acids

No.	Compound	RCC	RCS	BCC	BCS
Saturated fatty acid (µg/g)					
1	**C6:0**	68.60±4.93	148.17±5.04	59.36±1.65	1141.57±16.76
2	**C8:0**	12.51±0.35	32.67±0.97	68.94±2.38	275.95±7.61
3	C10:0	16.58±0.84	21.14±0.70	10.79±0.86	62.71±2.87
4	**C11:0**	7.22±0.32	11.64±0.24	2.83±0.32	11.72±1.17
5	**C12:0**	104.77±5.31	77.81±0.91	52.41±1.96	82.92±1.46
6	**C13:0**	26.64±0.63	22.24±0.41	18.01±0.42	32.56±0.81
7	**C14:0**	647.73±11.74	328.45±4.92	504.13±6.19	668.91±1.43
8	**C15:0**	5500.96±90.09	2206.63±12.99	3437.25±27.20	3328.16±87.70
9	C16:0	38414.06±286.31	12198.40±32.83	22754.51±310.53	27286.88±393.37
10	**C17:0**	1933.18±14.86	431.97±3.38	1000.47±28.29	2060.77±25.10
11	**C18:0**	4057.22±51.87	1125.55±12.60	1714.70±17.03	3313.34±21.39
12	**C20:0**	499.06±4.02	118.30±7.24	153.17±3.16	208.77±9.60
13	**C21:0**	177.94±3.91	172.42±3.46	189.95±7.04	159.22±10.42
14	**C22:0**	1005.60±15.88	405.136±9.92	776.26±26.11	685.34±26.58
15	**C23:0**	660.66±56.06	634.16±5.75	1805.91±32.68	1207.65±44.63

16	**C24:0**	1426.71±54.43	488.03±12.88	2908.04±67.16	2814.08±11.92
Trans unsaturated fatty acids (µg/g)					
1	C14:1t	25.06±1.08	19.67±1.76	21.91±1.62	97.97±7.19
2	**C15:1t**	45.87±3.67	43.95±4.40	34.33±2.46	77.11±8.25
3	**C16:1t**	70.31±4.86	53.69±4.70	55.88±4.44	133.84±11.39
4	**C17:1t**	235.28±6.42	186.36±17.00	150.60±4.07	291.42±8.51
5	C18:1n12t	342.51±12.72	421.86±8.67	254.38±1.85	948.89±28.63
6	C18:1n9t	185.05±2.57	165.56±10.74	234.26±3.90	368.11±14.37
7	C18:1n7t	136.60±4.23	148.00±8.48	89.95±1.49	276.78±7.74
8	C18:2n6t	117.95±8.72	101.63±2.58	81.78±4.01	186.16±5.45
9	**C20:1t**	133.34±3.31	86.54±3.91	82.41±3.27	179.59±3.16
10	C22:1n9t	128.46±12.08	138.42±7.42	117.42±2.14	219.39±3.52
11	C19:1n12t	ND	ND	ND	ND
12	C19:1n9t	ND	ND	ND	ND
Monounsaturated fatty acids (µg/g)					
1	C14:1	14.48±1.03	19.47±2.39	13.23±1.06	26.66±1.19
2	**C15:1**	55.43±7.58	49.79±4.18	40.78±2.30	74.82±4.14
3	**C16:1**	792.54±16.37	797.00±2.60	1649.53±24.95	703.50±33.20
4	**C17:1**	604.57±24.01	394.38±8.33	628.36±72.81	440.23±16.29
5	C18:1n12	5518.17±283.62	2187.24±111.10	6159.64±890.72	3971.72±351.34
6	C18:1nN9c	41705.70±201.66	13746.63±278.06	106148.21±973.26	18594.50±177.39
7	C18:1n7	5174.58±41.92	4596.26±51.44	3337.64±43.98	2713.32±32.66
8	C20:1	239.65±10.12	138.73±7.65	345.06±5.23	155.31±2.67
9	C22:1n9	327.93±7.66	287.10±13.12	247.85±8.43	324.52±16.38
10	C24:1	345.11±34.02	361.93±35.99	492.03±10.44	506.22±18.39
Polyunsaturated fatty acids (µg/g)					
1	C18:2n6	164024.74±2096.30	56918.17±1694.32	83024.11±1209.12	27072.35±362.11
2	**C18:3n6**	95.40±1.18	80.26±8.41	65.56±1.40	96.29±2.31
3	**C18:3n3**	307.71±11.12	205.75±7.08	243.78±16.76	152.16±8.34
4	**C20:3n6**	95.93±2.23	76.25±2.63	77.52±3.75	99.23±4.30
5	**C20:3n3**	66.11±1.71	58.34±7.77	52.53±3.07	85.05±0.81
6	**C20:4n6**	66.46±2.59	58.77±0.93	56.25±3.64	84.68±3.90
7	**C20:5n3**	78.80±6.58	57.59±6.25	48.38±3.60	95.10±6.63
8	**C22:4**	16.68±1.38	42.09±0.71	34.62±3.78	55.12±8.04
9	**C22:5n6**	15.89±0.42	60.95±3.92	52.52±6.97	143.87±19.19
10	**C22:5n3**	266.60±7.99	303.02±25.04	170.03±7.78	651.70±33.77
11	**C22:6n3**	131.70±3.44	109.11±8.81	125.22±9.78	204.01±9.49

* All values are expressed as mean±standard deviation of three replicates. The fatty acids content is presented as mg/g lipid. ND indicates that not detected.

These FAs were the highest in RCC. The other main SFAs were pentadecanoic (C15:0), heptadecanoic (C17:0), stearic (C18:0), and lignoceric acids (C24:0). Petroselaidic acid (C18:1n12t) was the highest trans FA in the Ga samples among all trans FAs. The total trans FAs content of these Ga samples ranged between 1 and 3 mg/g. Moreover, long-chain and very long-chain FAs were the main SFAs in the Ga samples. The very long-chain FAs content of the black Ga samples were higher than the red Ga samples, especially the canopy part of Ga.

The variations in Ga FA compositions could be due to the influence of the growing environment, especially environmental stress

and soil composition (Upchurch, 2008). The literature supports our findings that the red-purplish varieties of Ga contained palmitic, oleic, and linoleic acids as the main FAs (Lv et al., 2012). Palmitic acid and oleic acid are important FAs; they are involved in cell growth and apoptosis. Oleic acid can convert palmitic acid into inert triglycerides for storage.
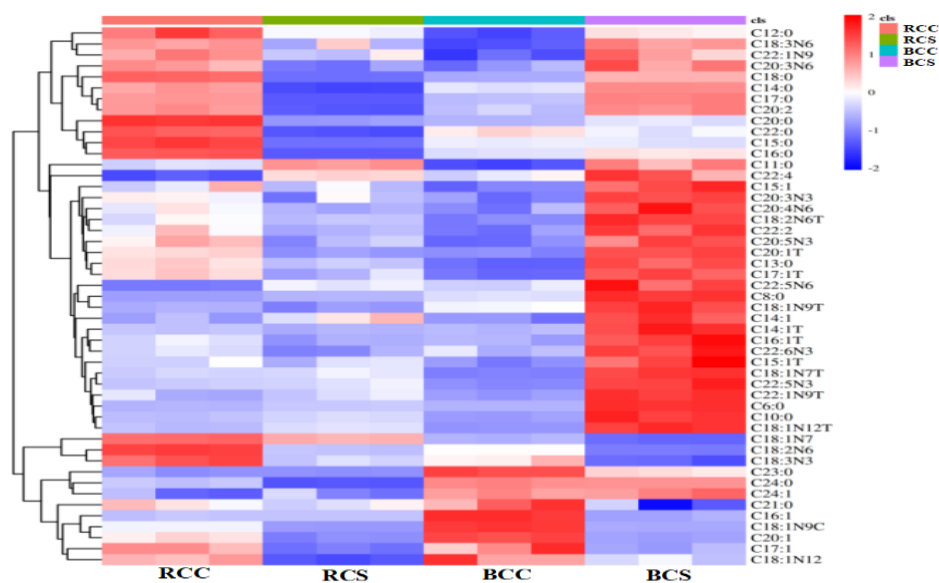
Figure 2. Overall heat map of fatty acids in the Ga samples.
*RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga

On the other hand, the lower amount of trans FAs and higher unsaturated FAs in the red Ga showed that it is a potent source of nutraceuticals for disease prevention. In this study, the types and parts of Ga are not only important for quality evaluation but also help to clarify their pharmacological activities.

3.3. Multivariate analysis of FA composition

The FA composition of Ga samples (RCC, RCS, BCC, and BCS) was analyzed based on the principal component analysis (PCA). It has been a popular multivariate statistical technique used to compare the FAs in the Ga samples. The PCA represented the similarity pattern of observations and variables by displaying them as points on a map (Abdi and Williams, 2010). As shown in Figure 3A, the PCA score plot reflects the distribution of the Ga samples. The distribution points of each sample are close to each other. It indicates that the FA composition and concentration in these samples are similar. The differences between the first and second principal components were 56.6% and 23.7%, respectively. The result also showed that 80.3%

of the variation can be explained by these two components. Moreover, less than 1/5 of the variation remained in the other components (Bro and Smilde, 2014). In this study, the Ga samples had R^2X (cum) of 0.803. The R^2X and PCA score plots were the main reference parameters for the model cross-validation, and R^2X was also the interpretability of the model. The R^2X (cum) of higher than 0.5 indicated that the cross-validation and interpretability of the test samples were good.

The model's independent variable explainability (R^2X), model dependent variable explainability (R^2Y), model predictability (Q^2), and PLS-DA score plot were used as parameters for the cross-validation of partial least squares discriminant analysis (PLS-DA) models. The results showed that the Ga samples had R^2X (cum), R^2Y (cum) and Q^2 (cum) of 0.972, 0.998, and 0.994, respectively. The differences between R^2 (cum), and Q^2 (cum) were between 0.004 and 0.022. The R^2 (cum) close to 1 showed that the experimental repeatability was high. It showed that the background noise was either absent or very small. There were also a

few abnormal samples in the model. The orthogonal partial least squares discriminant analysis (OPLS-DA) can evaluate the classification performance of the model by using the independent variable explainability (R^2X), model dependent variable explainability (R^2Y), model predictability (Q^2), and OPLS-DA score plot (Yao *et al.*, 2019). The OPLS-DA score plot is shown in Figure 3C. The R^2X (cum), R^2Y (cum), and Q^2 (cum) of the Ga samples was 0.771, 0.99, and 0.982. We observe that the differences between R^2 (cum) and Q^2 (cum) ranging from 0.008 to 0.0.211. The R^2 (cum) of OPLS-DA was significantly lower than that of the PLS-DA, which indicated that the its experimental repeatability was worse than the PLS-DA. However, its R^2 (cum) was higher than 0.5, and the Q^2 (cum) was 0.982. This results indicated that the experimental set was repeatable, and there were only a few abnormal samples in the model.

The metabolome data is multidimensional, and some variables are highly correlated. The traditional univariate analysis cannot rapidly and accurately mine the potential information in the dataset. Therefore, it is necessary to apply the principle of chemometrics and multivariate statistical methods in the metabolomic analysis for reducing and classifying the multidimensional data. As a results, the most applicable information can be mined and extracted.

3.4. ROC analysis

The ROC results showed that the area under curve (AUC) values of 10-trans pentadecenoic acid (C15:1t) and tricosanoic acid (C23:0) between RCC and RCS were $\leq$ 0.5. Their differential content was 44.96 and 637.10 µg/g, respectively. The finding suggests that the method used to identify these two FAs is ineffective to predict the occurrence of the events and has no predictive value. The AUC value of palmitoleic acid (C16:1) was 0.556, and its differential content was 797.49 µg/g, which indicates that the differential prediction accuracy of this FA was low. The AUC value of 10-pentadecenoic acid (C15:1), nervonoic acid

(C24:1), and brassidic acid (C22:1n9t) ranged from 0.7 to 0.9.
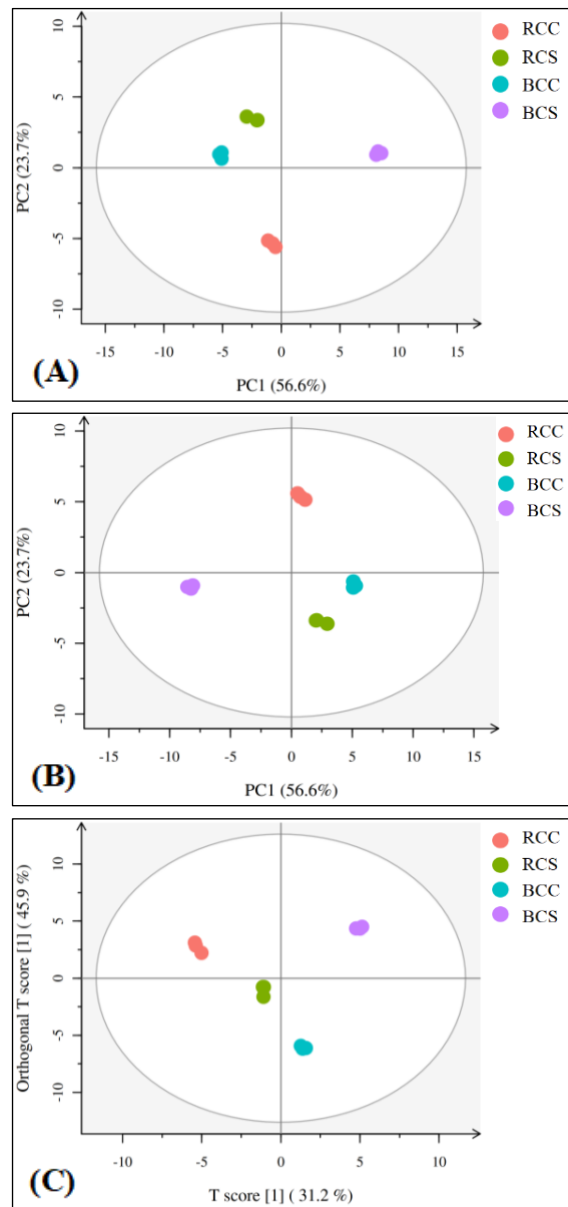
Figure 3. Multivariate statistical analysis diagrams of FAs in the Ga samples. (A) PCA, (B) PLS-DA, and (C) OPLS-DA score plots.

Their differential content was 48.42, 331.81, and 126.49 µg/g, respectively. The high AUC values indicated that the method used to identify these three biomarkers had some predictive accuracy. The AUC values of all remaining FAs were 1. It showed that the identification of these FAs was highly accurate.

The AUC values of pentadecanoic acid (C15:0) and nervonoic acid (C24:1) between

BCC and BCS were 0.889 and 0.778, respectively, and their differential content was 3436.73 and 505.25 μg/g, respectively. This result indicates that the method used has some predictive accuracy. The AUC values of the remaining FAs were 1, which shows that this method has high predictive accuracy.

The comparison of the AUC values of 10-heptadecenoic acid (C17:1) and docosahexaenoic acid (C22:6n3) between RCC and BCC was also performed. Their AUC values were 0.667, and their differential content was 619.46 and 125.84 μg/g, respectively. It showed that the predictive accuracy of identifying these two biomarkers was low. The AUC values of myristoleic acid (C14:1) and petroselinic acid (C18:1n12) were 0.778, and their differential content was 13.96 and 5531.62 μg/g, respectively. The AUC values of the remaining FAs were 1, which indicates that the identification of these biomarkers had high predictive accuracy. By comparing RCS and BCS, their AUC value of unndecanoic acid (C11:0) was 0.667, and its differential content was 12.10 μg/g, indicating that the identification of this biomarker had low predictive accuracy. The AUC value of heneicosanoic acid (C21:0) was 0.889, and its identification content was 170.86 μg/g. It indicates that the identification of this FA was predictively accurate.

The AUC is used to assess the sensitivity and specificity of the biomarker for predicting the occurrence of the event. The sensitivity and specificity of each metabolite are determined by the optimal threshold of the ROC curve (Carter *et al.*, 2016). If the AUC value is between 0.5 and 1.0, the AUC value closer to 1 denotes a higher prediction accuracy. The prediction accuracy is low when the AUC value ranges between 0.5 and 0.7; the prediction accuracy is moderate to high when the AUC value is between 0.7 and 0.9; the prediction accuracy is higher when the AUC value is above 0.9. The biomarker does not effect the occurrence of events and has no predictive value if the AUC value is 0.5. In this study, the GC-MS analysis and quantification of most FAs in the Ga samples was highly accurate, especially their palmitic, oleic, and linoleic acids. It is because the AUC values of these FAs were 1. These analytical data were precise and highly reliable, especially determining the principal FAs in the Ga samples.

3.5. β-carotene content

As shown in Figure 4, the RCC extract had the significantly highest β-carotene content (p<0.05), followed by BCS, BCC, and RCS extracts. There was no significant difference in β-carotene content among these Ga extracts, except for RCC extract (p>0.05). The results showed that the β-carotene in the extracts of black Ga stalk extract was somehow higher than that of the red Ga stalk extract.
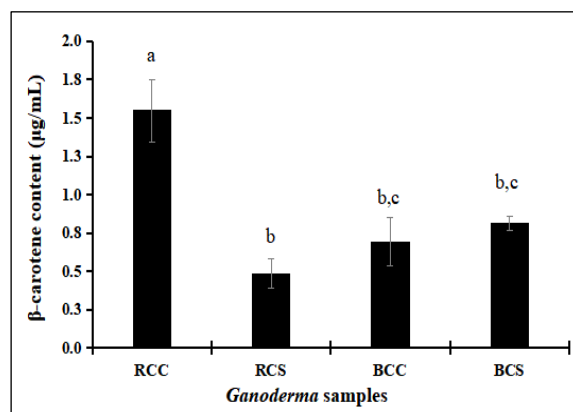
Figure 4. β-carotene content of the Ga lipids. *RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga.

β-carotene content in the RCS extract was lower than in the RCC extract, probably due to its larger canopy. The literature showed that the carotenoid-rich extracts, even stored in the dark at an ambient atmosphere of -20 °C, had a carotenoid decomposition rate of about 5% per day in the presence of antioxidants (Rodriguez-Amaya, 2010). As plant-based lipid is rich in carotenoids, it is a potent free radical scavenger (May, 1994). Carotenoids have been reported to be beneficial to human health. These colored compounds play a role in disease prevention besides their antioxidative effect (Khoo *et al.*, 2011). Therefore, the in vitro antioxidant activity of the Ga extracts could be attributed to the β-carotene content.

3.6. *In vitro* antioxidant activity

The antioxidant activities of the Ga extracts are depicted in Figure 5. Both DPPH radical scavenging activity and FRAP values were according to the electron transfer reaction pathway. The activities of these antioxidant assays were dose-dependent, with linear R^2 values greater than 0.9. The results showed that the EC_{50} values of the red Ga extracts were significantly lower than the black Ga extracts (p<0.05). It shows that the red Ga extract, especially RCC, had the highest antioxidant activity, followed by RCS, BCS, and BCC extracts. The low DPPH radical scavenging activity of BCC could be due to its small black canopy part, where it might contain a low amount of water-soluble carotenoids. Only BCC had an EC_{50} value greater than 5 mg/mL. The EC_{50} value of Vc was 5.63±0.09 µg/mL. The results also showed that the DPPH radical scavenging activity of Vc was about 1000 times better than the BCC extract.

The Ga extracts had the significantly highest FRAP values, except for the RCC extract (p>0.05). The FRAP value of the RCC extract was about one time lower than the FRAP values of the other Ga extracts. Vc had an FRAP value of 19.92±1.75 µM Fe^{2+}/g. The value was 50 times higher than those reported for the Ga extracts. The low FRAP value of the Ga extract is attributed to the antioxidants with a low reducing ability. On the other hand, the RCC extract had the highest DPPH radical scavenging activity with a low FRAP value. It shows that the DPPH radical scavenging activities of the Ga extracts are not dependent on their FRAP values. The contradicting results between these two antioxidant assays could be due to the antioxidant components in the extracts. Although RCC extract had a high β-carotene content with a high DPPH radical scavenging activity, its reducing activity was low. Therefore, β-carotene is a weak reducing agent.

As reported in the literature, the reducing ability of lycopene is higher than β-carotene (Edge *et al.*, 1998). The study reveals that lycopene is easily oxidized to protect the eye macular, but not for β-carotene. This result confirms that β-carotene was the main carotenoids in the Ga extracts. The high amount of β-carotene could be found in the spongy canopy of the Ga, and RCC had the most spongy proportion. The high reducing ability of the other Ga extracts could also be due to these lipid extracts having high amounts of lipid-soluble terpenoids. The high free radical scavenging activity of the Ga extracts makes them potent nutraceuticals for preventing diseases. The related pharmacological effects are immune regulation, anti-tumor, hypotensive, hypoglycemia, and anti-aging (Lin and Deng, 2019).
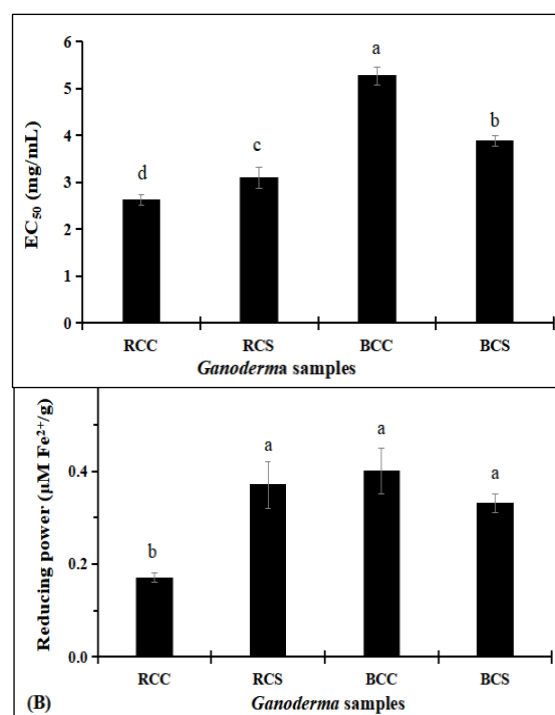
Figure 5. In vitro antioxidant activities of the Ga lipid extracts. (A) DPPH radical scavenging assay (EC50 value); (B) FRAP assay (reducing power). *RCC, canopy of the red Ga; RCS, stalk of the red Ga; BCC, canopy of black Ga; BCS, stalk of black Ga.

The free radical scavenging activity of the Ga extract could be involved the electorn transfer from an oxygen molecule of the hydroxyl group to the carotenoid structure with unpaired electrons or hydrogen during oxidative stress (Khoo *et al.*, 2017). The carotenoids in the Ga extracts could scavenge free radicals through hydrogen atom transfer reaction besides the electron transfer pathway (Gharib *et al.*, 2022).

The carotenoids also have antidiabetic effects besides their antioxidative ability (Sayahi and Shirali, 2017). Determining antioxidant activity in the carotenoid-rich extracts of Ga samples indicates that these extracts are potential edible fats with pharmacological effects.

4. Conclusions

The lipid content of the Ga canopies was higher than that of the stalk part. GC-MS analysis found 49 FAs in the Ga lipids, and their major FAs were palmitic acid, oleic acid, and linoleic acid. Multivariate analysis showed that the FA measurements were precise. The Ga lipids were composed of β-carotene as one of the main carotenoids. These carotenoid-rich extracts possessed antioxidant activities, especially the RCC extract, possessing the highest DPPH radical scavenging activity with a low FRAP value. The results also showed that the lipid extracts of black Ga samples had lower DPPH radical scavenging activity with high reducing ability, but these results were not comparable to Vc. Based on these findings, the red and black Ga are sources of functional lipids with β-carotene as their main bioactive substances. These lipids can also be used as functional excipients in dietary supplements and cosmetic products in the future.

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Acknowledgments

EFFECT OF ULTRASOUND ON THE THERMAL, STRUCTURAL, PASTING AND MORPHOLOGICAL PROPERTIES OF *MARANTA ARUNDINACEA* STARCH

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ABSTRACT

Starch extracted from maize, rice and potato is widely used in various sectors of different industries. However, other alternative sources have proved equally interesting for industrial applications, arrowroot being one of them. The starch of this rhizome has been studied, especially when subjected to modifications, such as physical by means of ultrasound, and its technological properties should be investigated. Therefore, the objective of this work was to evaluate the thermal, structural, morphological and paste properties of arrowroot starch modified by ultrasound. It was observed that the modification of arrowroot starch caused a decrease in the initial gelatinization temperature, and gradual reduction of thermal stability at temperatures of 270 ℃. In the analysis of the paste properties, the sonicated samples showed a greater tendency to retrogradation and syneresis, and higher final viscosity. Regarding structural properties, a decrease in relative crystallinity was observed. The morphology of the granules was little affected by the ultrasonic waves. Thus, it is stated that ultrasonic modification was able to alter some properties of arrowroot starch.

1.Introduction

Starch is the main carbohydrate reserve of plants. It is found mainly in seeds, roots, stems, tubers, leaves and fruits. The starch granule is composed of amylose and amylopectin chains, alternating in amorphous and crystalline regions, which influence the crystallinity of the granules (Lorenzo et al., 2022; Ai, Jane, 2015). The granules have different shapes and sizes, which vary according to the species and the developmental stages of the plant (Paes et al., 2019).

In the presence of water and with increasing temperature, starch undergoes the gelatinization process. This process causes changes in the structural organization and loss of crystallinity of the granules. As the granules absorb water, they swell and may deform, causing the amylose to leach out, forming a fluid paste. With decreasing temperature, a partial reorganization of amylose and amylopectin occurs, a step called retrogradation (Shenglin et al., 2023; Fan et al., 2018).

As far as its application is concerned, starch is widely used in the textile, chemical,

pharmaceutical, and paper industries. This is due to its versatility, easy availability, and low cost. In the food industry, starch is used as a gelling, thickener, emulsifier and encapsulant agent. This natural biopolymer is mostly obtained from corn, cassava, wheat, potato and, to a lesser extent, rice (Afolabi et al., 2012; Watershoot, Gomand, Fierens, 2015; Liu, Xu, 2019). Studies point to a search for new sources of starch with potential application in the industry. In this aspect, tropical countries have an advantage in relation to the main world producers of starch, located in temperate regions, due to the variety of tropical starch crops (Maniglia, Tápia-Blácido, 2016; Tarique et al., 2021). In this sense, arrowroot, classified as a rhizome, can be considered an unconventional source of starch, containing 25-30% of this polysaccharide.

Starch is rarely used in its native form due to limitations such as low stability to heating and shear, as well as higher tendency to retrograde. Thus, starch is modified to improve its characteristics, through chemical, enzymatic, or physical methods (Ai, Jane 2015; Hoover, 2010; Kupervaser et al., 2023). The application of ultrasound is a physical method that has been widely researched and applied in the food industry, as well as to modify starches. This technology is considered environmentally friendly, since it does not use chemicals, nor the generation of polluting waste (Sukja, 2017). Ultrasound consists of mechanical acoustic waves with a frequency above the threshold of human hearing (>15 - 20 kHz) (Vela, Villanueva, 2023; Sukja, Janroz, 2013). Ultrasonic treatment can promote changes in the morphology of starch granules through the phenomenon of cavitation, causing cracks, depressions, or pores on the surface. Such structural damage can modify the amorphous and crystalline parts of the starch, making it more permeable to water. Changes can also occur in the thermal stability as well as in the paste properties and crystallinity of the starch granules (Sukja, 2017, Zheng et al., 2013; Zhu, 2015). The effect of ultrasound on granules depends on the type of starch and the conditions

used such as: power, frequency, temperature, and treatment time (Zhu, 2015; Obadi et al., 2021). These changes can result in obtaining starches with different technological properties of industrial interest (Bernardo, Ascheri, Carvalho, 2016; Zheng et al., 2013).

Thus, the aim of this study was to evaluate the thermal, structural, morphological and paste properties of a commercial arrowroot starch modified by ultrasound.

2. Materials and methods
2.1. Materials
2.2.1.Samples

Arrowroot starch (HEMA, lot 1903LOJ) was purchased from a local market in Curitiba-PR, Brazil.

2.2.2.Starch Modification

The commercial starch sample was modified by ultrasound according literature (Yu et al., 2018) with some modifications: Solutions containing 12 g of starch and 200 mL of deionized water were subjected to ultrasonic treatment (Vibra-Cell 500W - Sonics & Material Inc, USA) with an ice bath to maintain a temperature of 25 °C in order to avoid gelatinization of the starch during the process. The probe (25 mm) was immersed in the solution and four tests at a constant frequency of 20 kHz were performed, with amplitudes of 50 % and 100 %, at times of 25 and 50 min (25' and 50'). After, the suspension was centrifuged (8000 rpm, 10 °C) and the starch was oven dried at 35 °C for 24 h. To perform the analyses, the samples were named as follows: commercial without modification (A), 25'50 % (B), 25'100 % (C), 50'50 % (D), 50'100 % (E).

2.2.3.Differential Scanning Calorimetry (DSC)

To obtain the differential scanning calorimetry curves of the native and modified starch fractions, the methodology described by Bet et al. (2018).

2.2.4.Thermogravimetry (TG)

To obtain thermogravimetric curves of native starch fractions and modified modifications, the methodology described by Bet et al. (2018).

2.2.5.X-Ray Powder Diffractometry (DRX)

The diffraction pattern of native and modified starches was determined by adapting the method described in Kuk et al. (2017).

2.2.6.Pasting Properties (RVA)

To obtain the mass profile of the samples, a viscoamylograph, model RVA-4 (Newport, Australia), was used. The methodology proposed by Ito et al. (2018) was used with some adaptations.

2.2.7.Field emission gun-scanning electron microscopy (SEM-FEG)

The diameter and shape of the starch granules were observed by using Scanning Electron Microscope with Field Emission Gun (SEM-FEG) model MIRA 3 (Tescan, Czech Republic). The diameter and shape of the starch granules were observed using a Scanning Electron Microscope with Field Emission Gun (SEM-FEG) model MIRA 3 (Tescan, Czech Republic). Using the methodologies described by Hornung et al., (2017) and Ito et al., (2018).

2.2.8. Statistical Analysis

Instrumental results were performed by analysis of variance (ANOVA) on data obtained in triplicate, considering the analysis of difference between samples with a confidence level of 95% (p<0.05). Variations between treatments were evaluated by Tukey's test (p≤0.05). All statistical analyses were performed using Action Stat software version 3.3 (Estatcamp, São Paulo, Brazil).

3.Results and discussions

3.1.Differential Scanning Calorimetry (DSC)

The DSC curves are represented in Fig. 1, in which the gelatinization of the native and modified arrowroot starch samples was evaluated. According to Biliaderis, Maurice (1980), the endothermic process of gelatinization occurs with starch granules in the presence of water when both are subjected to heating.

From Fig. 1, it is possible to observe a similarity between the endothermic curves representing the phenomenon of starch gelatinization. During the gelatinization process, the amorphous regions of the granules undergo hydration as they absorb water, resulting in disorganization of the crystalline structure and the release of hydroxyl groups. Table 1 shows the temperature range values obtained for gelatinization to occur.

Figure 1. DSC curves of native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

Table 1. DSC and Relative Crystallinity (RC) results for samples of native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

	To /°C	Tp /°C	Tc /°C	ΔH_{gel} /Jg^{-1}	RC (%)
A	61.8±0.1[a]	68.0±0.1[a]	75.2±0.3[ab]	6.84±0.2[c]	29.4 ± 0.8[a]
B	61.1±0.1[b]	67.7±0.0[b]	75.4±0.3[ab]	8.11±0.2[ab]	26.6 ± 0.7[b]
C	61.0±0.2[b]	67.5±0.0[c]	76.1±0.4[a]	8.25±0.3[ab]	27.1 ± 0.7[b]
D	61.1±0.1[b]	67.6±0.1[bc]	75.2±0.6[ab]	7.73±0.5[b]	27.0 ± 0.6[b]
E	60.7±0.2[c]	67.2±0.0[d]	75.0±0.2[b]	8.51±0.3[a]	27.1 ± 0.6[b]

*Values with the same letter subscribed in the same column do not show statistical difference between them by the Tukey Test (p<0.05). T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), ΔHgel (gelatinization enthalpy).

There was a decrease in the onset temperature (T_o) in all samples after modification (Table 1). Sample (E), which was treated with the highest amplitude (100 %) and treatment time (50 min), showed the lowest values of T_o and T_p, starting the gelatinization process at a lower temperature when compared to the native sample. T_o showed a reduction after the treatments, while for Tc there was no significant difference between the treated samples. There was an increase in ΔH_{gel} after the treatments, with a statistical difference between the commercial sample and the samples treated with longer time (50') and vibration amplitudes of 50 and 100%. Wang et al. (2018) obtained a higher gelatinization enthalpy (12.96±0.25 J g^{-1}) for commercially available native arrowroot starch.

Gelatinization temperatures illustrate the stability of starch crystallinity, while enthalpy is related to the energy required for crystal melting (Lopez-Rubio et al., 2008). Ultrasound treatment can cause distortions in the amorphous and crystalline regions of starch granules, which can result in the modification of the granular structure, thus altering its initial characteristics such as crystallinity, enthalpy, and gelatinization temperatures (Zhu, 2015; Ye, et al., 2023; Guo et al., 2022; Jambrak, 2010).

3.2.Thermogravimetry and Derivative Thermogravimetry (TG/DTG

The TG/DTG curves of commercial arrow starch are illustrated in Fig. 2, where it is observed that arrowroot starch shows three main mass losses. The ultrasonically treated samples showed similar curves with only slight shifts in the temperatures of each mass loss as shown in Table 1.
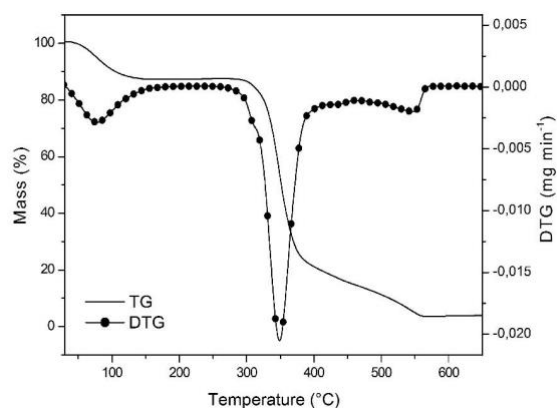
Figure 2. TG/DTG curves of untreated arrowroot starch.

The first mass loss, commonly observed in starches, is associated with the dehydration of the sample (Beninca et al., 2019). According to Table 2, the native sample showed the highest mass loss (13.2 %), and sample (C) exhibited the lowest water loss (8.6 %). It is suggested that ultrasound application may result in starches that are more resistant to dehydration, i.e. with the water fraction more strongly bound to the starch structure (Valencia et al., 2012).
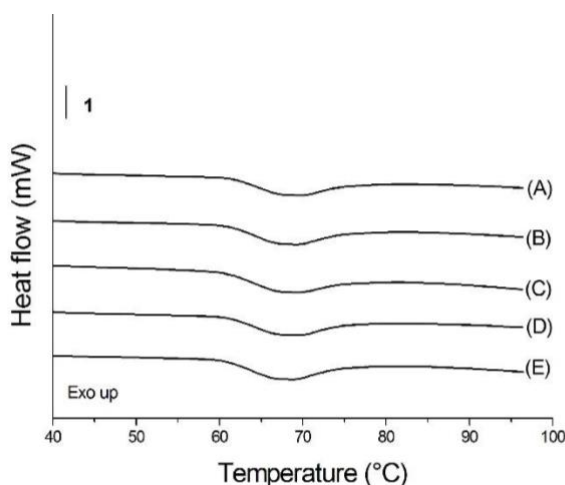
Table 2. TG/DTG results for samples of: native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 %(C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

Thermal event	1st			Stability	2nd			3rd		
Sample	Δm, %	ΔT, °C	Tp, °C	ΔT, °C	Δm, %	ΔT, °C	Tp, °C	Δm, %	ΔT, °C	Tp, °C
A	13.2	30-150	81	150-280	69.1	280-422	348	15.0	422-572	502
B	10.5	30-160	81	160-277	72.5	277-421	345	14.2	421-590	509
C	8.6	30-150	83	150-274	74.4	274-409	341	13.3	409-590	494
D	9.7	30-158	84	158-270	74.7	270-417	338	14.2	417-582	497
E	9.1	30-159	86	159-267	73.5	267-416	341	15.2	416-585	501

Δm (mass loss %), ΔT (Difference between the Initial and Final Temperatures °C) of each step (thermal event), Tp (peak temperature °C).

After the first event, a period of stability was identified before the onset of thermal decomposition. Native arrowroot starch showed higher thermal stability, withstanding a temperature up to 280 °C. According to Minakawa et al., (2019), the higher thermal stability of native starches is related to the more compact semi crystalline structure and the high degree of polymerization of native starches. Thus, more ordered structures require more energy to be thermally degraded.

In the second mass loss, it was observed that at temperatures above 267 °C there was a high thermal degradation of the starches, verified by the highest mass decrease in Fig. 2. Sample (A) presented the lowest mass loss in this thermal event (69.1 %), and sample (D) is related to the highest of them (74.7 %). Similar results were reported by Sandoval Gordillo et al., (2014) for native arrowroot starch. According to Valencia et al., (2012), in this step, the thermal degradation of organic compounds of starch, such as fibers and lipids, as well as amylose and amylopectin chains occurs

The native sample presented the highest initial degradation temperature (280 °C), and the starch sonicated with 100 % amplitude for 50 minutes (sample E), exhibited the lowest temperature (267 °C). It is observed that, inversely to the time and amplitude of the treatments, the sonication of the samples caused a decrease in the resistance of the starches to thermal degradation.

The third loss is related to the oxidation of the matter which results in the formation of ash Costa et al., (2013). At this stage, the samples presented mass losses between 13.3 and 15.2 %. Such values may be related to the high content of inorganic residues in arrowroot, such as phosphorus, sodium, and potassium, and to a lesser extent, iron, magnesium, zinc, and calcium Pérez et al., (2005).

3.3. X-Ray Powder Diffractometry (XRD)

At the molecular level it is possible to verify, through X-ray diffraction analysis, the structural characterization of starch, determining the degree of crystallinity through the ratio between amorphous and crystalline regions (Lacerda et al, 2014; Colman, Demiate Schnitzler, 2014). The crystalline material of the granules has typical X-ray diffraction pattern shapes, based on the packing configuration of amylopectin and variations in the water content of the molecule (Chrungoo, Devi, 2015; Pérez, Bertoft, 2010). According to (Pérez, Bertoft, 2010), most cereal

starches have an A-type pattern, some tuber, rhizome and amylose-rich cereals a B-type pattern, and most legume starches a C-type pattern.

The diffraction patterns of native and sonicated arrowroot starches (Fig. 3) are characteristic of type A. A similar result was reported by Wang et al., 2018.

Figure 3. Diffractograms of native commercial arrowroot starch samples (A), commercial modified 25'50 % (B), commercial modified 25'100 %(C), commercial modified 50'50 % (D), commercial modified 50'100 % (E), obtained by X-ray diffraction.

After ultrasonic modification, slight changes were observed in the peaks at 17° and 18° (2θ). This means that the ultrasonic treatment did not severely affect the starch granule structure. However, the modification promoted a decrease in the relative crystallinity (RC) of the samples (Table 1). These results indicate that the application of ultrasonic waves may have caused changes in the ordered structure (amylopectin) and disorder of the double helices of the starch granule, thus decreasing the relative crystallinity (Dar et al., 2018).

3.4. Pasting Properties (RVA)

Starch in the presence of water and increased temperature undergoes a structural change in the granule, with the gelatinization process occurring in a certain temperature range (Mesquita et al., 2016). Viscoamylographic analysis analyzes the thermal behavior of starches during heating and cooling cycles (Wang et al., 2018). Figure. 4 shows the viscoamylographic curves of native and sonicated commercial arrowroot starch samples.

Figure 4. RVA curves of native commercial arrowroot starch samples, commercial modified 25'50 %, commercial modified 25'100 %, commercial modified 50'50 %, commercial modified 50'100 %.

The temperatures of the arrowroot starch samples ranged between 65.8 and 67.2 °C (Tab. 3). A similar result was found by Maniglia, Tapia-Blácido (2016) for native arrowroot starch (67.1 °C). It was observed that the ultrasound treatment with amplitude of 100 % after 25 minutes (sample C), provided the reduction in the temperature (65.8 °C) of starch paste formation, possibly due to a decrease in the resistance to swelling of the granules. This data corroborates with the Tp observed by DSC (Table 1).

Table 3. RVA results for samples native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

Sample	Peak viscosity (mPa^{-1})	Breakdown (mPa^{-1})	Final Viscosity (mPa^{-1})	Setback (mPa^{-1})	Peak Time (min)	Pasting Temperature (°C)
A	2960±1.0[c]	2161±1.0[c]	1590±1.0[e]	791±1.0[e]	6.1±0.1[a]	66.7±0.0[b]
B	2922.7±0.6[e]	2132±1.0[e]	1616±1.0[d]	826±1.0[d]	5.9±0.0[b]	67.2±0.1[a]
C	3101±0,0[a]	2251±1.0[a]	1694.6±0.6[b]	845±1.0[b]	5.9±0.0[b]	65.8±0.1[c]
D	2925.3±0.6[d]	2148.3±0.6[d]	1636±1.0[c]	860±1.0[a]	5.9±0.0[b]	66.7±0.1[b]
E	3093±1.0[b]	2224±1.0[b]	1706±1.0[a]	837.3±0.6[c]	5.9±0.0[b]	66.7±0.1[b]

*Values with the same letter subscribed in the same columns do not show statistical difference between them by the Tukey Test ($p < 0.05$).

The use of ultrasound resulted in an increase in setback and final viscosity values in all samples. The setback is the ratio of the final viscosity to the minimum viscosity and is directly proportionally related to the tendency for retrogradation and syneresis (Ai, Jane, 2015; Sheglin et al., 2023; Zórtea-Guidolin, 2017). Thus, sonication of the samples resulted in pastes with a higher tendency to retrogradation and syneresis compared to the native sample (A).

In the treatments with 100 % amplitude, (C) and (E), an increase in the peak viscosities and a breakdown were recorded. In this case, despite the increase in peak viscosity, there was a decrease in the ability of the gel to resist both shear stress and heating. On the other hand, in the treatments with 50 % amplitude, (B) and (D), a decrease in these two parameters was observed.

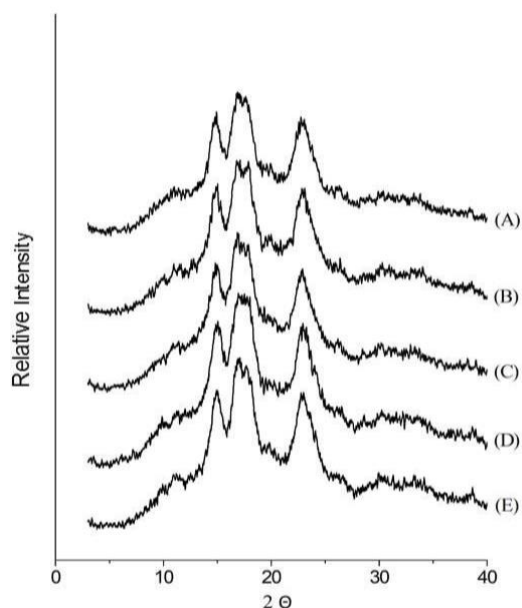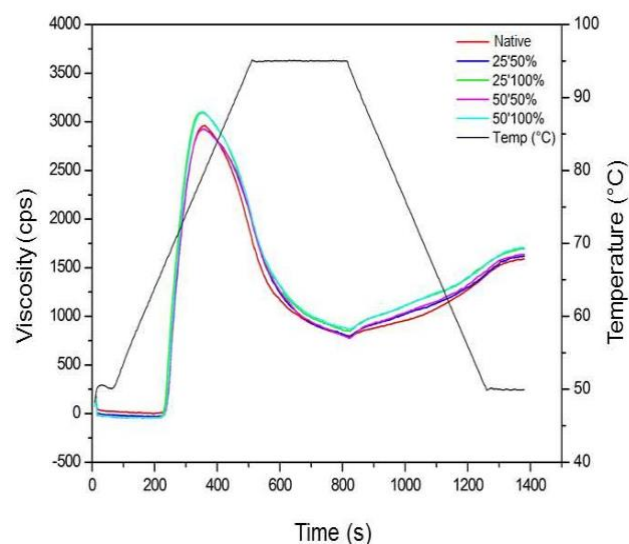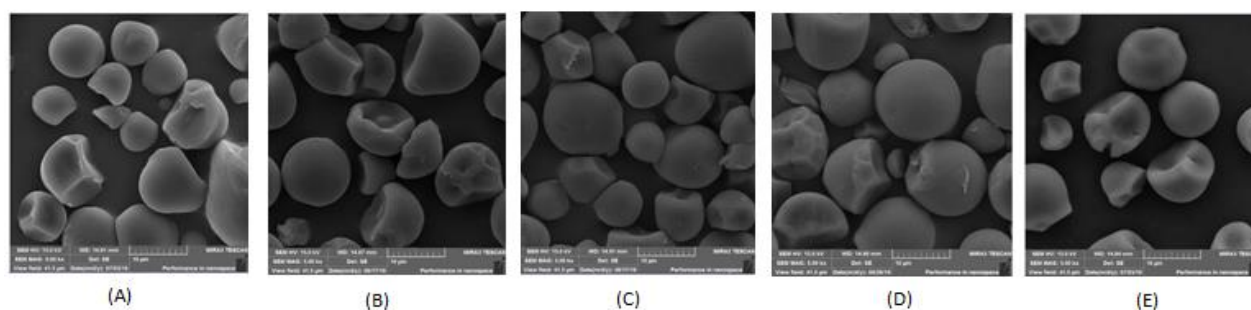**Figure 5.** Microimages (5000 X) of unmodified commercial arrowroot starch samples (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

3.5. Field emission gun-scanning electron microscopy (FEG - SEM)

Scanning electron microscopy is an important technique to verify the structure, morphological characteristics, and size determination of starch granules (Paes et al., 2019).

Native arrowroot starch presents a mixture of circular and oval granules with a diameter ranging from 9 to 42 µm (Maniglia, Tapia-Blácido, 2016). Suastegui-Baylón et al., (2021) point out the small number of circular granules and the predominance of the bean shape. In this study, commercial arrowroot starch exhibited a predominantly circular shape (Fig. 5) and a granule diameter between 6 and 13 µm. Similar values were recorded by Astuti et al., (2016) in native arrowroot starch.

The morphological characteristics of arrowroot starch granules were little affected by the treatments. A similar result was found by Kang et al., 2016, in which the application of ultrasound to maize and rice starches caused some small cracks and depressions that were not very evident on the surface. Obadi, Xu, 2021, suggests that the effects of an ultrasound on starch granules depend on the intensity of the treatment, in addition to factors such as power, frequency, temperature, treatment time and the properties of the starches.

4. Conclusions

The application of ultrasound to modify arrowroot starch caused some changes in the properties of the starches. Regarding thermal properties, the sonicated samples showed lower initial gelatinization temperature compared to the native sample. The use of ultrasound also resulted in starches with a lower thermal stability compared to the native sample, withstanding temperatures up to 270 °C. Regarding paste properties, the modified samples showed a higher tendency for retrogradation and syneresis, although higher viscosity was achieved, especially when higher amplitude and sonication time were used. The analysis of the morphological properties revealed that the structures of the starch granules were little altered by the ultrasonic waves, however, there was a decrease in the crystallinity of the sonicated samples.

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Acknowledgments

A REVIEW ON NUTRITIONAL COMPOSITION AND PHARMACOLOGICAL EFFECTS OF GUAVA; (*Psidium Guajava* L.)

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ABSTRACT

Psidium guajava L. (*Psidium guajava*) is well known fruit for its nutritional and medicinal uses, knowing as *super fruit* attributable to its promising dietary significance. *Psidium guajava* fruit, leaves, seeds and bark exhibits various chemical constituents including β-carotene, ascorbic acid, Flavonoids, Guaijavarin, Guajivolic acid, Galangin, Tannins, linoleic acid, linolenic acid, carofenoid. *Psidium guajava* is used as ethnomedicine for Curing wounds, lowering blood glucose level, treating ulcer, curing tooth pain, diarrhea, treating digestive problems, gastroenteritis, dysentery, rheumatic pain around the world. Leaves and fruits of *Psidium guajava* have promising pharmacological effects such as antioxidant, anticancer, antidiabetic and anti-inflammatory, aiding its ethnomedicinal uses.

1. Introduction

The *Psidium guajava* L. belongs to genus *Psidium,* family *Myrtaceae*, which is native to tropical and subtropical regions of the world. Different types of *Psidium guajava* are available around the world including common types such as apple guava, yellow-fruited cherry guava, strawberry guava, and red apple guava. *Psidium guajava* fruits consist of a green skin in immature stages, yellow skin in ripening with white, yellow or pinkish flesh (Parvez *et al*., 2018: Sing *et al*., 2019). These fruits are sweet, tangy and mostly eat in raw forms when fruits are ripe or semi ripe. The fruit has the unique fragrance when it's ripe which is more or less similar to the fragrance in lemon rind. This attributes to the availability of carbonyl compounds in the *Psidium guajava* fruits (Shehanaz, 2013).

Psidium guajava is not just a fruit but a source of nutrients including vitamin A, ascorbic acids, pantothenic acid, niacin, minerals including Na, Fe, Ca, P, carotenoids such as lycopene and β carotene. *Psidium guajava* fruit is a rich source of vitamin C which is four times higher than the oranges (Shehanaz, 2013). Availability of various bioactive compounds in *Psidium guajava* fruits attributes for its promising health benefits. But these bioactive compounds vary with the maturity stage, soil conditions, variety, climatic conditions and geographic location (Chauhan *et al*., 2015).

Psidium guajava leaves and bark is used in indigenous medicine as a treatment for gastroenteritis, dysentery and colic pain of the intestine. Leaves of *Psidium guajava* are rich in flavonoids such as quercetin (Sing *et al*., 2019; Chauhan *et al*., 2015). Several ethno-medicinal uses of *Psidium guajava* plant have been reported. Amazonia, Cuba, India, Trinidad, Philippines, Mexico and Peru have used *Psidium guajava* plants parts as ethno-medicine for dysentery, diarrhea, epilepsy, itch, piles, scabies, skin sores, sore throat, stomachache, wounds and as an antiseptic and astringent. In

India leaves and bark of *Psidium guajava* are used to cure diarrhea, dysentery, vomiting and sore throats, and to regulate menstrual cycles (Kamath *et al*., 2008).

Psidium guajava fruits are used to produce different food products including juice, nectar, jelly, jam, syrup, canned fruits, puree, fruit bar, ready to serve beverage, dehydrated products, flavoring agent in candies, cakes, biscuits, chocolate bars and also as an additive to other fruit juices or pulp (Kanwal, Randhawa, and Iqbal, 2016).

1.1. Taxonomical classification of *Psidium guajava L.*

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-Class	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	Psidium
Species	*Psidium guajava*

2. Plant morphology

Psidium guajava exists as a shrub or a small evergreen tree with branches, usually growing 1-6 m tall (Sing et al., 2019) . Bark of the *Psidium guajava* is copper or reddish brown in color smooth, thin and easily flakes off. Flakes cover the greenish new bark, therefore it gives a spotted appearance to the plant with combination of green and brown color. Branches of the *Psidium guajava* are twisted, bearing opposite leaves with a 4 – 10 mm long petiole. Some younger branches contain hairs in its stem. Leaves of the *Psidium guajava* are ovate-elliptic or oblong-elliptic in shape with an obtuse or acute apices and obtuse bases, dark green in color. Lower side of the leaves contain hair when young. Flowers of *Psidium guajava* are fragrant, white in color and consist four to five white petals with large number of pale-yellow anthers (Sing *et al*., 2019; Vikrant, Thakur and Kashyapa, 2012).

The shape of the *Psidium guajava* fruits are round, pear or ovoid in shape and consist of remaining sepals (Calyx lobe). Peel of the fruit is sour and bitter in taste, and is green in color. Once it matures, when the fruit gets ripen, the flesh turns in pink, yellow or white in color. Fruit contains edible seeds in its flesh. Based on the variety nature of seeds can be different such as very hard to soft and chewable (Vikrant, Thakur and Kashyapa, 2012).

3. Nutritional Composition

Psidium guajava consists of different nutrients including vitamin, minerals, fiber, fat and protein. Nutritional composition can be vary based on the variety, geographical region and climatic conditions. *Psidium guajava* considered as a super fruit due to the presence of vitamin C, vitamin A, Niacin, Riboflavin and various minerals including potassium (Joseph and Priya, 2011). Single fruits of *Psidium guajava* contain higher amount of vitamin C, which is four times higher than the oranges (Vikrant, Thakur and Kashyapa, 2012; Joseph and Priya, 2011). *Psidium guajava* has 68 kcal per 100g which is considered as low energy (Table 1). Minerals including K, Ca, Mg, Na, S, P, fe, Cu, B, Zn were reported in the *Psidium guajava* fruits which was obtained from 128 *Psidium guajava* accessions sampled from four regions of Kenya K is the prominent mineral in the *Psidium guajava* accounting to a mean value of 293.7 mg/100 g FW. Based on the color of the flesh amount of minerals present in the *Psidium guajava* fruit varies. w white-fleshed *Psidium guajava* fruits exhibited more protein content and some minerals (K, Mg, Na, S, and B) than the red-fleshed ones (Chiveu *et al*., 2019).

Table 1. Nutritional Composition of *Psidium guajava* Fruit (Source: USDA)

Proximates	Minerals	Vitamins	Lipids
Water 80.8 g Energy 68 kcal Protein 2.55g Total lipid (fat) 0.95g Carbohydrate, by difference 14.32g Fiber, total dietary 5.4g Sugars, total 8.92g	Ca 18mg Fe 0.26mg Mg 22 mg P 40 mg K 417 mg Na 2 mg Zn 0.23 mg	Vitamin C, total ascorbic acid 228.3 mg Thiamin 0.067 mg Riboflavin 0.04 mg Niacin 1.084 mg Vitamin B-6 0.11 mg Folate, DFE 49 µg Vitamin A, RAE 31 µg Vitamin A, IU 624IU Vitamin E (alpha-tocopherol) 0.73mg Vitamin K (phylloquinone) 2.6 mg	Fatty acids, total saturated 0.272 g Fatty acids, total monounsaturated 0.087 g Fatty acids, total polyunsaturated 0.401 g

Table 2. *P. guajava* fruit composition

Fruit Composition	Amounts Recorded	References
Ascorbic acid	136.5 -220.4 10 mg/100 g FW and 360 mg/100 129.5 mg/100 g and 247.9 mg/100 g	Chiveu, et al. (2019), Gull et al. (2012)
TSS (Brix)	11% 7.64 %to 11.87% 9.35-11.88%	El-Sisy (2013), Sylvia and Mitchell (2008), Adrian et al. (2015)
Glucose	0.95 ± 0.08 g/ 100ml 1.11 g/100 g	Sanz et al. (2004), Chiveu, et al. (2019)
Fructose	2.74 g /100ml 2.81 g/100g 5.64 -7.64 g/100ml of juice	Sanz et al. (2004), Chiveu, et al. (2019)
Sucrose	6.2 -7.2 g/100ml of juice 0.57 g/100ml	Bulk et al. (1996), Sanz et al. (2004)
Protein	0.76 to 1.85%. 1.52%	Sylvia and Mitchell (2008), Chiveu, et al. (2019)
Acidity (as citric acid)	0.52 to 1.67%.	Sylvia and Mitchell (2008),

Psidium guajava has 68 kcal per 100g which is considered as low energy (Table 1). Minerals including K, Ca, Mg, Na, S, P, Fe, Cu, B, Zn were reported in the *Psidium guajava* fruits which was obtained from 128 *Psidium guajava* accessions sampled from four regions of Kenya. K is the prominent mineral in the *Psidium guajava* accounting to a mean value of 293.7 mg/100 g FW. Based on the color of the flesh amount of minerals present in the *Psidium guajava* fruit varies. White-fleshed *Psidium guajava* fruits exhibited more protein content and some minerals (K, Mg, Na, S, and B) than the red-fleshed ones.

Psidium guajava fruits contain the second richest vitamin C content among the all fruits (Chen *et al*., 2006). Ascorbic acid content in *Psidium guajava* fruits in Kenya vary among 10 mg – 360 mg /100g of fruit Weight (Chiveu *et al*., 2019). In Pakistan ascorbic acid content in pulp and peel of fully ripe *Psidium guajava* varies between 29 mg /100g and 247mg/100g (Gull *et al*., 2012). Ascorbic acid composition of fully ripe *Psidium guajava* varies geographically (Chiveu *et al*., 2019).

Correlation between temperature of the area and the ascorbic acid content of fruits were recorded whereas higher values were observed in high temperature regions and lower values were observed in moderate and colder regions. Converse finding was reported in Thailand whereas higher ascorbic acid content exhibited in winter season (15t). In addition to the temperature, an increase in precipitation was also recorded as a reason for the increase in the ascorbic acid content (Chiveu *et al*., 2019; Taipong *et al*., 2005).

TSS values of *Psidium guajava* are demonstrated in Table 2. Fifteen *Psidium*

guajava genotypes grow in the similar conditions demonstrated TSS as a 9.04-14.07 % (El-Sisy, 2013). In another two studies reported TSS values of *Psidium guajava falls in between* 9.35%-11.88% (Chiveu *et al*., 2019; Patel *et al*., 2011).

Further TSS values were changed with their regions and climatic conditions. TSS exhibited positive correlation between temperature and negative correlation with rainfall of the geographical region (Marsh *et al*., 1999).

Major sugar components in *Psidium guajava fruits* at the ripen stage are fructose and sucrose (Sanz *et al*., 2004; Bulk *et al*., 1999). During the fruit development total and individual sugar contents were increased. Once fruit reached to maturity, fructose, glucose and sucrose account 20%-48%, 14%-59% and 21%-45% to total sugar content respectively. Development of fruit from immature to ripen stage fructose built within the fruit varies rapidly hence fructose to glucose ratio is very low (Bulk *et al*., 1999).

Moreover, fructose to glucose ratio was recorded as 0.35 in *Psidium guajava* fruits (sucrose (Sanz *et al*., 2004; Bulk *et al*., 1999). It is known to be fructose resulted lower level of post-prandial hyperglycemia which would result from the same intake of glucose. Therefore, fructose to glucose ratio of *Psidium guajava* gives high level of dietary significance.

4. Medicinal and Pharmacological Effects of Psidium guajava

Various plant parts of the *Psidium guajava* have been used for different medicinal purposes around the world. Availability of various constituents in plant parts gives medicinal value to plant. Ethno medicinal applications of *Psidium guajava* are demonstrated in Table 3.

4.1. Antimicrobial activity

Effect of *Psidium guajava* in many pathogenic microorganisms *Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus spp., Shigella spp. and Escherichia coli* were examined (Adrees *et al*., 2010). These microorganisms cause infections in intestine of humans and inhibitory activity of extracts of *Psidium guajava* leaves and roots were analyzed against these pathogens (Adrees *et al*., 2010). Gallocatechin in *Psidium guajava* leaves demonstrated antimutagenic activity against *E. coli*. Leaves and bark extracts of *Psidium guajava* exhibited in vitro toxic action against different microorganisms including bacteria (Chah *et al*., 2006).*Psidium guajava* fruit and leaf extract have been used to determine its effectiveness against the acne causing bacteria *Propionibacterium acnes* (Qadan *et al*., 2005). *Psidium guajava* extracts were checked against tea tree oil, doxycycline and clindamycin antibiotics which have proven to cure acne. Results demonstrated that leaf extracts of *Psidium guajava* were effective against acne casing bacteria *Propionibacterium acnes* (Qadan *et al*., 2005). Activity of *Streptococcus mutans* were inhibited by active flavonoid compound - quercetin-3-Oalpha-l-arabinopyranoside (guaijaverin) in leaves of *Psidium guajava* which has high antiplaque activity.

Antimicrobial activity of *Psidium guajava* linked to the availability of guajaverine and to psydiolic acid (Joseph and Priya 2011).

Root extract of *Psidium guajava* consist of different antibacterial compounds. Four antibacterial compounds have been separated using column chromatography and antifungal activity was revealed in ripe fruit of *Psidium guajava* against *Arthrinium sacchari* and *Chaetomium funicola* strains (Prabu *et al*., 2006; Arima and Danno, 2002; Sato *et al*., 2000).

Table 3. Ethno medicinal applications of *Psidium guajava*

Parts of the *Psidium guajava*	Ethno medicinal applications	Reference
Leaf	Curing wounds, lowering blood glucose level, treating ulcer, curing tooth pain, diarrhea, treating digestive problems, gastroenteritis, dysentery, rheumatic pain, used to force out placenta after childbirth, curing plethora, treating inflammations, treatment for diabetic Mellitus, treating cold, treating rashes and itchiness, sore throat, treatments for worms, treating bacterial infections, treating vaginal discharge problem, to purifying blood, treating constipation, febrifuge, astringent, skin infections, mouth swelling	Sylvia and Mitchell (2008), Jansen and Mendez (1990) Leonti et al. (2001) Martinez and Barajas (1991), Lara and Marquez (1996), Oh et al. (2005), Pontikis (1996)
Fruit	Treatment for dysentery, astringent, treating skin problems respiratory sufferings, wounds, treatment for fever, treating dehydration, vaginal bleeding, cough, treating inflammations, ulcers, diarrhea, and digestive problems.	Sylvia and Mitchell (2008) Henrich et al.(1998) Jansen and Mendez.1990 Leonti et al.(2001), Smith and Nigel (1992)
Bark	Treatment for dysentery, astringent, treating skin problems. Treatment for diarrhea and amoebic sufferings	Sylvia and Mitchell (2008) Cabieses (1993) Tona et al. (1998)
Flower	Treating indigestion, cholera, diarrhea, sore throat, and vaginal discharge sufferings	Sylvia and Mitchell (2008) Cabieses (1993) Tona et al. (1998) Cybele at al. (1995)
Root	Treatment for wounds, diarrhea and ulcer	Sylvia and Mitchell (2008) Smith and Nigel (1992)

4.2. Cancer treatment

Different phytochemical constituents in different parts of *Psidium guajava* attributed to its cancer prevention properties. Several studies reveal various chemicals found in the *Psidium guajava* leaves have effects upon human cells such as Psiguadials A, B, and guajadial which inhibit the growth of the Prostrate cancer cell lines. Young leaves of *Psidium guajava* has contain high concentration of isoflavonoids and polyphenols which inhibit the angiogenesis and cell migration processes (Sato *et al*., 2010)

Ascorbic acid, Apigenin and Lycopene in the fruits of *Psidium guajava* have anticarcinogenic effects. Ability to prevent breast cancers by preventing cell proliferation of

cancer cell lines was reported in *Psidium guajava* fruit extract (Oh *et al*., 2005). Antiproliferative activity of *Psidium guajava* fruits was tested against the human lung cancer cells (A549), human breast cancer cells (MCF-7), human hepatoma cells (HepG2) and human colon cancer cells (HT-29) through the MTT assay (Chen *et al*., 2015). As a result of it different phytocompounds such as Catechin, Galangin, Homogentisic acid, Gallic acid, Kaempferol and Cyanidin 3-glucoside were recognized as an antiproliferative agents.

Antitumor potential of leaves of *Psidium guajava* extracts was recorded along three cancer cell lines: PC3 (prostate), A549 (lung), and BT549 (breast) cancer (Alhamdi *et al*., 2019). Results demonstrated *Psidium guajava* extract effectively inhibited the three types of cancer cells from proliferation. *Psidium guajava* leaves have cytotoxic effects on HSC-2 cell line of human oral cancer (Pakpahan *et al*., 2003). Essential oils form the leaves have cytotoxic impact on PC-3 cell lines which was able to suppress tumors (Sato *et al*., 2000).

4.3. Antioxidant activity

Antioxidants are the compounds which prevent or delay the oxidative damage of macromolecules caused by reactive oxygen species at low concentrations (Arshiya, 2013).

Several researchers have studied about leaf, fruit, seeds and bark of *Psidium guajava* as potential sources of natural hydrophilic and lipophilic antioxidants (Taipong *et al*., 2005; Taipong *et al*., 2006). Ascorbic acid, vitamin A, vitamin E, polyphenols, carotenoids, polysaccharides and flavonoids are the major antioxidants and free radical scavengers (Chauhan *et al*., 2015; Joseph and Priya, 2011. Vitamin C and polyphenols are hydrophilic antioxidants while carotenoids such as β-carotene and lycopene are lipophilic antioxidants . The carotenoid content is high in *Psidium guajava* fruit compared to other fruits. Hydrophilic antioxidants are responsible for 99% of antioxidant activity (Taipong *et al*., 2005). The leaf and fruit of *Psidium guajava* are rich in strong polyphenol antioxidant compounds (Braga *et al.,* 2014). The

polyphenols such as quercetin, narigin, catechins, rutin, caffeic acid, gallic acid and chlorogenic acid, are the strong antioxidants in *Psidium guajava*. The antioxidant content of *Psidium guajava* depends on various factors like variety, maturity, climate, soil, composition, geographic location and storage conditions (Chauhan *et al*., 2015). The young *Psidium guajava* leaves showed higher antioxidant activity than matured leaves (Nantitanon *et al*., 2010)

The ultrasonication and homogenization were the best antioxidant extraction techniques for pink-flesh *Psidium guajava* fruits (Nantitanon *et al*., 2010; Musa *et al*., 2011). Further, using the pure solvents for the antioxidant extractions were inefficient and is recommended to use 50% acetone in combination with aqueous than methanol and ethanol for efficient extraction. Another study was reported that ultrasonication extraction method yielded high amounts of phenolic antioxidants in *Psidium guajava* leaves and showed highest antioxidant activity (Nantitanon *et al*., 2010). Moreover, they reported that hot water was the best solvent to extract active antioxidants. Thaipong *et al*., 2005 used methanol for extraction of hydrophilic and lipophilic antioxidants from white and pink fleshed *Psidium guajava* fruits. Qian et al (2004) extracted the antioxidants effectively from dried ground leaves of *Psidium guajava* with 50% aqueous ethanol. The variety of *Psidium guajava* , the polarity of the solvent used (Musa *et al*., 2011) and type of extraction method (Nantitanon *et al*., 2010) may influence the extraction efficiency of antioxidants (Musa *et al*., 2011). The Folin–Ciocalteu index (FCI), ferric-reducing antioxidant power assay (FRAP), 2,2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), the oxygen radical absorption capacity (ORAC) and 1,1-diphenyl-2-picrylhydrazyl free radical-scavenging capacity (DPPH) were used to determine the antioxidant activity of *Psidium guajava* (Musa *et al*., 2011). Thaipong et.al (2005) suggested that FRAP technique was best for determining antioxidant activity of *Psidium guajava* fruit since it was simple, showed high

reproducibility, rapid performance and highest correlation with total phenolics and vitamin C. The antioxidant activity of phenolic compounds depends on the position and degree of hydroxylation of the ring structure and their molecular structures (Qian *et al.*,2004).Sato et al (2000) reported in their review that the fresh *Psidium guajava* skin has higher total phenolic content than the pulp.

The *Psidium guajava* leaves contain a broad spectrum of flavonoids, ascorbic acid and polyphenols (Gayathri and Kiruba, 2014; Joseph and Priya, 2011). Braga et al (2014) found that the total phenolic and flavanol contents of ethanol extracts of *Psidium guajava* leaves were 766.08 ± 14.52 mg/g and 118.90 ± 5.47 mg/g respectively and the extract showed effective antioxidant activity of 87.65% in the DPPH assay. Qian et al (2004) noted that the total phenolic content of dried *Psidium guajava* leaves was 575.3 ±15.5 mg (GAE)/g in dry basis and 82.74%. The antioxidant activity was observed for DPPH assay conducted with the ethanolic leaf extract at a concentration of 0.5 mg/ml and the 50% radical scavenging activity (EC50) of ethanolic leaf extract against DPPH was 54 mg/ gram of DPPH. Nantitanon et al (2010) reported that 24.30 ± 0.50 mM/mg of total phenolic content was recorded for the hot water extract of *Psidium guajava* young leaves (Qian *et al.*,2004). The antioxidant activity was increased with the increasing concentration of leaf extract. It was reported that the polysaccharides in *Psidium guajava* leaves can be used as antioxidant additive in food industry (Kumar *et al.*, 2021).

The *Psidium guajava* fruits showed more hydrophilic antioxidant property than lipophilic antioxidant property due to presence of higher phenolic and vitamin C content than carotenoids. Further they stated that the *Psidium guajava* fruits with white flesh showed high hydrophilic antioxidant activity due to presence of high phenolic content (33.3 µM TE/g FW) than the pink flesh fruits (ranging from 15.5 to 30.4 µM TE/g FW). The lipophilic antioxidant activity also higher for white flesh fruits compared to pink flesh fruits while pink flesh fruits had higher vitamin C content than white flesh fruits (Thaipong *et al.*, 2005). Omayio et al (2019) have reported that the red-orange colored *Psidium guajava* fruits have higher levels of polyphenols, carotenoids and pro-vitamin compared to yellow-green varieties.

Moreover, spectrophotometric analysis at 470 nm showed that the carotenoids (β-carotene) were absent in white flesh *Psidium guajava* s and 0.78 to 2.93 mg/100 g were present in pink flesh *Psidium guajava*. Lycopene protects the skin from UV rays and the lycopene content of pink *Psidium guajava* s is twice the lycopene content of tomato (Arshiva, 2013).

Chen and Yen (2007) have reported that the *Psidium guajava* leaf extract and *Psidium guajava* fruit extracts showed 94.4%-96.2% inhibition of linoleic acid oxidation at a concentration of 100 µg/ml. Further they stated that the *Psidium guajava* leaf extracts had strong antioxidant properties than dried *Psidium guajava* fruit extract and based on the chromatogram, the ferulic acid (phenolic acid) was responsible for the antioxidant activity of *Psidium guajava* leaf and dry fruit extracts. Daily consumption of *Psidium guajava* is good for human health in preventing diseases such as cancers and cardiovascular diseases arising from oxidative stress (Thaipong *et al.*, 2006).

4.4. Antidiabetic activity

The diabetes mellitus is one of the major and serious health problem in the world today (Mazumdar *et al.*, 2015; Rawi *et al.*, 2011). It is a carbohydrate metabolism disorder of endocrine system which causes the rapid increase of blood glucose level due to insulin secretion deficiency (Rawi *et al.*, 2011; Mazumdar *et al.*, 2015)**.** Santosh Mazumdar et al (2015) has predicted that the number of people suffering from diabetes was 171 million in the year 2000 and it will be increase to 366 million in 2030 and Sharma also estimated that 366 million diabetes patients will be there in 2030 (Sharma *et al.*, 2012) but Liu et al (2015) reported that 552 million will be diagnosed with diabetes mellitus by the year 2030. The drugs such as biguanides, sulfonylurea and thiazolidenediones are presently available to

treat diabetes mellitus but the use is restricted due to the side effects, secondary failure and pharmacokinetic properties (Rawi *et al*., 2011)**.** In traditional folk medicine, the plants rich in phytochemicals and secondary metabolites are used to treat diabetes for many years (Mazumdar *et al*., 2015**;** Sharma, 2012). According to ethnobotanical report, it was reported that about 800 plants species possess the antidiabetic potential (Rawi *et al*., 2011).

Many researchers have reported that the bioactive compounds in *Psidium guajava* have strong antidiabetic potential (Luo *et al*., 2019)**.** The flavanoids, steroids, saponins, polysaccharides (Joseph and Priya, 2011) and polyphenols (Kanwal *et al*., 2016; Sisy El, 2018) in various parts of *Psidium guajava* (fruit, seed, leaf and bark) are responsible for the antidiabetic activity (Rawi *et al*., 2011; Mazumdar *et al*., 2015). Bioactive compounds *in Psidium guajava* such as myrciaphenone B, flavonol glycosides, casuarictin and tellimagrandin , catechin and geraniin, quercetin and cyanidin-3-O-β-glucoside are responsible for the inhibition of carbohydrate-hydrolyzing enzymes and geraniin, gallic acid, naringenin, vescalagin, morin, quercetin, epicatechin and catechin are responsible for anti-glycation activity **.** *Psidium guajava* is a rich source of dietary fiber, which helps to reduce sugar levels in diabetic patients (Parvez, 2018; Cerio, 2016)**.**

The *Psidium guajava* leaf extract has been used traditionally in folk medicine to treat diabetes in East Asia (Mazumdar *et al*., 2015; Luo *et al*., 2019) and other countries like North America, (Luo *et al*., 2019**)** Japan and Africa (Deguchi and Miyazaki., 2010) Deguchi and Miyazaki (2010) stated in a review there was no enough evidences on antidiabetic activity of *Psidium guajava* leaf extract in clinical trials and the therapeutic mechanisms and safety remain uncleared. However, in 2000 the Japanese Ministry of Health, Labour and Welfare approved and recommended the *Psidium guajava* leaf tea which containing aqueous *Psidium guajava* leaf extract for the pre-diabetes patients under ''Foods for Specified Health Uses' (FOSHU) and in both developed and developing countries and it is

suggested to ingest consecutively with every meal as an alimentotherapy for better relief in prediabetic and diabetic patients. Another study revealed that the aqueous *Psidium guajava* leaf extract at a dose of 200 and 400 µg/ml showed effective antioxidant activity and improved insulin resistance by increasing the glucose uptake in normal and high glucose induced insulin-resistant mouse FL83B cells in western blot analysis and significantly enhanced the glycogen content by modulating the insulin signaling pathway (Mazumdar *et al*., 2015)**.** A review study revealed that the alpha amylase enzyme inhibition activity of *Psidium guajava* methanol extract was dose-dependent, 0.2 ml of plant extract showed only 27.8% inhibition while 1 ml extract showed 96.3% inhibition (Parvez *et al*., 2018).

Researchers found that the phytochemicals in *Psidium guajava* triggers the glucose metabolic enzymes in liver tissues (Mazumdar *et al*., 2015). Santosh Mazumdar et.al (Mazumdar *et al*., 2015) reported that the freshly prepared ethanolic *Psidium guajava* leaf extract at doses of 0.5 and 1.0 g/kg efficiently reduced the blood glucose level and lipid profile levels in the oral glucose tolerance test conducted with diabetic albino rats. Another study also stated that the leaf extract of *Psidium guajava* inhibited the increase of plasma glucose level in alloxan-induced diabetic rats at 250 mg/kg in oral glucose tolerance test (Rawi *et al*., 2011; Mukhtar et al., 2004). Shukla and Dubey also found that the ethanolic and aqueous leaf extracts of *Psidium guajavanj* (300mg/kg of body weight) showed the mean percentage blood glucose reduction of 18.88% and 9.19% respectively for diabetic albino rats and they stated that ethanolic extract was better than aqueous extract (Shukla and Dubey, 2009). Polysaccharides extracted from *Psidium guajava* leaves significantly lowered the fasting blood sugar, total triglycerides, total cholesterol, glycated serum protein and creatinine in an experiment conducted with diabetic mice (Luo *et al*., 2019). A research study reported that the *Psidium guajava* leaf extract individually or combination with mango leaf extract (dose of 250 mg/kg body weight) showed an effective

hypoglycemic activity and antidiabetic activity in streptozotocin- diabetic albino rats (Rawi *et al.*, 2011)**.**

It was reported that the *Psidium guajava* fruit extract significantly reduced the blood glucose level in diabetic condition and protects the pancreatic tissues including islet beta cells against lipid peroxidation (Mukhtar *et al.*, 2009). (Table 4).

Table 4. Chemical constituents present in plant parts of guava

Parts of *Psidium guajava*	Chemical presents	Reference
Fruit	Anthocyanin, Pectin, Lycopene, β-carotene, β-cryptoxanthin, Phosphoric acid, Oxalic acid, Mallic acid, Galaturonic acid, Aspartic acid, Ascorbic acid, Oleanolic acid, Psidium guajava coumaric acid, Ellagic acid, Pantothenic acid, Phenolic acids, Homogentisic acid, Gallic acid, Ursolic acid, Arjunolic acid, Oleandolic acid, Guajavanoic acid, Glucuronic acid, Flavonoids, Phenolic compounds (phenol triterpenes), benzaldehyde, Niacin, Quercetin, Saponin with oleanolic acid, Morin-3-O-α-L-lyxopyranoside, , Morin-3-O-α-Larabopyranoside, Catechin Cectin Guaijavarin, Guajivolic acid, Galangin, Tannins, linoleic acid, linolenic acid, carofenoid, hexanal, γ-butyrolactone, (E)-2-hexenal, (E, E)- 2,4-hexadienal, (Z)-3-hexenal, (Z)- 2-hexenal, (Z)-3-hexenyl acetate, 3-caryophyllene, nerolidol, 3-phenylpropyl acetate, 3-penten-2-ol and 2-butenyl acetate, Carboxyl compounds, Kaempferol, Cyanidin 3-glucoside, Apigenin, Limonene, β-Caryophyllene, Aliphatic Alcohols, Aliphatic Ketones, Aliphatic Aldehydes, Aliphatic ketones, Aliphatic esters, Terpene alcohol, Terpene Aldehydes, Terpene Ketones, Terpene Esters, Terpene oxides, Lactone, Furan, Acetal, Benzothiazole, β-caryophyllene hydrate, diacetyl, diethylene glycol, 5,6-dihydro-2H-pyran- 2-carboxaldehyde, 2,4-dimethyl 1,3-dioxane, monobutyl ether- 3,4-dihydro-8-hydroxy-3- ethyl-2-benzo-1H-pyran-1-one, isopentana, methylpyrazine, octyl methyl ether, Aromatic esters, Ketones and Aldehydeds, Essential oils [α-Pinene,β-Pinene, δ-2-Carene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, 1, 8-Cineole, cis-β-Ocimene, γ-Terpinene,	Shehanaz, (2013) Martinez and Barajas (1991) Oh et al. (2005), Pontikis (1996) Chiari-Andreo et al. (2017), Biswas et al. (2019) Huang et al. (2011), Bontempo et al. (2012), Oliveira et al.(2010), Thuaytong and Anprung (2011) Radha and Chandrasekaran (1997) Li et al.(1999) Liang et al. (2005) Mercadante et al.(1999), Zhu (1997)

	Terpinolenea, Carvacrol, α –Terpineol, α-Copaene, α-Gurjunene, β-Caryophyllene, β-Copaene, β-Gurjunene, Aromadendrene, allo-Aromadendrene, α-Humulene, γ-Gurjunene, Aromadendrene, α-Humulene, β-Selinene, α-Selinene, Valencene, δ-Cadinene, α-Calamenene, α-Calacorene, Germacrene B, Viridiflorol, Spathulenol, β-Caryophyllene-oxide, Daucol], Protocatechuic acid, Guavin B, Amritoside, Araban, Ascorbigen, Asiatic acid, Leucocyanidin, Mecocyanin, Rubixanthin, Criptoflavin, Neochrome, Lutein, Phytofluene, Copaene, Quercetin 3-α-L- arabinofuranoside, Quercetin-3-β-galactoside, Quercetin 3- β-D- galactoside, Avicularin. Amritoside, Ethyl octanoate, 3-phenylpropanol, (E)-2-hexanal, α-Humulene, Benzaldehyde, Butanal, Octonol, Ethyl octanate, 6-methyl-5-hepten-2-one	
Leaves	Kaempferol, 3-O-xylosyl-rutinoside, Schottenol ferulate, 3-Methoxysinensetin, Quercetin 3-O-diglucoside, 3-O-acetilrhamnoside, 3-O-xylosyl-rutinoside, 3-O-xyloside and 3-O-(6''-malonyl-glucoside), Sesamolinol 4'-O-β-D-glucosyl (1->6)-O-bD-glucosid, Esculin, 3-Sinapoylquinic acid, flavonoids, tannins triterpenoids, saponins, sterols, alkaloids, Essential oils [α-Pinene,β- Pinene, δ-2-Carene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, 1, 8-Cineole, c is-β-Ocimene, t rans-β-Ocimene, rans-β, γ-Terpinene, Terpinolenea, Carvacrol, α –Terpineol, α-Copaene, α-Gurjunene, β-Caryophyllene, β-Copaene, β-Gurjunene, Aromadendrene, allo-Aromadendrene, α-Humulene, γ-Gurjunene, Aromadendrene, α-Humulene, β-Selinene, α-Selinene, Valencene, δ-Cadinene, α-Calamenene, α-Calacorene, Germacrene B, Viridiflorol, Spathulenol, β-Caryophyllene-oxide, Daucol], Gallic acid, Catechin, Epicatechin, Quercetin, Rutin, Vitamin E, Heneicosane, Pyrogallol, palmitic acid, Caryophyllene oxide, Alloaromadendrene, Sitosterol, α-Bulnesene, Squalene, Avicularin, Apigenin, Hyperin, Myricetin, Epigallocatechin gallate, Caffeic acid, Kaempferol-3-arabofuranoside, Isoquercitrin, Chlorophyll, Alkaloids,	Bulk et al. (1996), Lara and Marquez (1996) Oh et.al. (2005) Chiari-Andreo et al. (2017) Gayathri and Kiruba (2014) Kim (2011), Bijauliya et al. (2018) Ashraf et al. (2016) Wang et al. (2010) Jassal and Kaushal (2019) Soliman et al. (2016) Liu et al. (2014) Wang et al. (2017) Wang et al. (2019) Kim et al. (2016) Seddiek et al. (2020) Diaz-de-Cerio et al. (2016) Abu-Bakr et al. (2003) Chen et al. (2006) Pino et al. (2002) Paniandy et al. (2000)

	Saponins, Anthraquinones, Tannins, Terpenes, Flavonoids, Coumarins, Proanthocyanidins, Protocatechuic acid, Guavin B, , Amritoside, Araban, Ascorbigen, Methyl cinnamate, Eucalyptol, Azulin, Asiatic acid, Ferulic acid, Leucocyanidin, Mecocyanin, Rubixanthin, Criptoflavin, Neochrome, Lutein, Phytofluene, Quercetin 3-α-L- arabinofuranoside, Quercetin-3-β-galactoside, Quercetin 3- β-D- galactoside, Avicularin, Amritoside	Pino et al. (2001) Mercado-Silva et al. (1998) Jordan et al. (2003) Lozoya et al. (2002) Hsin-Chun et al. (2007)
Flower	Quercetin, Leucocyanidin, Kaempferol, Quercetin 3-α-L- arabinofuranoside, Kaempferol-3- glucoside, Psidium guajava coumari acid, Obtusinin	Mercadante et al. (2005), Begum et al. (2002a), Begum et al. (2002b), Begum et al. (2004c), Jordan et al. (2003), Pelegrini et al. (2008), Vargas et al. (2010)
Roots	Obtusinin, Gallic acid, Tannin, Leukocyanidins, sterols, tannic acid, Ellagic acid	Jordan et al. (2003)
Seeds	Glycosids, Carotenoids, Phenolic compounds, Flavonoid compounds, Flavonol glycoside, quercetin-3-O-β-D-(2"-Ogalloyglucoside)-4'-O-vinylpropionate, cylated flavonol glycoside, Arjunolic acid, Jacoumaric acid, 1-O-3,4-dimethyl-phenylethyl-4-o-3,4-dimethoxy cinnamoyl-6-o-cinnamoyl-β-D-glucopyranase, Mecocyanin, cylated flavonol glycoside	Pelegrini et al. (2008) Michael et al. (2002) Salib et al. (2004)
Bark	Polyphenols, Ellagic acid, Resins	Michael et al. (2002), Salib et al. (2004), Ryu et al. (2012), Rahim et al. (2010)

5.Conclusions

Psidium guajava fruits are very promising for its dietary significance. The whole fruit can be eaten in raw form as well as in many processed products like jam, RTD beverages, Psidium guajava enriched dairy products like cheese, yoghurt and jellies which are available in the market. The leaves of the Psidium guajava are edible and used as traditional medicine due to extensive therapeutic properties. Fruits and leaves of Psidium guajava exhibits different chemical constituents aiding its pharmacological effects such as antioxidant, anticancer, antidiabetic and anti-inflammatory properties. Evaluation of pharmacological activities have been carried out by in-vitro using animals whereas recorded human trials are minimum. But the plants have been extensively studied for its chemical components, fruit leaves bark, seeds, roots and

flowers have also been evaluated for its chemical composition. Fruits and leaves have proven its dietary significance but further research will be required to prove its nutraceutical and pharmacological properties further.

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COMPARISON OF THE NUTRITIONAL PROPERTIES OF ROASTED NIXTAMALIZED AND NON-NIXTAMALIZED IPB VAR 6 CORN AS COFFEE SUBSTITUTE

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ABSTRACT

Coffee is a widely consumed beverage known for its stimulating and cognitive effects. However, the undesirable effects attributed to its consumption have led to an increased interest in coffee substitutes. Coffee substitutes made from roasted plant materials are gaining popularity due to their distinct flavor profiles and potential health benefits. This study compared the nutritional properties of roasted nixtamalized and non-nixtamalized IPB var 6 corn, a corn variety known for its higher protein quality, tryptophan, and lysine content, as a coffee substitute. Twelve treatments were analyzed to evaluate the effects of nixtamalization on the proximate and mineral content of corn. Based on the results obtained from this study, significantly higher moisture, ash and iron content was obtained from nixtamalized treatments. Although significantly higher crude fiber, crude fat, zinc, and potassium content were observed from non-nixtamalized treatments. The differences in the roasting time and temperature in terms of the nutritional properties of the roasted IPB var 6 corn treatments were also determined. At increased roasting temperature and time, the ash, and mineral content were observed to be higher. While varying effects of different roasting time and temperature to the moisture, crude fat, crude protein, and crude fiber were also observed. The non-nixtamalized roasted at 240ºC for 45 min obtained the highest crude fiber, zinc, and potassium content. While Treatment 12 (nixtamalized; 240ºC for 45 min) obtained the highest ash, moisture, nitrogen free extract, and iron content.

1.Introduction

Caffeine is a stimulant that provides physical capacity, and cognitive function (Ruxton, 2008). It is one of the most widely consumed compounds in the world and is usually found in common food and beverages like cacao, chocolates, sodas, and tea, as well as coffee (Rodak et al., 2021; Mahoney et al., 2019). Although excessive consumption of caffeine can cause dehydration, headache, anxiety, and sleep disturbances (Ruxton, 2008). Coffee, the most common source of caffeine, is a dark-colored sweet-bitter aromatic beverage brewed from roasted coffee beans (Reyes and Cornelis, 2018; Samoggia and Riedal, 2018). It is considered as a household staple in the Philippines, especially to lower socioeconomic classes. This beverage, due to its caffeine content, provides a

stimulating effect to humans and enhances consumers' alertness, vigilance, and cognitive abilities. Previous studies also proved that coffee consumption lowers the risk of having high blood pressure and adult-onset diabetes when consumed moderately (Cornelis, 2019; Anese, 2016). But, since it contains caffeine, side effects such as insomnia, nervousness, nausea, diuresis, and palpitations may be experienced (Cornelis, 2019; Barahona, 2002; Waizenegger et al., 2011). The Food and Drug Administration (FDA) recommends a maximum of 400 milligrams a day of caffeine for healthy adults which is equivalent to 4-5 cups of coffee. Excessive coffee intake can lead to gastroesophageal reflux, palpitations, and hypertension, as well as anxiety and sleep disturbances. Some studies also suggest that frequent coffee consumption can hinder the growth and development of young individuals which may also contribute to long term consequences like kidney-related diseases and anxiety disorders (Torres-Ugalde et al., 2020).

Due to the potential risk of caffeine from drinking coffee, several non-coffee products called coffee substitutes became available which are usually made from ingredients like barley, rice, soybean, and chicory root (Anese, 2016). Coffee substitutes are non-coffee products which mimic the taste of coffee (Mostafa et al., 2021). These substitutes are processed and prepared the same way as coffee, whereas raw materials are roasted, grounded, and brewed (Anese, 2016). Although limited information is available regarding these products, studies should be conducted to evaluate their potential benefits in terms of nutrition, food safety, consumer acceptability and marketability of these beverages.

Corn, as the second most important crop in the Philippines, is used to produce valuable products like starches, syrups, oil, and snacks according to the Philippine Department of Agriculture Regional Field Office 3 (DA-RFO3) utilizing this crop, specifically its kernels in introducing and developing another high value product can be considered since it is easily propagated in the country. IPB var 6 corn, a white flint-type corn variety developed by the Institute of Plant Breeding in the University of the Philippines Los Baños (IPB-UPLB), was used in the recent product developments such as corn flour and rice-corn blends. It is known to have higher protein quality, tryptophan, and lysine content compared with other corn variants (Sunico et al., 2020; Salazar et al., 2016). It is also known for its resistance to lodging and promising ear-fill characteristics. With the mentioned properties of IPB var 6 corn, using it as a raw material to produce a coffee substitute might be considered. Moreover, recent studies also proved that a pre-treatment process called nixtamalization, done by steeping a raw material in an alkaline solution, can increase the quality of the corn. This process also changes the starch properties, improves the protein quality, and increases the bioavailability of calcium, niacin, and iron (Arendt and Zannini, 2013; Suri and Tanumihardjo, 2016). It can also reduce phytic acid levels and aflatoxins present in corn (Guzmán-de-Peña, 2010). With the benefits provided by nixtamalization, it can be reasonable to be used in the production of a corn coffee substitute.

This study focuses and limited on the comparison between roasted nixtamalized and non-nixtamalized IPB var 6 corn in terms of proximate (moisture, crude fat, ash, crude protein, crude fiber, and nitrogen-free extract), and mineral (zinc, potassium, iron, calcium, and magnesium) content. It also focuses and limited on determining the differences of the roasting temperatures (200°C, 220°C, and 240°C) and time (30 and 45 minutes) in each of the mentioned parameters, as well as in determining the treatment that will achieve the highest nutritional properties.

2. Materials and methods
2.1. Raw Materials
Dried IPB var 6 corn kernels was obtained from the Institute of Plant Breeding, University of the Philippines Los Baños (IPB-UPLB). The corn samples were stored in a freezer prior to use in nixtamalization, roasting, and further analyses. The chemical reagents needed for the

analyses were analytical grade reagents, while distilled water was used in all experiments unless otherwise specified.

2.2. Place and Time of Study

The nixtamalization and production of roasted nixtamalized and non-nixtamalized IPB var 6 corn was conducted in a kitchen setup in Olongapo City, Philippines. The proximate (moisture, ash, and crude fat) were analyzed in the Institute of Food Science and Technology, University of the Philippines Los Baños (IFST-UPLB). While the total nitrogen, crude fiber, and mineral content analyses were conducted in Department of Agriculture Regional Field Office 3 (DA-RFO3) Regional Feed Chemical Analysis Laboratory (RFCAL) and Regional Soils Laboratory (RSL). All laboratory analyses were done in the year 2022.

Table 1. Roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments

Treatments	Nixtamalization	Time (min)	Temperature (ºC)
Treatment 1	Non-nixtamalized	30	200
Treatment 2			220
Treatment 3			240
Treatment 4		45	200
Treatment 5			220
Treatment 6			240
Treatment 7	Nixtamalized	30	200
Treatment 8			220
Treatment 9			240
Treatment 10		45	200
Treatment 11			220
Treatment 12			240

2.3. Nixtamalization of IPB var 6 corn

The IPB var 6 corn was nixtamalized using an ecological method used by Sunico her colleagues with some modifications. One kilogram of dried IPB var 6 corn was cooked at 95°C for 30 minutes with two liters of distilled water having 1% (w/w) calcium carbonate (Sunico et al., 2020). The cooked grains were steeped in the same solution for 16 hours at room temperature before decanting the liquid. After that, the nixtamalized kernels were dried in an oven- drier at 50-55°C for 12 hours minimum and were packed in sealed polyethylene bags before roasting (Sunico et al., 2020; Rodriguez-Mendez et al., 2013; Das et al., 2016).

2.4. Production of Roasted IPB var 6 Corn Treatments

Different treatments of roasted nixtamalized and non-nixtamalized IPB var 6 corn as shown in Table 1. were produced using a coffee roaster. Then, the roasted kernels were ground using a multi-functional high-speed disintegrator and were packed in a laminated aluminum-polyethylene container per treatment. Each container was stored in a freezer prior to use in the analyses.

2.5. Proximate Analyses

The methods used in obtaining the moisture content, crude protein, crude fat, crude fiber, and nitrogen-free extract content of the roasted IPB var 6 corn treatments were analyzed based on the standard conventional methods in AOAC (2005).

The moisture content was determined using the oven-drying method, whereas approximately 1g of sample was weighed in tared crucibles and placed in an oven at 100 ± 5 °C for at least 5 hrs. Afterward, the crucibles were placed into the desiccator and cooled to room temperature before weighing. The procedure was repeated until the weights of the two previous readings are constant. The percent moisture content (%MC) of the sample was calculated using the percent of the weight loss. For the ash content, the crucibles with the dried samples from the moisture content analysis were placed in a muffle furnace and ignited at 550oC for 5 hrs. Then, the muffle furnace was turned off and cooled overnight. The crucibles were then placed in an oven at 100 ± 5 °C for at least 2 hrs. After that the crucibles were transferred to a desiccator to cool before weighing. The drying procedure was repeated until the weights of the two previous readings are constant. The percent ash content (%Ash) of the sample was calculated using the percent of the weight remaining. The crude fat content was obtained by placing one gram of the sample inside a pre-weighed folded filter paper and dried in an oven at 105 °C for 2 hours and then transferred to a desiccator to cool for 30 mins before weighing. The samples were placed in the extraction chamber of a Soxhlet set-up using petroleum ether as the extracting solvent. The fat in the samples were extracted for 16 hours, after that, the samples were removed from the extraction chamber, air-dried under the fume hood for about 15 minutes, and then transferred inside the oven (100 ± 5 °C) for at least 30 mins. The samples were cooled in a desiccator for 30 mins before weighing. The percent crude fat content (%Crude fat) was calculated using loss in weight. The crude fiber analyses were obtained using the method for crude fiber by ANKOM Technology using filter bags. The samples were weighed and inserted in the filter bags, sealed, and labeled. Before conducting the analysis, the filter bags containing the samples were soaked in petroleum ether for 10 minutes to remove fat. Then the filter bags were air-dried. The air-dried filter bags were inserted in the vessel of the ANKOM crude fiber analyzer, and the extraction of non-fiber components were done automatically. After extraction, the bags were soaked in acetone for 3 to 5 minutes and allowed to be air dried. The air-dried bags were also dried using an oven at 102oC for 2 to 4 hours and cooled for weighing. Percent crude fiber (%Crude Fiber) is calculated using the loss in the weight of the organic matter. The Kjeldahl method was used to obtain the total nitrogen, whereas the crude protein content of the samples was obtained by multiplying a protein factor to the total nitrogen. The sample was weighed and transferred into the Kjeldahl digestion flask. Then a catalyst and concentrated H_2SO_4 were added. The mixture was digested in the digestion set-up which was heated until it became colorless. The Kjeldahl digestion flask with the digested sample was cooled and transferred into distillation setup. The Kjeldahl flasks were washed with distilled water and the combined washings were transferred to a distillation flask. A receiver flask containing 4% boric acid solution and mixed indicator was placed at the end of the condenser, while 40% NaOH solution was added to the sample in the distillation flask through a valve. The setup was distilled, and the distillate was titrated with 0.1 N standard HCl solution up to the first appearance of faint orange color. A blank titration was done to correct any nitrogen that may be present on the reagents. The total nitrogen of the samples was calculated, as well as the percent crude protein (%Crude Protein) using the protein factor 6.25. Lastly, the percent nitrogen-free extract (%NFE) content of the roasted IPB var 6 corn treatments were calculated by subtracting the %MC, %Ash, %Crude Fat, %Crude Protein, and %Crude Fiber to 100%.

2.5. Mineral Content

The magnesium, iron, calcium, and zinc content analyses of the roasted IPB var 6 corn treatments were obtained using acid digestion and Flame Atomic Absorption Spectroscopy (FAAS) method. While the potassium content of the treatments was obtained using acid digestion and Flame Atomic Emission Spectroscopy

(FAES). The different corn coffee treatments were ashed and dissolved in an acid and were diluted to 50 mL using deionized water. The solutions were measured using Flame Atomic Absorption Spectroscopy (FAAS) at 285 nm, 510 nm, 422 nm, and 213nm for magnesium, iron, calcium, and zinc, respectively. While the potassium content of the IPB var 6 corn coffee treatments were obtained using the same method but using Flame Atomic Emission Spectroscopy at 766 nm.

2.6. Statistical Analyses

The data obtained from the proximate and mineral analyses were statistically treated to determine the differences and significance of the results. The Student's T-test was used to determine significant differences between nixtamalized and non-nixtamalized treatments. Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) were used to determine significant differences between the temperature (200ºC, 220ºC and 240ºC), while Student's or Welch's T-test was used to compare the roasting time (30 and 45 minutes).

3.Results and discussions
3.1. Proximate Analyses

Proximate analysis is an analytical system which determines the moisture, ash, crude fat, crude protein, and crude fiber as well as nitrogen-free extract (NFE). It is important because it gives an estimate on the composition of a food product and serves as a basis for nutritional data and government regulation compliance. Table 2. shows the results of the proximate analyses (moisture, ash, crude fat, fiber, total nitrogen, protein, and nitrogen free extract) on the roasted IPB var 6 corn treatments. Generally, significantly higher moisture and ash content were recorded for nixtamalized (Treatments 7 to 12) as compared with the non-nixtamalized (Treatments 1-6) treatments. While most of the non-nixtamalized treatments were found to be significantly higher in terms of crude fiber, and crude fat. Moreover, varying significant differences were observed on the crude protein.

Moisture Content. Moisture content is the measure of the water content in food, unlike water activity, moisture content is the measure of the totality of the water in the product, not just the free or unbound water. Determining the moisture content of a product is important because it can affect the appearance, texture, sensory qualities, consumer acceptability, and shelf-life stability during processing and storage (Saloko et al., 2019). It was observed that the moisture content of the roasted IPB var 6 corn treatments is within the range of 0.167% to 1.650%. It was also observed that most nixtamalized treatments have significantly higher moisture content (P≤0.05) compared with non-nixtamalized treatments. Moreover, the results show that roasting temperatures 200ºC, 220ºC, and 240ºC are significantly different from each other (P≤0.05) in terms of moisture content as shown in Figure 1. While, in terms of roasting time, it was observed that nixtamalized treatments roasted at 200ºC and non-nixtamalized treatments roasted at 220ºC when roasted for 30 minutes have significantly higher moisture content (P≤0.05) compared when roasted for 45 minutes.

Crude Fat. Crude fat estimates the fat content of a food sample which is done by separation of fat in the food matrix using a non-polar solvent and estimating the fat content using the weight loss. It is an accurate indicator of coffee quality because it contributes to the aroma and flavor of the coffee product (Koshima et al., 2020). Measuring crude fat is important to include in the nutrition label of the product to inform consumers the estimated amount of energy they can acquire from the fat content of the product as well as to comply with the regulations.

Table 2. Proximate composition* of roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments[1].

Proximate composition	Roasting Time (min)[2]	Temperature (ºC)					
		200		220		240	
		Non-nixtamalized	Nixtamalized	Non-nixtamalized	Nixtamalized	Non-nixtamalized	Nixtamalized
Moisture, %	30	1.144 ± 0.193^{b}	1.650 ± 0.127^{aA}	0.622 ± 0.022^{bA}	0.705 ± 0.017^{a}	0.167 ± 0.032^{bB}	0.420 ± 0.094^{aB}
Ash, %		1.461 ± 0.088^{b}	1.491 ± 0.060^{a}	1.433 ± 0.087^{b}	1.535 ± 0.061^{a}	1.434 ± 0.014^{b}	1.544 ± 0.051^{a}
Crude Fat, %		6.986 ± 0.115^{aA}	5.973 ± 0.143^{bA}	6.383 ± 0.103^{a}	5.714 ± 0.129^{bA}	6.298 ± 0.095^{aA}	5.536 ± 0.105^{bA}
Crude Protein, %		9.13 ± 0.13^{b}	9.63 ± 0.19^{a}	9.00 ± 0.06^{aB}	9.00 ± 0.06^{a}	9.50 ± 0.19^{aA}	8.88 ± 0.13^{b}
Crude Fiber, %		3.7 ± 0.2^{a}	3.2 ± 0.3^{b}	3.6 ± 0.4^{aB}	4.0 ± 0.3^{a}	4.9 ± 0.3^{aB}	4.4 ± 0.2^{b}
NFE, %		77.6	78.1	79.0	79.0	77.7	79.2
Moisture, %	45	1.153 ± 0.087^{b}	1.451 ± 0.054^{aB}	0.176 ± 0.019^{bB}	0.634 ± 0.077^{a}	0.496 ± 0.027^{bA}	1.292 ± 0.177^{aA}
Ash, %		1.441 ± 0.017^{b}	1.513 ± 0.099^{a}	1.438 ± 0.031^{b}	1.551 ± 0.042^{a}	1.448 ± 0.017^{b}	1.592 ± 0.047^{a}
Crude Fat, %		6.217 ± 0.116^{aB}	3.984 ± 0.195^{bB}	6.166 ± 0.171^{a}	3.827 ± 0.112^{bB}	5.569 ± 0.197^{aB}	3.317 ± 0.124^{bB}
Crude Protein, %		8.94 ± 0.13^{b}	9.56 ± 0.19^{a}	9.50 ± 0.19^{aA}	8.88 ± 0.13^{b}	9.13 ± 0.13^{aB}	8.75 ± 0.13^{b}
Crude Fiber, %		4.2 ± 0.4^{a}	3.5 ± 0.2^{b}	4.3 ± 0.4^{aA}	3.8 ± 0.3^{a}	6.1 ± 0.3^{aA}	4.7 ± 0.3^{b}
NFE, %		78.0	80.0	78.4	81.3	77.3	80.3

*In each row, results are mean ± SD of triplicate analysis except for NFE.

[1] Mean ± SD followed by different small letters within rows represent significant differences between nixtamalized and non-nixtamalized treatments (P ≤0.05) compared using Student's T-test.

[2] Mean ± SD followed by different capital letters within columns represent significant differences between 30 and 45 minutes of roasting time (P ≤0.05) compared using Welch's or Student's T-test. NFE- nitrogen free extract.
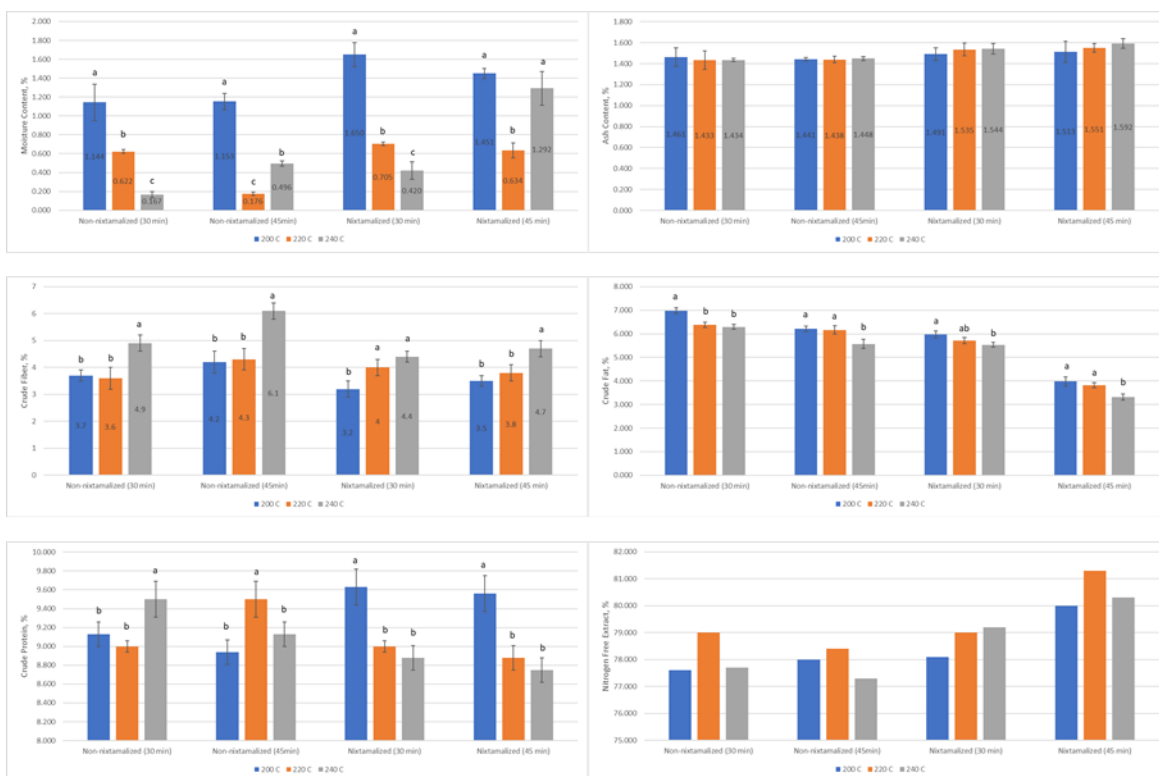
Figure 1. Proximate composition of roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments. Different small letters represent significant differences between roasting temperatures 200°C, 220°C and 240°C (P ≤0.05) compared using ANOVA and Tukey's HSD.

The crude fat content of the roasted IPB var 6 corn treatments ranges from 3.317 to 6.986 % as shown in Table 2. It was observed that non-nixtamalized treatments have significantly higher (P≤0.05) crude fat content compared with the nixtamalized treatments. The data also shows that roasting temperatures 200°C, 220°C, and 240°C have varying significant differences from each other (P≤0.05) in terms of crude fat as shown in Figure 1, whereas higher crude fat was observed on treatments roasted at 200°C. In terms of roasting time, results show that most of the treatments roasted for 30 minutes have significantly higher crude fat (P≤0.05) than the treatments roasted for 45 minutes.

Ash Content. The ash content is the estimate of the mineral content and other inorganic materials in a food. The ash content of food sample is done by igniting it in a muffle furnace to vaporize water and volatile substances and convert organic matter into carbon dioxide and water, whereas the remaining substance is referred as the ash which is composed of mineral oxides, sulfates, phosphates, chlorides, and silicates (Ismail, 2017). The ash content of the roasted IPB var 6 corn treatments ranges from 1.433% to 1.592%, as is shown in Table 2. All the nixtamalized treatments have significantly higher ash content (P≤0.05) compared with the non-nixtamalized treatments. While no significant differences were observed between the roasting time (P>0.05) and temperatures (P>0.05) in terms of ash content as shown in Figure 1.

Crude Protein. Crude protein analysis is used to estimate the protein composition of a food product, known methods used are Kjeldahl and Dumas that measures the total nitrogen of a product and a protein conversion factor is used to obtain the crude protein value (Chang and Zhang, 2017). Similar to crude fat, measuring crude protein is important to include in the

nutrition label of the product to inform consumers the estimated amount of energy they can acquire from the protein content of the product as well as to comply with the regulations. Using the conversion factor of 6.25, the crude protein was found to be at a range of 8.88 to 9.63%, whereas varying significant differences (P≤0.05) were found between nixtamalized, and non-nixtamalized roasted corn treatments as shown in Table 2. The crude protein of nixtamalized treatments at 200ºC was significantly higher (P≤0.05) than non-nixtamalized treatments. While at 220ºC and 240ºC, it was observed that most of the non-nixtamalized treatments have significantly higher crude protein (P≤0.05). In terms of the roasting temperatures, 200ºC, 220ºC, and 240ºC have varying significant differences from each other (P≤0.05) as shown in Figure 1. The results also show that non-nixtamalized treatments roasted at 240ºC have significantly higher crude protein (P≤0.05) when roasted for 30 minutes compared with 45 minutes. While non-nixtamalized treatments roasted at 220ºC have significantly higher crude protein (P≤0.05) when roasted for 45 minutes.

Crude Fiber. The crude fiber content estimates the indigestible carbohydrates in food. It involves continuous extraction of food samples using hot sulfuric acid and hot sodium hydroxide solutions leaving some residues that are the crude fiber content of the food sample. Crude fiber is also important to include in the nutrition label of the product to inform consumers the estimated amount of fiber they can acquire from the product as well as to comply with the regulations. It is referred to as the type of dietary fiber that remains as a residue after acid and alkali treatment. It is also known to help in digestion and serve as a prebiotic material for bacterial growth in the gut. The crude fiber content of the roasted IPB var 6 corn treatments ranges from 3.2% to 6.1% as shown in Table 2. Most of the non-nixtamalized treatments have significantly higher crude fiber (P≤0.05) compared with nixtamalized treatments. The results also show that roasting temperatures 200ºC, 220ºC, and 240ºC have

varying significant differences from each other (P≤0.05) in terms of crude fiber as shown in Figure 1, whereas higher crude fiber was observed on treatments roasted at 240ºC. In terms of roasting time, results show that non-nixtamalized treatments roasted at 220oC and 240ºC have significantly higher crude fiber (P≤0.05) when roasted for 45 minutes compared with 30 minutes.

Nitrogen Free Extract. Nitrogen free extract (NFE) is a rough estimate of the carbohydrate content of a product which was obtained by subtracting the sum of the percentages of moisture, crude protein, crude fat, crude fiber, and ash from 100. It refers to the non-fibrous carbohydrates in a food material which is composed mostly of simple sugars and starches. Nitrogen free extract results of roasted IPB var 6 corn treatments are shown in Table 2. which ranges from 77.3 to 81.3%.

3.2. Mineral Content
The determination of mineral content is important in determining the nutritional value of a food product, as well as to determine if the food product has mineral components that comply with regulations (Ward and Legako, 2017). Table 3. shows the potassium, zinc, iron, calcium, and magnesium content of the roasted IPB var 6 corn treatments. A general observation is that significant differences between nixtamalized and non-nixtamalized treatments are found in terms of potassium, zinc, and iron content. While there were no significant differences in terms of magnesium and calcium content.

Zinc. Zinc, even though the human body needs only small amounts, has a huge role especially in enzymatic and other biochemical reactions in the human body. It also has an important role in DNA and cell functions, protein building, tissue regeneration, and the immune system. It was observed that the zinc content of the roasted IPB var 6 corn treatments ranges from 29.31 to 44.72 ppm, where the non-nixtamalized treatment roasted at 240ºC for 45 minutes obtained the highest value. It was also observed that non-nixtamalized treatments have

significantly higher zinc (P≤0.05) compared with nixtamalized treatments as shown in Table 3. Moreover, in terms of roasting time, it was observed that treatments roasted for 45 minutes obtained significantly higher zinc (P≤0.05) than the treatments roasted for 30 minutes. In terms of roasting temperature, no significant differences (P>0.05) between 200°C, 220°C, and 240°C were observed on non-nixtamalized treatments roasted for 30 minutes. Although, in other treatments higher zinc content was obtained from treatments roasted at 240°C as shown in Figure 2.

Potassium. Potassium is an important essential mineral needed by the body to function; one of its roles is maintaining the fluid balance inside our cells. It was observed that the potassium content of roasted IPB var 6 corn treatments ranges from 0.22 to 0.38 %. It was also observed that significantly higher potassium content (P≤0.05) was obtained from non-nixtamalized treatments compared with nixtamalized treatments as shown in Table 3. In terms of roasting temperatures, no significant differences (P>0.05) between 200°C, 220°C, and 240°C were observed on nixtamalized treatments. Although higher potassium content was observed from non-nixtamalized treatments roasted at 240°C as shown in Figure 2.

Iron. Iron is known to be an important component of hemoglobin in blood that carries oxygen around the body. It was observed that the iron content of roasted IPB var 6 corn treatments ranges from 26.06 to 36.11 ppm, and the majority of the nixtamalized treatments have significantly higher (P≤0.05) iron compared with the non-nixtamalized treatments as shown in Table 3. It was also observed that treatments roasted for 45 minutes obtained significantly higher iron (P≤0.05) than the treatments roasted for 30 minutes. The data also shows that roasting temperatures 200°C, 220°C, and 240°C are significantly different from each other (P≤0.05) in terms of iron as shown in Figure 2, whereas higher iron was observed on treatments roasted at 240°C.

B var 6 corn treatments ranges from 0.04 to 0.05 %, although no significant differences were observed in the calcium contents of roasted nixtamalized and non-nixtamalized treatments as shown in Table 3. Moreover, no significant differences were observed from the roasting time (P>0.05) and temperatures (P>0.05) as shown in Figure 2.

Magnesium. Magnesium plays an important role in several enzymatic reactions in the body, as well as other processes such as protein building, maintenance of the bones, regulation of blood sugar and pressure, and muscle and nerve functions. It was observed that the magnesium content of roasted IPB var 6 corn treatments ranges from 0.04 to 0.06 %, and that there are no significant differences (P>0.05) between the roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments in terms of magnesium content as shown in Table 3. It was also observed that treatments roasted for 45 minutes obtained significantly higher magnesium (P≤0.05) than the treatments roasted for 30 minutes, while no significant differences were observed from the roasting temperatures (P>0.05) as shown in Figure 2.

3.3. Effects of Ecological Nixtamalization on the Roasted IPB var 6 Corn Treatments

Nixtamalization is a well-known pre-treatment for milled corn products. This process involves cooking and steeping corn in an alkaline solution, softening its pericarp, and altering the grain structure releasing the bound nutrients (Sunico et al., 2020, Wacher, 2003). Studies show that nixtamalization enhances the nutritional value of corn not only the protein quality, but also increases the amounts of calcium and niacin bioavailability, reduction of phytic acid levels, and increases iron digestibility and bioavailability (Suri and Tanumihardjo, 2016). This process is also known to eliminate 97% to 100 % of aflatoxins from grains contaminated with mycotoxin (Guzmán De Peña, 2010).

Table 3. Mineral composition* of roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments[1].

Mineral	Roasting Time (min)[2]	Temperature (ºC)					
		200		220		240	
		Non-nixtamalized	Nixtamalized	Non-nixtamalized	Nixtamalized	Non-nixtamalized	Nixtamalized
Zinc, ppm	30	30.93 ± 0.05^{aB}	29.31 ± 0.04^{bB}	31.08 ± 0.48^{aB}	30.97 ± 0.30^{aB}	31.13 ± 0.59^{aB}	31.08 ± 0.03^{aB}
Potassium, %		0.30 ± 0.02^{a}	0.24 ± 0.02^{b}	0.35 ± 0.01^{a}	0.24 ± 0.01^{b}	0.36 ± 0.01^{aB}	0.25 ± 0.01^{b}
Iron, ppm		26.06 ± 0.02^{bB}	26.40 ± 0.01^{aB}	26.95 ± 0.43^{aB}	27.02 ± 0.07^{aB}	27.49 ± 0.01^{bB}	29.45 ± 0.02^{aB}
Calcium, %		0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}
Magnesium, %		0.04 ± 0.01^{a}	0.05 ± 0.02^{a}	0.04 ± 0.01^{aB}	0.05 ± 0.02^{a}	0.05 ± 0.02^{a}	0.06 ± 0.01^{a}
Zinc, ppm	45	31.58 ± 0.28^{aA}	31.20 ± 0.05^{bA}	39.29 ± 0.12^{aA}	35.11 ± 0.08^{bA}	44.72 ± 0.14^{aA}	42.53 ± 0.03^{bA}
Potassium, %		0.33 ± 0.02^{a}	0.22 ± 0.01^{b}	0.34 ± 0.01^{a}	0.23 ± 0.01^{b}	0.38 ± 0.01^{aA}	0.23 ± 0.02^{b}
Iron, ppm		30.60 ± 0.02^{bA}	30.97 ± 0.03^{aA}	31.53 ± 0.05^{bA}	34.04 ± 0.01^{aA}	35.12 ± 0.02^{bA}	36.11 ± 0.01^{aA}
Calcium, %		0.04 ± 0.01^{a}	0.05 ± 0.02^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.02^{a}
Magnesium, %		0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{aA}	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}	0.06 ± 0.01^{a}

In each row, results are mean ± SD of triplicate analysis.

[1] *Mean ± SD followed by different small letters within rows represent significant differences between nixtamalized and non-nixtamalized treatments (P ≤0.05) compared using Student's T-test.*

[2] *Mean ± SD followed by different capital letters within columns represent significant differences between 30 and 45 minutes of roasting time (P ≤0.05) compared using Welch's or Student's T-test.*
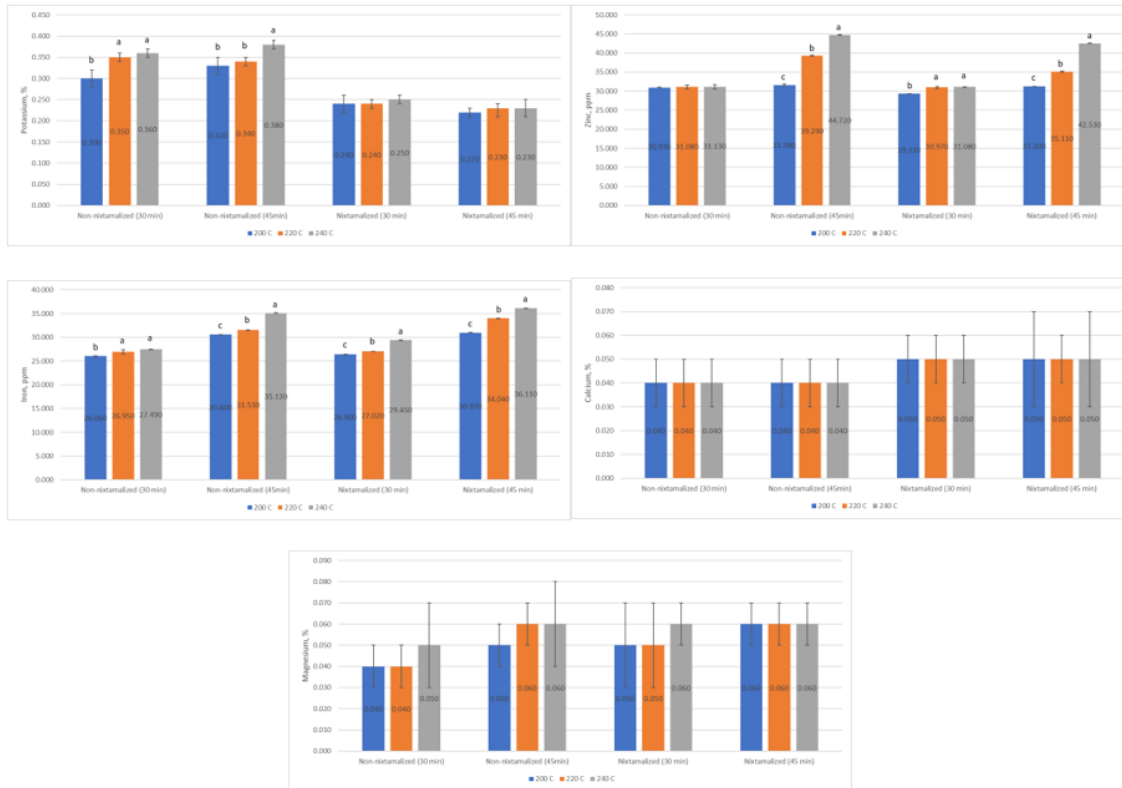
Figure 2. Mineral composition of roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments. Different small letters represent significant differences between roasting temperatures 200ºC, 220ºC and 240ºC (P ≤0.05) compared using ANOVA and Tukey's HSD.

Other than traditional and classical method, one way of nixtamalizing a product is by ecological nixtamalization, which makes use of calcium salts like food grade calcium carbonate, in this type of nixtamalization changes in the nutritional and functionality of corn is more observed. The effects of ecological nixtamalization on the nutrient content, mineral content, antioxidant, and phytochemical properties of nixtamalized and non-nixtamalized roasted IPB var 6 corn treatments were determined as shown in Table 2 and Table 3.

Generally, various advantages and disadvantages of nixtamalization were observed on the treatments. Considering the process of nixtamalization, whereas the dried IPB var 6 corn kernels were introduced to an aqueous solution containing calcium carbonate, and having it cooked and steeped, there is a partial gelatinization that occurred and have altered the

intrinsic properties of the kernels including its water retention capacity.

This explains why the results show that most of the nixtamalized treatments obtained significantly higher moisture content (P≤0.05) than non-nixtamalized treatments. While the incorporation of calcium carbonate during nixtamalization may have contributed to the higher ash content of the roasted nixtamalized IPB var 6 corn treatments. For crude fat content, the results show that non-nixtamalized treatments have significantly higher (P≤0.05) crude fat content compared with nixtamalized treatments, which agrees with the study of Maureen et al. (2020). It is possible that during the steeping process some organic compounds including crude fat from the kernels have seeped out to the aqueous solution which was discarded after the nixtamalization process. On the other hand, the nixtamalization process also has a

decreasing effect on the crude fiber content of the roasted IPB var 6 corn kernels, which can be explained by the partial degradation of the seed coat of the kernels, leaving a higher percentage for the other components. The varying significant differences between the crude protein of nixtamalized and non-nixtamalized treatments has been observed to be different with each roasting parameter. Hence, nixtamalization may not be the only step in the whole process that influences the crude protein content.

In terms of mineral content, the results suggest that nixtamalization has a lowering effect on the potassium and zinc content of the roasted IPB var 6 corn kernels. According to some studies, the steeping step during nixtamalization contributes to the significant losses in the zinc and potassium content of the corn during processing which can explain the results (Sunico et al., 2020; Bressani et al., 2002; Morales and Zepeda, 2017). Unlike what was observed on the potassium and zinc content, the iron content of the nixtamalized roasted IPB var 6 corn kernels are higher than the non-nixtamalized treatments. Based on Similarly, Sunico and her colleagues (2020), observed nixtamalization had influenced the increase in the iron concentration, whereas they explained that it is due to the removal of the pericarp on the corn kernels leaving the endosperm and the germ, which are the parts of a kernel with high iron concentration. Contrary to earlier studies about nixtamalized products, no significant differences were observed in the calcium contents of roasted nixtamalized and non-nixtamalized treatments. One possible reason for this is that the limitation of the equipment used in the analysis, whereas limited significant figures were obtained which is not enough to have a significant difference between nixtamalized and non-nixtamalized treatments even though the numerical values are higher on the nixtamalized treatments. Another reason is possible losses of calcium carbonate during the steps in production such as decantation, drying, roasting, and grinding. The same possible reasons were also sought to explain the results

for the magnesium content, which results show that nixtamalized and non-nixtamalized treatments are not significantly different from each other.

3.4. Differences of Roasting Time and Temperature in terms of the Nutritional of the Roasted IPB var 6 Corn Treatments

During roasting, different chemical reactions like Maillard reaction, caramelization, and pyrolysis are favored giving distinctions in chemical, physical, and sensory properties of coffee and coffee substitutes (Anese, 2016). The differences of roasting time and temperature in terms of the nutritional parameters of the Roasted IPB var 6 Corn Treatments were shown in Figure 1. and Figure 2. In the present study, it was observed that the moisture content of the treatments has a decreasing trend at increasing roasting temperature and time. Although all treatments roasted at 240°C, a higher temperature, were observed to have a significantly higher moisture content when roasted for 45 minutes compared with treatments roasted at lower roasting time and temperature. Theoretically, it is expected that the moisture content of the treatments roasted at higher temperature and longer time are lower, which was observed on most of the results except for some treatments mentioned. A possible reason for the observed phenomenon is the changes in the intrinsic properties of the corn kernels, specifically hygroscopicity when roasted at very high temperatures (Nakilcioğlu-Taş and Ötleş, 2019). While the roasting time and temperatures were not observed to have an influence on the ash content of treatments, which disagrees with the previous studies. According to Saloko and his colleagues (2019), the ash content increases during roasting because the non-mineral components such as moisture, fat, protein, fiber, and carbohydrates decrease during processing. The observed increase in the ash content from the previous studies might not be observed in the present study due to the limited range of the roasting time and temperatures used. In terms of crude fat, crude protein, and crude fiber the results

shows that roasting temperatures 200ºC, 220ºC, and 240ºC have varying significant differences from each other (P≤0.05), hence the effect of roasting temperature on crude fat and crude protein cannot be established. For the roasting time, results show that most of the treatments roasted for 30 minutes have significantly higher crude fat (P≤0.05) than the treatments roasted for 45 minutes. In the study conducted by Oboh and his colleagues (2010), roasting have led to an increase of the crude fat in corn kernels, which they have discussed to be caused by the break down the bonds between the fat and matrix, releasing the oil reserve more efficiently. Unlike the present study, the corn kernels in the study of Oboh and his colleagues (2010) were roasted at a lower temperature (120ºC to 130ºC). It is possible that crude fat content in the present study might have volatized during the roasting, which may be the reason why crude fat was observed to decrease at increasing roasting time and temperature. From the same study, they also have observed that roasting can cause a significant decrease in the crude protein and crude fiber content of corn (Oboh et al., 2010). Although, the effect of roasting time for crude protein and crude fiber in the present study cannot be also established due to varying significant differences observed.

In terms of mineral content, the results show that roasting for a longer time led to a higher zinc, potassium, magnesium, and iron content of the treatments. Based on the study of Oboh and his colleagues (2010), significant increase in the mineral content like calcium, sodium, magnesium, and zinc were observed after roasting the corn. Although it was observed from the present study that roasting temperature has no effect on the zinc, potassium, and magnesium content. Moreover, unlike what was observed by Oboh and his colleagues (2010), it was observed from the present study that there is an increasing amount of iron content when the roasting temperature is increased as observed. In terms of calcium there was no significant differences were observed from the roasting time (P>0.05) and temperatures (P>0.05), which

does not agree with the previous studies (Oboh et al., 2010).

3.3. Ideal Roasting Time and Temperature of Roasted IPB var 6 Corn Treatments each Parameter

The nutritional properties of roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments varies on each parameter. In terms of proximate analyses, highest ash (1.592%), moisture (1.292%), and nitrogen free extract (80.3%) was obtained from Treatment 12 (nixtamalized; 240ºC for 45 min), although this treatment also obtained the lowest crude fat and crude protein. While Treatment 1 (non-nixtamalized; 200ºC for 30 min) and Treatment 5 (non-nixtamalized; 240ºC for 45 min) obtained the highest crude fat (6.986%) and crude fiber (6.1%), respectively. Treatment 7 (nixtamalized; 200ºC for 30 min), on the other hand obtained the highest crude protein (9.63%). For mineral content, Treatment 5 stood out in terms of zinc (44.72 ppm) and potassium (0.38%), while Treatment 12 obtained the highest iron (36.11 ppm). Nixtamalized treatments (Treatment 7 to 12) obtained the highest calcium content (0.05%). Moreover, the highest magnesium content was also obtained from nixtamalized treatments which ranges from 0.05% to 0.06%. In terms of antioxidant and phytochemical properties of roasted nixtamalized and non-nixtamalized IPB var 6 corn treatments.

4. Conclusions

Coffee is one of the most popular and widely consumed beverages globally due to its stimulating and cognitive effects. However, undesirable effects attributed to its consumption, such as palpitations and sleep disturbances, have led to increased interest in coffee substitutes. Coffee substitutes made from roasted plant materials are gaining popularity due to their distinct flavor profiles and potential health benefits, including cereal-based coffee substitutes. The effects of ecological nixtamalization on the nutrient content, mineral content, antioxidant, and phytochemical

properties of roasted IPB var 6 corn treatments were determined. Based on the results obtained from this study, in terms of nutrient content, significantly higher moisture, ash and iron content was obtained from nixtamalized treatments. Although significantly higher crude fiber, crude fat, zinc, and potassium content were observed from non-nixtamalized treatments. The differences in the roasting time and temperature in terms of the nutritional properties of the roasted IPB var 6 corn treatments were also determined. At increased roasting temperature and time, the ash, and mineral content were observed to be higher. While varying effects of different roasting time and temperature to the moisture, crude fat, crude protein, and crude fiber were also observed. The non-nixtamalized roasted at 240ºC for 45 min (Treatment 5) obtained the highest crude fiber (6.1%), zinc (44.72 ppm), and potassium (0.38%) content. While Treatment 12 (nixtamalized; 240ºC for 45 min) obtained the highest ash (1.592%), moisture (1.292%), nitrogen free extract (80.3%), and iron (36.11 ppm). For crude fat and crude protein, the highest results were obtained from Treatment 1 (non-nixtamalized; 200ºC for 30 min) (6.986%) and Treatment 7 (nixtamalized; 200ºC for 30 min) (9.63%), respectively. Moreover, nixtamalized treatments (Treatment 7 to 12) obtained the highest calcium content (0.05%). Moreover, the highest magnesium content was also obtained from nixtamalized treatments which ranges from (0.05% to 0.06%).

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THE EFFECT OF USE OF HYDROCOLLOIDS IN DIFFERENT TYPES AND RATIOS ON THE QUALITY OF GLUTEN-FREE BREADS

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ABSTRACT
Hydrocolloids are used as gluten substitutes in gluten-free bread formulation due to providing the improvement on textural, moisture, viscosity and overall quality properties. Although the effect of hydrocolloids on the final product varies, the chemical structure-amount of hydrocolloid used, process parameters and interactions with other components are very important. In our research, the specific volume, moisture, color, texture (hardness, chewiness, elasticity, cohesiveness) and organoleptic properties of gluten-free breads (konjac gum (CG) and xanthan gum (XG), hydroxypropyl methyl cellulose (HPMC), carboxymethyl cellulose (CMC)) effects were investigated. As a result of analytical and sensory analyzes, it was determined that the use of konjac gum at increasing concentrations significantly affected the quality and the consumability of gluten-free breads ($p<0.05$). In this research, the effects of different types of hydrocolloids on gluten-free breads, that were planned to be developed for celiac patients, were investigated and it was determined that the konjac gum among these hydrocolloids which is widely used in the food industry but has a limited usage in gluten-free bread production, significantly improves the physicochemical, textural and sensory properties of the samples.

1.Introduction

Celiac is a chronic inflammatory intestine disease caused by gluten consumption in genetically susceptible individuals (Ballestero-Fernande et al., 2021). This disease is a syndrome that occurs when the alcohol-soluble proteins of rye or barley and the gliadin fraction of wheat gluten damage the small intestinal mucosa. (Benazir et al., 2019). Gluten-free diet is the only effective treatment method that allows the clinical symptoms of celiac patients to disappear and thus the small intestine mucosa to return to its normal structure (Marciniak et al., 2021) and the digestive system to be healthy (Caio et al., 2019). Today, the products for the consumption of celiac patients (bread, pasta, biscuits, cake, pudding) are on the market

shelves (Larretxi et al., 2020). Among gluten-free products, bread is one of the foods that is highly preferable and forms an important part of the diet (Houben et al., 2012). However, gluten-free breads have poor nutritional, technological and sensory properties compared to wheat bread due to gluten deprivation (Cappelli et al., 2020). In order to improve the quality of gluten-free breads, some food additives (enzymes, emulsifiers, some polysaccharides), especially hydrocolloids, are added to product formulations as gluten substitutes (Conte et al., 2019; Bender and Schönlechner 2020; Aguiar et al., 2022).

Hydrocolloids are described as water-dispersible and/or soluble, thickening polymeric carbohydrates (Yücel, 2009). In addition to their

high water holding capacity, it is reported that they also behave like gluten when mixed with water (Houben et al., 2012). The addition of hydrocolloids to gluten-free bread formulations may have different effects on intermediate and final products (Yano et al., 2017). The hydrocolloids effects are; the increasing viscosity, foam stability, flocculation and the cohesion (Mir et al., 2016; Rai et al., 2018), the improving viscoelastic properties and increasing gas holding capacity in dough structure. (Nishinari et al., 2018) They also have features such as reducing moisture loss, maintaining general quality such as texture, specific volume, crust structure and sensory properties, and extending the shelf life of bread (Jnawali et al., 2016). The effects of hydrocolloids on gluten-free bread quality vary depending on their molecular mass-structure-amount, chain length and bonds, and interactions with other components such as starch (Vidaurre-Ruiz et al., 2019; Clapasson et al., 2020). In researches in the recent years, xanthan gum (XG), guar gum, carob gum, hydroxypropyl methyl cellulose (HPMC), carboxymethyl cellulose (CMC), methyl cellulose (MC) are the leading hydrocolloids used to produce better quality gluten-free bread. It has been emphasized that they increase the quality and improve sensory properties of the final products (Lazaridou et al., 2007; Morreale et al., 2018; Vidaurre-Ruiz et al., 2019). There are studies on gluten-free bread quality of the single and combined use of hydrocolloids, which are frequently mentioned in the literature, but there are insufficient studies on the effects of plant-based konjac gum on gluten-free bread qualities.

Konjac gum (CG) has important technological properties due to its high water holding capacity, gelling property and improving dough rheology (Wang et al., 2017). Demirkesen et al (2010) reported in their study that the use of xanthan gum (XG) in combination with konjac gum caused a synergistic interaction each other in the dough structure and this effect improved the textural properties of gluten-free products.

In our research, the effects of using different types of hydrocolloids (CG, X, HPMC, CMC) in increasing concentrations (2%, 4%, 6%) on the physicochemical, textural and sensory properties of gluten-free breads were investigated. With this research, it was aimed to produce gluten-free bread suitable for the consumption of celiac patients, and at the same time, it was aimed to reveal the effects of konjac gum, which has limited studies in the literature.

2. Materials and methods
2.1. Materials
2.1.1. Samples

Corn starch (10.65% moisture, 0.13% ash, 0.18% crude fat, 0.4% protein, 88.65% total carbohydrate), granulated sugar, sun flower oil, salt, compressed yeast (Saccharomyces cerevisiae), milk powder purchased from local market. The hydrocolloids Konjac gum (CG) Xanthan (XG), hydroxypropylmethyl cellulose (HPMC) and carboxymethyl cellulose (CMC) obtained from Tunckaya and Demeter Chemical substances companies (Turkey). Gluten-free wheat starch (11.4% moisture, 0.11% ash, 0.17% crude fat, 0.35% protein, 87.97% total carbohydrate), purchased from Demeter Chemical (Turkey). This starch has a certificate analysis and the gluten content is given as 10 ppm (according to the International Food Standard Gluten Content should be less than 20 ppm).

2.1.2. Gluten-free bread production

In gluten-free bread production, for each formulation 70% of the water (Table 1) was put into the kneading bowl of the mixer (KitchenAid, USA), and after the salt and sugar were dissolved in water, compressed yeast was added and mixed for 30 seconds. Subsequently, the other solid components were taken into the kneading bowl and the remain of the water was added, and the mixing process was continued for 5 minutes after obtaining a homogeneous mixture in the 3rd cycle. Doughs prepared and poured into 165 g molds (inner part; 14.3x7.9 cm, bottom part; 12.9x6.4 cm and inner depth; 5.7 cm) were fermented for 70 minutes at approximately 30°C in the fermentation cabinet

set. Afterwards, the doughs were baked in the oven at 210°C for 30 minutes. After the breads were cooled on wire racks at 25°C, they were stored in polyethylene bags until analysis were performed. Analysis were made 6 hours after baking (Tumer, 2018).

Table 1. Main ingredients and production formulations of gluten-free bread

Ingredients (%)	Formulations											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Konjac	2.0	0	0	0	0	0	0	0	0	0	0	0
	0	4.0	0	0	0	0	0	0	0	0	0	0
	0	0	6.0	0	0	0	0	0	0	0	0	0
XG	0	0	0	2.0	0	0	0	0	0	0	0	0
	0	0	0	0	4.0	0	0	0	0	0	0	0
	0	0	0	0	0	6.0	0	0	0	0	0	0
HPMC	0	0	0	0	0	0	2.0	0	0	0	0	0
	0	0	0	0	0	0	0	4.0	0	0	0	0
	0	0	0	0	0	0	0	0	6.0	0	0	0
CMC	0	0	0	0	0	0	0	0	0	2.0	0	0
	0	0	0	0	0	0	0	0	0	0	4.0	0
	0	0	0	0	0	0	0	0	0	0	0	6.0
Corn Starch	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Gluten-free wheat starch	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Sugar	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Sunflower Oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
SSL	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Compressed Yeast	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Powder Milk	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Water	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

2.2. Methods
2.2.1. Moisture Analysis

The moisture content of gluten-free breads was calculated gravimetrically. The samples were kept in an oven at 130 °C for 5 hours to calculate their % moisture values (Ozturk and Mert, 2018). Six replications were carried out for moisture content analysis.

2.2.2. Specific Volume

The weights of the gluten-free breads were determined and the volume was measured on the basis of displacement with rapeseed. The average of these obtained values was taken and the volume was divided by the weight, and the specific volume used to determine the quality of the breads was obtained (Zorzi et al., 2020). Six replications were carried out for the analysis.

2.2.3. Color Analysis

Gluten-free bread color intensity measurements were determined by Konica Minolta CM-5 (Japan) calorimeter. This instrument makes the three-dimensional color measurement, L* (lightness) on the Y axis; 0=black to 100=white, for example light-darkness, a on the X-axis; green (-a), red (+a), b on the Z axis; yellow (+b), blue (-b) indicate color size or location (Ozkoc and Seyhun 2015). Six replications were carried out for the analysis.

2.2.4. Texture Analysis

The textural properties of the gluten-free breads were determined using the TA.XT-plus Texture Analyzer (Stable Micro Systems, UK). Two slices of 12.5 mm thick bread that did not contain the crust were analyzed by stacking them on top of each other. The slices were pressed down at a speed of 1 mm/s using a 50 mm cylindrical probe and pressed until 50% deformation was applied to the breads, and the load was kept on the breads for 30 seconds. Hardness, chewiness, cohesiveness and springiness values were measured (Kiumarsi et

al., 2019). This analysis was made in six replications.

2.2.5. Sensory Analysis

In sensory evaluation trials, breads were presented to panelists whole and sliced (thickness 15 mm), coded with three-digit random numbers. Panelists, consisting of 13 faculty members from Çukurova University's Department of Food Engineering, evaluated crust color, inner color, taste and odor, texture, chewiness and general acceptability parameters for gluten-free breads. Before the sensory panel, panelists were given training on the criteria they were asked to evaluate. A hedonic scale from 1 (very poor) to 5 (very good) was used for sensory analysis (Mudgil et al., 2016).

2.5. Statistical analysis

The data obtained in the study were evaluated using the SPSS 20 (SPSS Inc., USA) statistical program, ANOVA analysis of variance ($p < 0.05$). Significant differences were determined using the Duncan multiple comparison test.

3.Results and discussions
3.1. Gluten-free Bread Analyses
3.1.1. Specific Volume Analysis

The specific volume values of the gluten-free bread samples produced with different hydrocolloid rates are given in Table 2. According to the analysis results, the specific volume values vary between 2.42 cm3 /g and 5.54 cm3 /g. Whilst the highest specific volume was obtained from gluten-free breads with 6% konjac gum (F3), the lowest value was determined in 2% CMC (F10). In our research, it was determined that the specific volume of gluten-free bread samples increased statistically with the addition of hydrocolloid (CG, X, HPCM and CMC) at different rates (2, 4, 6%) ($p < 0.05$). The reason for this overall increase may be related to the structure of the hydrocolloids of high capasities on water

holding, retaining moisture and stabilizing. Andresson et al. (2011) investigated that the effect of different hydrocolloids in the production of corn starch (100%) based gluten-free bread, in the research, HPMC, MC and guar gum were used at the rates of 2-2.5-3 % and they stated that 2% HPMC had the best gluten-free bread properties. Lazaridou et al. (2007) added XG and CMC at different rates (1% and 2%) in gluten-free bread formulation, the researchers emphasized that while no change was observed in the volume of breads with XG addition at the rate of 1%, there was an increase in the bread volume due to the increase in the hydrocolloid ratio. Chakraborty et al. (2020) reported that the effect of XG was the highest compared to other hydrocolloids in their study, in which they examined the effect of adding 2%-5% guar, XG, CMC, HPMC to corn flour-based gluten-free bread formulations on bread volume ($p < 0.05$). In the literature, it has been determined that the effects of different hydrocolloids sources on gluten-free bread quality have been investigated. But; among these sources, studies on the effects of konjac gum on bread quality are limited. In our study, it was determined that konjac gum increased the specific volume of gluten-free breads compared to other hydrocolloids (X, HPMC, CMC) ($p < 0.05$).

Laignier et al. (2021) reported in their research that konjac gum used in gluten-free product formulation improves the expansion ability of dough and provides volume increase by keeping the CO_2 (g) in its structure during baking. Konjac gum provided the stability to the cells against various processing conditions and gas expansion by both creating an interface at the boundaries of the gas cells and increasing the specific volume of breads (Sutrisno et al. 2021). Due to its techno-functional properties, konjac gum plays an important role in increasing the specific volume of gluten-free bread.

Table 2. Specific volume, moisture and texture properties of gluten-free breads

Sample No	Specific Volume	Moisture	Hardness	Chewiness	Elasticity	Cohesiveness
F1	3.26±2.22[f]	46.12±0.83[b,c]	963.47±2.67[d]	1208.21±3.33[d]	0.99±0.01[d,e]	0.72±0.01[b]
F2	4.60±5.03[b]	47.38±0.09[b]	709.09±9.73[e]	759.28±1.34[f]	0.99±0.00[d,e]	0.72±0.00[b]
F3	5.54±4.66[a]	47.52±0.78[b]	570.85±9.14[f]	752.25±16.22[f]	1.02±0.02[c,d]	0.73±0.01[a]
F4	4.09±2.23[e]	43.91±0.72[d]	542.05±7.92[f]	957.31±2.82[e]	0.99±0.01[d,e]	0.72±0.01[b]
F5	4.27±1.19[d]	44.87±0.93[c,d]	1066.94±29.04[c]	937.97±12.11[e]	1.01±0.01[c,d]	0.74±0.00[a]
F6	4.42±2.20[c]	46.89±1.38[b]	1447.60±34.92[b]	759.65±4.85[f]	1.06±0.02[c]	0.74±0.03[a]
F7	2.99±3.47[h]	46.15±1.27[b,c]	957.03±5.12[d]	2147.36±58.14[b]	0.96±0.00[e]	0.73±0.00[a]
F8	3.18±1.34[g]	47.99±1.18[b]	1174.54±11.75[c]	1237.48±30.73[d]	0.99±0.00[d,e]	0.74±0.05[a]
F9	3.21±1.84[g]	49.62±0.79[a]	1497.39±55.24[b]	723.34±28.63[f]	0.99±0.00[d,e]	0.74±0.03[a]
F10	2.42±3.30[j]	44.36±1.31[d]	1052.06±3.92[c]	1251.09±99.80[d]	1.03±0.06[c,d]	0.72±0.01[b]
F11	2.71±2.43[i]	46.62±0.86[b]	1457.57±63.29[b]	1516.37±37.32[c]	1.26±0.00[b]	0.72±0.00[b]
F12	2.97±2.67[h]	46.69±0.15[b]	2569.02±3.29[a]	2678.5±4.73[a]	2.14±0.04[a]	0.73±0.01[a]

The differences between the values indicated with same letter in the same column in the table are significant in accordance with the 0.05 confidence limit.

3.1.2. Moisture Analysis

As seen from Table 2, the moisture content in gluten-free breads varied between 43.91% and 49.62%. In Table 2, it was determined that the gluten-free breads with the lowest and the highest moisture content were 2% X (F4) and 6% HPMC (F9), respectively. In our research, it was determined that the moisture content of the samples increased depending on the increase in the hydrocolloid concentration, regardless of the source of the hydrocolloids. The increase in moisture content is thought to be related to the constant dough consistency and the water holding capacity of hydrocolloids (Rosell et al., 2001; Guarda et al., 2004). Mohammadi et al. (2014) reported that the use of hydrocolloids in the gluten-free bread formulations due to their high water holding capacity causes an increase in the amount of moisture in the bread. Gambus et al (2007) reported that the addition of XG at increasing concentrations to corn starch-potato starch based gluten-free breads significantly increased the moisture content of the samples compared to the control group (p<0.05). This was explained by the fact that XG was able to stabilize starch gels, thus reducing starch retrogradation and maintaining the moisture content of the samples (Brennan et al., 2004). Calle et al (2020) added HPMC, XG and guar gum into the gluten-free product formulation. The moisture value of the samples was determined as 58.74% in the application of HPMC use only and 58.80% in the equal use of

HPMC, XG and guar gum, and the results were found to be statistically significant (p<0.05). Maghaydah et al (2013) investigated the effect of using equal proportions of XG, pectin and carrageenan on the moisture content of rice and corn flour added gluten-free breads and the researchers found the moisture values of the samples as 1% XG-1% carrageenan 43.09%, 1% XG-1% pectin 43.31% and 1% carrageenan-1% pectin 40.66% and reported that they found a significant difference compared to the control group (p<0.05) so, this difference has been attributed to the hydrophilic nature of the hydrocolloids. As seen above; our research is in line with the other corresponding researches and the use of hydrocolloid caused an increase in the moisture content of the gluten-free breads.

3.1.3. Texture Analysis

When the texture analysis results of gluten-free breads were examined, it was seen that the lowest hardness value was determined in the bread groups with 6% konjac gum (F3) addition, while the highest hardness value was determined in the bread groups with 6% CMC addition (F12). When the samples were examined among themselves, it was determined that the hardness values increased with the increase in the hydrocolloid concentration (Table 2). Naji-Tabasi and Mohebbi (2015) investigated the effect of using hydrocolloid in gluten-free formulation in terms of bread quality and they reported that XG reduces the hardness value of breads and delays the staling during storage due

to its improved moisture retention ability. It has been reported by researchers that the addition of hydrocolloid into the gluten-free bread formulation reduces the hardness of the bread by increasing the amount of water in the dough and consequently the moisture content of the bread due to the high water holding capacity of hydrocolloids (Zhao et al., 2021). According to Renzetti and Rosell (2016), HPCM and XG are frequently used as gluten substitutes in different formulations in gluten-free bread formulation. During cooking, these water-soluble polymers with high surface activity maintain homogeneity and stability and do not cause any negative effects on the textural parameters of the final product. In general, they improve the quality of gluten-free breads and contribute to the development of products with high specific volume and low hardness (Mccarthy et al., 2005; Djordjevic et al., 2018). As for the chewiness parameter, it was determined that the chewiness value decreased statistically as the hydrocolloid usage rate increased in the gluten-free bread groups except for CMC (2%-6%) ($p < 0.05$). Liu et al. (2018) examined the effects of 0.5%, 1%, 2% usage rates of CMC, XG and HPMC on the chewiness of gluten-free breads and stated that the addition of XG reduced the chewiness, while HPMC did not affect it and the use of CMC increased the chewiness value. Similarly, Patil and Arya (2019) reported that the use of increasing concentrations of hydrocolloid (XG, guar gum) reduces the chewiness of gluten-free breads and this is due to the ionic interactions detected between proteins and hydrocolloids that provide the formation of hydrophilic complexes (Rosell et al., 2007). The elasticity data of the samples varied in the range of 0.96 N-2.14 N (Table 2), it was determined that the elasticity values increased with increasing usage rates regardless of the type of hydrocolloids ($p < 0.05$). Similarly, Mohammadi (2014), Encina- Zelada (2018) and Bravo-Nunez (2019) reported that different types of hydrocolloids used as gluten substitutes increase the elasticity of breads. As seen in Table 2, it was determined that the cohesiveness values of the gluten-free breads varied between 0.72 and 0.73 and the

cohesiveness increased significantly as the hydrocolloid concentration in the product formulation increased ($p < 0.05$). Belorio and Gomez (2020) added 2% HPMC, XG and psyllium to corn starch-based gluten-free breads and determined the cohesiveness values as 1.011, 0.964 and 0.974, respectively. While no significant difference was observed between XG and psyllium added breads, a difference was observed in HPMC added breads ($p > 0.05$). This was associated with the significant effect of hydrocolloids on the cohesiveness of starches. In addition, the change in HPMC gels after firing was also revealed as the reason for the high cohesion. Bravo-Nunez (2019) determined the cohesiveness values of 2% HPMC-added gluten-free breads on different storage days (1st and 5th), respectively, as 0.47 and 0.66, and reported a significant difference between the values. ($p < 0.05$). The cohesion values determined in the literature are within the ranges determined in our research.

3.1.4. Color Analysis

The L* parameter, which expresses the lightness of the crust values of the breads, varies between 83.78-58.99 (Table 3). When the crust color values of gluten-free breads were examined, an increase was observed in the lightness values of the samples with konjac gum, HPMC and CMC added with the increase in the use of hydrocolloid. This increase in L* value was associated with the reduction of Maillard reaction rate by affecting the water distribution of the hydrocolloid added into the product formulation (Torbica et al., 2010). Sciarini et al. (2010) investigated the effects of XG and guar gum on gluten-free breads produced using rice flour and corn starch and it has been observed that the use of both gums separately and in combination caused the darkest the color of the bread crust. Marco et al. (2008) examined the effect of HPMC which is used at different rates as a water retainer in rice flour-based gluten-free breads and it was determined that the breads using 4% had the highest brightness value on the crust. Belorio and Gomez (2020) reported that the addition of 2% HPMC into gluten-free bread formulation caused a reduction in L* value and

it was reported by the researchers that the detected decrease was caused by the Maillard reaction and sugar caramelization. In our research, crust a* value of breads varied between 0.21 (2% HPMC) - 8.81 (6% Konjac) and b* value ranged from 14.90 (4% XG) to 32.85 (4% CMC). The increases detected are supported by previous researches that are associated with the source, type, and increased concentration of the hydrocolloids used in the product composition (Gonzales et al., 2010). Kringel et al (2017) determined the a* value of the samples with 2% methylcellulose added to the rice flour-based samples in gluten-free bread production as 4.02. Similarly, when Mancebo et al. (2017) added 2% HPMC to the bread formulation, they observed a significant increase in a* value compared to the control group and reported that the crust color darkened. Martinez and Gomez (2017) found the crust b* value to be 19.32 as a result of the addition of HPMC as a hydrocolloid at a usage rate of 2.2% in corn starch-based gluten-free bread production, the crust b* value of 26.08 as a result of the addition of 2% CMC to rice flour-based gluten-free breads, Kringle et al. (2017) and Paciulli et al. (2016) investigated the effects of 2% HPMC and 2% guar gum added on the color values of corn starch-based gluten-free breads and they reported that the addition of hydrocolloids increased the b* value (p<0.05).

Table 3. Instrumental color properties of gluten-free bread (n=3)

Sample Number	Hunter Color Values		
	L*	a*	b*
F1	58.99±0.15[i]	2.17±0.31[g]	18.65±0.84[c,d]
F2	74.61±0.69[e]	3.50±1.12[b,c]	29.68±1.49[a,b]
F3	83.75±0.54[a]	8.81±0.23[a]	25.41±2.78[a,b,c]
F4	76.92±0.56[d]	1.40±0.35[f,g]	22.67±3.04[b,c,d]
F5	70.54±0.56[f]	2.65±0.40[c]	14.90±3.69[d]
F6	62.97±0.51[h]	4.53±2.28[b]	31.21±0.55[a,b]
F7	81.40±0.74[b,c]	0.21±0.09[e,f]	16.45±0.28[d]
F8	81.76±0.19[c]	0.65±0.91[d]	19.62±12.6[c,d]
F9	82.44±0.35[b]	1.54±0.56[f,g]	25.15±0.65[a,b,c]
F10	68.60±0.73[g]	2.98±0.18[c]	28.38±1.64[a,b]
F11	68.99±0.80[g]	3.60±0.18[b,c]	32.85±0.38[a]
F12	70.58±0.35[f]	3.20±0.17[b,c]	31.04±0.66[a,b]

The differences between the values indicated with same letter in the same column in the table are significant in accordance with the 0.05 confidence limit.

3.1.5. Sensory Analysis

Sensory evaluation scores of the effects of different hydrocolloids in different ratios on gluten-free bread quality are given in Table 4. Appearance plays an important role in consumers' preference for the bakery products. In our research, an increase in crust color was observed due to the increase in hydrocolloid concentrations. When the sensory analysis data of hydrocolloids (Konjac gum, XG, HPMC and CMC) were compared among themselves, a statistical difference was observed (p<0.05). It has been observed that the breads with the addition of Konjac gum are mostly liked by the consumers in terms of crust color. When bread crumb color, taste and odor parameters were examined, similar results were obtained with crust color. When evaluated in terms of general acceptability, konjac gum added gluten-free breads received the highest score by the panelists, as in other parameters. The use of Konjac gum in bakery products improves the quality characteristics of the final product by improving its sensory and physicochemical properties (Sutrisno et al., 2021).

Table 4. Sensory properties of gluten-free breads

Sample Number	Sensory Properties					
	Crust Color	Crumb Color	Taste	Odor	Chewiness	Overall Acceptability
F1	3.48±0.36[a]	3.22±0.01[b]	3.21±0.01[c,d]	3.36±0.01[a]	3.22±0.01[a]	4.03±0.01[a]
F2	3.50±0.1[a]	3.27±0.01[b]	3.22±0.01[b]	3.39±0.01[a]	3.27±0.01[a]	4.04±0.01[a]
F3	3.50±0.1[a]	3.37±0.01[b]	3.31±0.01[a]	3.46±0.01[a]	3.40±0.01[a]	4.11±0.01[a]
F4	3.11±0.01[b]	3.11±0.01[b]	3.16±0.01[d]	3.30±0.00[a]	3.11±0.01[a,b]	3.79±0.00[a]
F5	3.13±0.02[b]	3.13±0.01[b]	3.18±00[c,d]	3.33±0.00[a]	3.13±0.01[a,b]	3.79±0.03[a]
F6	3.15±0.04[b]	3.17±00[b]	3.20±0.01[c,d]	3.41±0.01[a]	3.17±0.01[a,b]	3.82±0.02[a]
F7	2.98±0.01[c]	2.54±00[b]	2.32±0.02[f]	2.65±0.01[b]	2.54±0.00[c]	2.17±0.01[b]
F8	2.99±0.01[c]	2.42±0.02[b]	2.10±0.01[h]	2.39±0.01[b]	2.42±0.02[c]	2.10±0.01[b]
F9	2.99±0.01[c]	2.28±00[b]	2.08±0.01[h]	2.10±0.01[b]	2.28±0.00[c]	2.04±00[b]
F10	2.80±0.01[c,d]	3.01±0.01[b]	2.65±0.09[e]	3.01±0.01[a]	3.01±0.01[c]	3.61±0.01[a]
F11	2.82±0.02[c,d]	3.04±00[b]	2.31±0.01[f]	3.02±0.01[a]	3.04±0.00[c]	3.66±0.00[a]
F12	2.81±0.01[c,d]	3.07±0.01[b]	2.16±0.01[g]	3.04±0.01[a]	3.07±0.01[c]	3.85±0.00[a]

The differences between the values indicated with same letter in the same column in the table are significant in accordance with the 0.05 confidence limit.

4. Conclusions

Hydrocolloids are an indispensable part of the gluten-free product industry. Especially in the bakery industry, the interest in these ingredients is constantly increasing due to their properties such as increasing the volume of the products, improving the texture and delaying staling and increasing the shelf life in gluten-free product formulations to which they are added. In this research, the effects of adding different hydrocolloids (Konjac gum, XG, HPMC, and CMC) at different rates (2, 4, 6%) into the combination of corn starch (70%) and gluten-free wheat starch (30%) on gluten-free bread quality were investigated. When the analyzes were examined, it was determined that konjac gum was more effective in increasing the moisture due to increasing concentrations and improving the textural properties (especially hardness) of the samples due to gluten deprivation. When gluten-free breads were evaluated in terms of sensory, it was determined that the group that was most liked by the panelists and provided product development was F3 (Konjac gum 6%). When evaluated in general, it has been determined that konjac gum is the best hydrocolloid for developing gluten-free breads and can be used for the production of high quality products that can be accepted by the consumers. Konjac gum has been used in the food industry of many countries in the recent years due to its high level of glucomannan content and the wide ability to cultivate the plant

from which it is obtained. However, when the literature is examined, it has been determined in our research that the use of konjac gum as a hydrocolloid in gluten-free bread composition is limited. In this context, it is thought that our study is pioneering and that konjac gum can be used as a different hydrocolloid alternative in many gluten-free products..

5.References

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EFFECT OF PACKAGING MATERIALS AND STORAGE TIME OF CONCENTRATE ORANGE JUICE ON CHEMICAL PROPERTIE AND ANTIOXIDANT ACTIVITY

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ABSTRACT

A study was conducted to determine the effects of packaging materials, and storage time on physicochemical and antioxidant properties of commercial concentrate orange juice largely consumed in Algeria (Ramy). The juice was packaged in different packaging materials (glass, cardboard and plastic bottles) and stored for three months at room temperature. Total soluble solids, pH, titratable acidity, vitamin C, and antioxidant activity (DPPH method) were evaluated in freshly juice, and after 1,2, and 3 months of storage. The results showed that total soluble solids, pH, vitamin C and antioxidant activity decreased with storage time under different storage time, irrespective of packaging materials. On the contrary, the acidity increased during storage. The juice in glass bottles did not show significant changes until the end of storage. It was conducted that glass bottles packing proved to be most suitable for orange juice storage, which maintained the better quality and antioxidant properties loss than rest of the packing materials.

1.Introduction

Orange (Citrus sinensis L) of the family Rutaceae is considered the most popular fruit. It is also well known as a rich source of antioxidants including ascorbic acid (vitamin C). A large portion of the citrus fruit produced worldwide is used in processed juices and other beverages, from among which orange juice is the most esteemed (Klimczak et al, 2007). Citrus fruits are known to be a source of bioactive molecules such as ascorbic acid, carotenoids, flavonoids, and phenolic compounds that all of them were found to be health promoting (Abeysinghe et al, 2007 ; Ghasemi et al, 2009 ; Manthey and Grohmann, 2001). In addition, some authors previously recognised that phenolic compounds and ascorbic acid were the key constituents of orange juices responsible for their antioxidant activity (Rekha et al, 2012). Packaging is an important aspect in the food processing industry as it serves the important fonctions of containing the food protecting against chemical and physical damage while providing information on product features, nutritional status and ingredient information (Anin et al, 2010). Various packaging materials such as high-density polyethylene, polypropylene, and glass are commonly used for packaging of fruits juices (Marsh and Bugusu, 2007). Different packaging materials influence the quality of the stored products differently. Therefore, the study of the effect of packaging materials on the quality parameters during storage is essential. In this study, orange juice was stored in plastic, cardboard and glass bottles at ambient temperatures. The aim of this study was to determine effects of packaging materials and storage time on chemical changes and antioxidant properties of orange juice.

2. Material and methods
2.1. Samples, packaging and storage condition

Three different materials packaging of commercial orange juice made from concentrate largely consumed in Algeria (Ramy) were used in the current study. The effect of packaging materials on the chemical and antioxidant properties of orange juice at different storage intervals was studied. The orange juice was packed in three different materials (glass, cardboard, and plastic bottles), chemical characteristics and antioxidant properties were monitored at 1, 2 and 3 months after storage. The fresh orange juice was bought from the local market and brought to the laboratory. The packed juices in different packaging were stored under room temperature. The experiment was replicated in triplicate for each treatment to adjust any uneven variation for chemical properties. After every one month, the samples from each packaging were collected for chemical analysis. The data were recorded for the juice quality characteristics including pH value, TSS (Brix), TA, ascorbic acid content, and antioxidant activity.

2.2. Total soluble solids (TSS), pH and titratable acidity (TA)

Total soluble solid in (Brix) was measured using a refractometer calibrated with distilled water at 20°C. The pH was carried at room temperature with a pH metre. Titratable acidity (TA) was determined by titrating 10 ml of the juice mixed with 3 drops of phenophtalein indicator against 0.1 N NaOH until the end-point at pH 8.2. The results were converted to citric acid and expressed as g/l of citric acid. All measurements were done is triplicate.

2.3. Ascorbic acid content

Ascorbic acid concentration was measured according to Klein and Perry, 1982. Orange juice (1 ml) was mixed with 10 ml of 1% metaphosphoric acid and then sonicated in an ice bath for 4 min. The samples were then centrifuged at 4000 x g for 5 min. Supernatants (10 ml) were pipetted into a tube and mixed with 9 ml of 2,6 dichloropheno lindophenol. The mixture was incubated in the dark for 10 min and the absorbance was measured at 515 nm using spectrophotometer. Results were expressed as mass of ascorbic acid equivalents per volume of orange juice, µg/ml.

2.4. Radical scavenging activity (DPPH assay)

The radical scavenging activities of the orange juice against 2,2- diphenyl-1-picrylhydrazyl radical were determined by UV-spectrophotometer at 517 nm by a slight modified method described by Brand-Wiliam et al, 1995. 1.95 ml of 0.1 mM DPPH of methanolic solution was added into 50 µl of the orange juice. The mixture was thoroughly mixed and kept in a dark place for 30 mn. The DPPH radical scavenging activity was calculated as follows : DPPH radical scavenging activity % = 100 (1- AS/AC), where AC is the absorbance of the DPPH radical without any antioxidant as control. AS is the absorbance reading of DPPH added to sample at 517 nm. Methanol was used as a blank. The antioxidant capacity of each sample was expressed as the amount of sample necessary to inhibit the initial DPPH.

2.5. Statistical analysis

Analyses were conducted in triplicate means and standard deviations were calculated by the Excel software (2007 version). One way ANOVA was applied to the different storing date using SPSS version 15 windows. Tukey's method was applied for comparisons of means; while differences were considered significant at $p < 0.05$.

3. Results and discussions
3.1. TSS content analysis

Important conditions and function of food packaging materials are that they should meet the aim of containing the food protect against chemicals, physical damage, provide information on product features, nutritional status and ingredient information (Anin et *al*, 2910). The concentrate orange juice did not

show a statitical change TSS content (p< 0.05) with all packaging materials, while the TSS content was markedly small difference in the juice when packed in cardboard bottles and plastic bottles. The range of TSS content from the first to end storage were (12 to 10.72), (12 to 10.07) and (12 to 9.91) Brix in the glass, plastic and cardboard respectively.. On the other hand, the TSS decreased with prolonging the storage period (fig 1). This indicates that

the glass bottles were the most suitable packing for fruits juice under room temperature of storage. Similar results on the effect of packing materials on the total soluble solids have been reported in previous studies (Janse, 1994) investigated the physicochemical properties of orange juice and found changes in TSS due to different packing materials and storage conditions as well as storage duration..
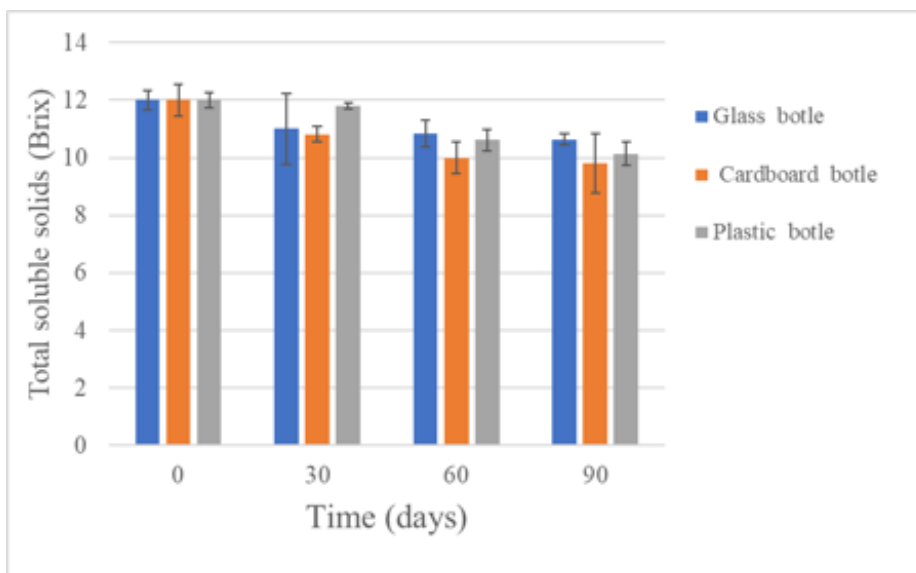

Figure 1. Effect of packaging materials and storage time on TSS of concentrate orange juice.

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3.2.pH / Titratable acidity analysis

Measurement of pH is an index for determining food quality especially during storage. The pH for concentrate orange juice in all packaging materials showed no significant decrease (p< 0.05) at the beginning of the storage (Fig.2). The pH for juice stored in glass and cardboard bottles ranged from 3.82 to 3.72 at room temperature, while in plastic packing, the pH ranged from 3.85 to 3.64. Similar decrease in pH with storage period were reported by (Muhammad et al, 2011) in apple pulp, (Wisal et *al*, 2013) in strawbery juice and (Durani et *al*, 2010) in apple pulp. The packaging materials and storage time has no significant effect on the titratable acidity of orange juice (fig 3). In this study, the TA value varied from 9.99 to 10.75 g/l and 9.79 to 10.77 in the glass and cardboard bottles packing respectively and 9.02 to 11.71

g/l under plastic bottles packing after three months of storage at ambient temperature. Similar results were also reported by Goyal and Srinivasan (Wisal et *al*, 2013). Increased acidity might be the production of organic acids, which can lead to reduction in pH and total soluble solids, and an increase in titratable acidity (Rivas et *al*, 2006). Acidity is a very important chemical predicate for conservation of fresh produce and food products by consumers as well as for the food industry, because it makes the food more resistant to deterioration by microorganisms and allows more flexibility in the addition of sugar, which is of particular importance in preparing ready-to drink beverages (Dell'ort Morgado et *al*, 2010). Decrease in pH value and increase in total titratable acidity during the storage period of 90 days may also be due to activity of some acid producing bacteria such as

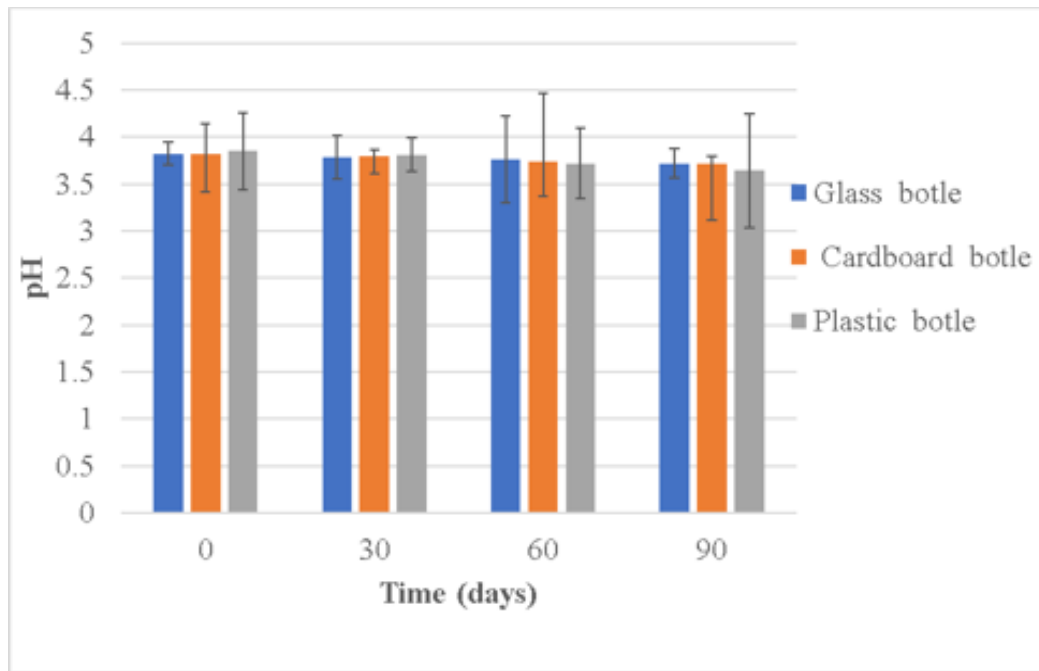*Alicyclobacillus acidoterrestris* as suggested by (Hussain, et *al*, 2011).

Figure 2. Effect of packaging materials and storage time on pH of concentrate orange juice.
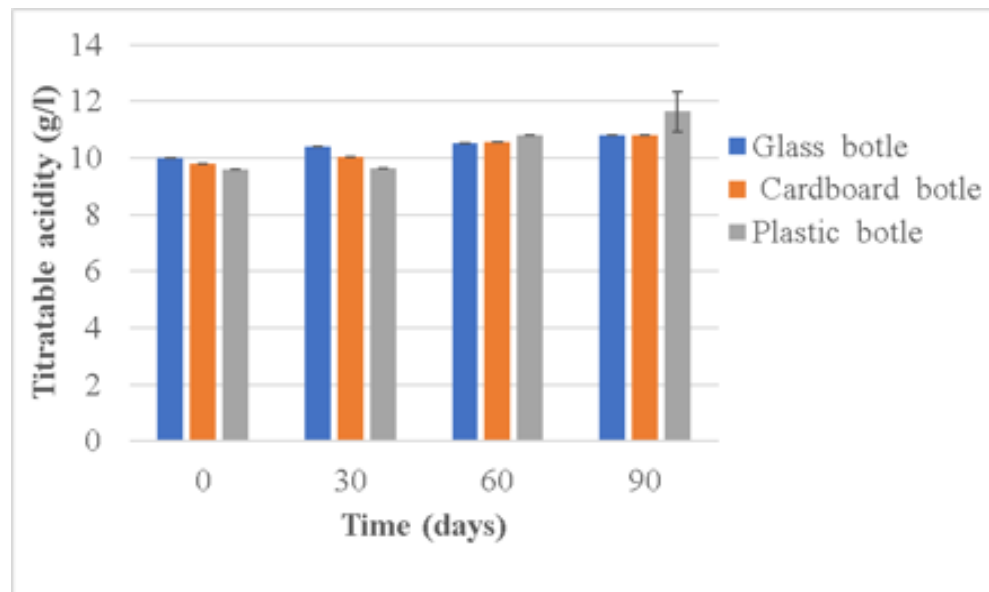

Figure 3. Effect of packaging materials and storage time on titratable acidity of concentrate orange juice.

3.3.Ascorbic acid analysis

Orange juice is a rich source of ascorbic acid, which is an important antioxidant (Rapisarda et al, 1999) and its concentration is also a significant indicator of orange juice quality. Modification in ascorbic acid could be a good indicator for enzymatic or non enzymatic degradative reactions taking place during processing or storage of the fruit (Skrede, 1996). According to (Fig.4), a significant decrease ($P < 0.05$) is observed in ascorbic acid content of all the experimental

packages during storage at ambient temperature after 90 days compared to day 1, and its content was within a range of 0.687 to 0.432 and 0.702 to 0.418 and 0.708 to 0.323 g/l ascorbic acid with cardboard, glass and plastic bottles respectively. Vitamin C content of the concentrate orange juice decreased significantly ($P < 0.05$) with increased storage period. The juice stored at cardboard packaging showed lower losses of vitamin C (37 %), while the values for its degradation in glass and plastic packaging were (40 and 54 %) respectively. while plastic packaging have a lower barrier to oxygen, causing a loss of some ascorbic acid for oxidation, because vitamin C

can easily be oxidized in the presence of oxygen by both enzymatic and non enzymatic catalyst (Jawaheer et al, 2003). Tamuno and Onyedikachi, 2015 reported that polyethylene packaging was not as effective in preserving vitamin C as the bottles. Also, Similar to the effect observed in the work, Alaka et al, 2003 and Berlinet et al, 2003 reported that the ascorbic acid decreased in different packaging materials..

This indicates that polyethylene (S) packaging material was not as effective in preserving vitamin C as the bottles. This is because light might have penetrated it causing vitamin C to leach out.
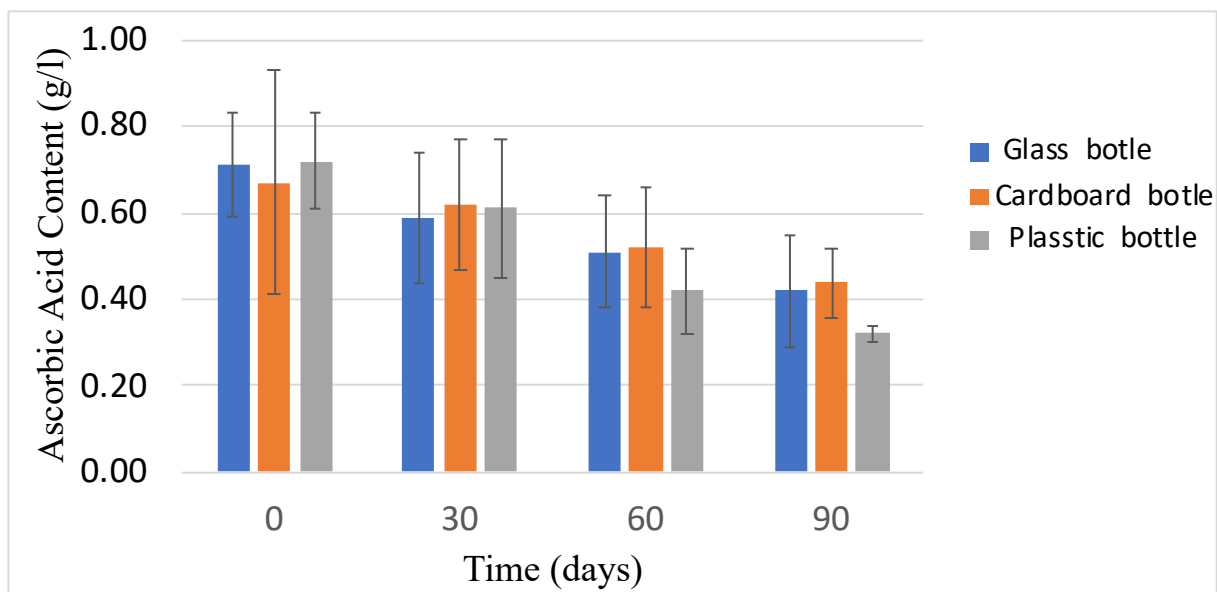

Figure 4. Effect of packaging materials and storage time on ascorbic acid content of concentrate orange juice.

3.4. Antioxidant activity analysis

Several studies reported a high correlation between phenolic content and antioxidant activity, however, other authors suggest that ascorbic acid is a powerful antioxidant in fruits and can give to the antioxidant potential of juices (Kuskoski et al, 2005 ; Reddy et al, 2010).The antioxidant activity of concentrate orange juice was evaluated using DPPH free

radical scavenging and its shown in (Fig. 5). The values of antioxidant activity showed a reduction during juice stored in different packaging materials after 90 days at ambient temperature. The initial radical scavenging capacity was over 76 % for all the packaging materials, The reading of over 76 % remained after two months of storage for glass and cardboard and one month for plastic packaging.
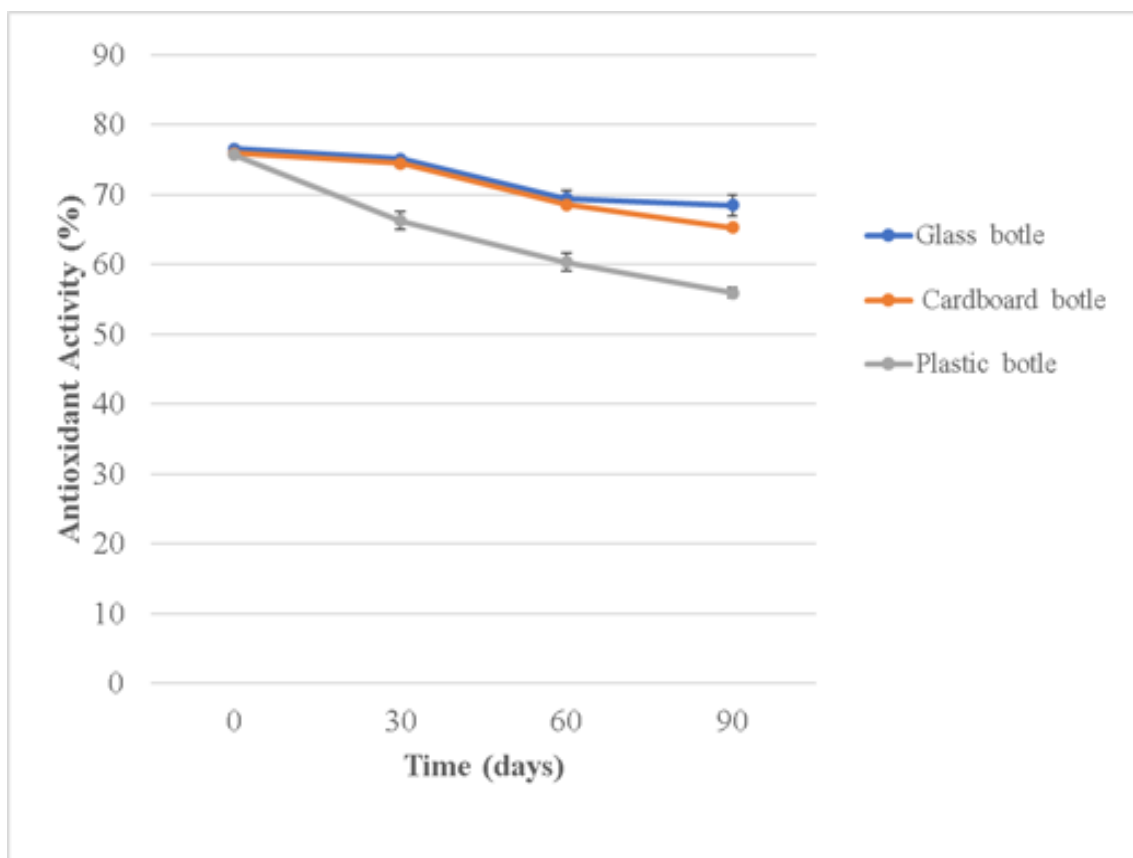
Figure 5. Effect of packaging materials and storage time on antioxidant activity of concentrate orange juice.

After one month, antioxidant activity of juice in plastic packaging decreased significantly to compared to the initial and one month. The decrease values of antioxidant activity content were observed up to 90 days of juice stored in three packaging materials ranged from 76.54 ± 5.1% to 68.42± 4.6% in glass bottles, 75.87±5.6 % to 65.22± 3.6 % in cardboard bottles and 75.68±5.3 % to 55.86±7.2 % in plastic bottles. For the first one month of shelf life, packaging materials did not affect the content of these bioactive responsible of antioxidant activity, whereas at three months, antioxidant capacity decreased significantly in juice with plastic bottle. The losses antioxidant activity value was lower at the end in juice stored in glass bottles packaging corresponding to 8.12 %. These results are in agreement with those reported in our previous study (Malecka et al, 2003). Walkowiak-Tomczak, 2007) has found that increase of oxygen, pH and temperature during storage reduced the antioxidant activity of the fruit concentrate. The result indicated that the decrease in the antioxidant activity may be linked to a decrease content of ascorbic acid in juice during storage. It is proved that the antioxidant activity was correlated to the concentration of ascorbic acid. Packaging in glass bottles and storage at ambient temperature should be encouraged as it efficient protect of vitamin C and antioxidant capacity of concentrate orange juice products. These results suggest that the juice sample studied should be consumed within the first month of storage on glass and cardboard packaging.

4. Conclusions

The concentrate orange juice presented some chemical changes during 3 months of storage in different packaging materials at ambient temperature. The most affected compounds were vitamin C and antioxidant

activity observed in plastic bottles. The juice stored at glass packaging showed lower remarkable losses of vitamin C and antioxidant as compared to plastic and cardboard packaging. It is concluded that ragardless the glass bottles packing proved to be most suitable for concentrate orange juice storage, which maintained the better juice quality and bioactive loss than rest of the packing materials.

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Acknowledgments

RESPONSE OF SECONDARY STRUCTURAL COMPONENTS OF EGG WHITE PROTEINS TO COLD AND THERMAL EXTREMITIES IN WATER/DEUTERIUM OXIDE MIXTURES

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ABSTRACT

Temperature and water influence proteins' stability and function. This study investigated the response of Amid I secondary structural components (SSC) of egg white proteins to cold (-80 °C) and thermal (100 °C) extremities in water and deuterium oxide (D_2O) mixtures by using FT-IR, DSC, and SEM analyses. Notably, D_2O enabled SSCs exhibit similar profiles at temperature extremities. Latent heat of melting (ΔH_m) raised by 9.5% at 100 °C, while it lowered by 106.8% at -80 °C. Heat capacity (C) increased by 0.9% and 42.2% at 100 and -80 °C, whereas melting temperature (T_m) decreased by 1.7% and 80.5% at 100 and -80 °C. SEM imaging showed flaky structures with different shapes, dimensions, and fissures. Statistical evaluation indicated that there was a strong positive correlation among SSC (*p=0.0001*), ΔH_m (*p=0.00008*), and C (*p=0.00001*) changes, except for T_m values (*p=.558182*). Overall, D_2O contributed to protein stability at 100 and -80 °C by controlling the unfolding process, possibly by an enthalpy-dependent mechanism. Therefore, it can be used as a reference solvent to establish kinetic models with/without enzymatic, physical, or chemical approaches for improved protein stability.

1. Introduction

The process optimization is based on scale-up rules and optimization paths assuming (near) equilibrium. However, the foods do not always rely on this assumption of (near) equilibrium (Burbidge and le Révérend, 2016). Adapting proteins to extreme physical conditions requires complicated and diverse intermolecular interaction alterations (Zhang et al., 2021). Many extrinsic and intrinsic stressors influence their stability, and to our knowledge, no current solutions have satisfied this requirement satisfactorily (Yousefi and Abbasi, 2022). Protein stability determines whether a protein stays in its native folded conformation or a denatured state. Its estimation plays an essential role in food design and the fate of food processing by opening the way to improved food products (Goldenzweig and Fleishman, 2018). Proteins' folded and unfolded states are related to protein stability, and α-helices and β-sheets are essential determinants of folded protein structure (Baronio and Barth, 2020). Protein unfolding caused by heating is referred to as "thermal denaturation," whereas it is so-called "cold denaturation" in the case of cooling. Protein goes from a naturally folded state to a random coil in an aqueous solution with rising

temperature. In contrast, cold denaturation is transitioning to a denatured state with decreasing temperature (Ballauff, 2022). Heat denaturation is typically an everyday experience because it can degrade many systems. However, lowering the temperature, "cold" generally slows down processes to eventually stabilize a system (Sanfelice and Temussi, 2016). Moderating proteins' stability or instability at temperature fluctuations remains speculative, at least to some extent (Weiss et al., 2018).

Water is a complex substance with various unusual properties due to its ability to form hydrogen bonds. Therefore, it plays an important role in governing proteins' structure, stability, dynamics, and function (Sen et al., 2009). Deuterium oxide (D_2O) is an isotopic form of regular water with relatively higher density (1.107 g/mL), melting (3.82 °C), and boiling (101.4 °C) temperatures. It forms hydrogen bonds stronger than in typical aqueous environments, resulting in stronger interactions among structural proteins and sticking them with one another together (Schnauß et al., 2021). Therefore, it can be used for isotopic labeling of salt micelles, oleosomes, carbohydrates, and deuterated alcoholic beverages and the stability of globular proteins, cells, and tissues (Pica and Graziano, 2017). In addition, several works investigated its impact on the peptization of some amino acids such as Phe, His, Pro, Cys, and Met (Fulczyk et al., 2019), and lipid oxidation of corn oil and linoleic acid (Oh et al., 2017; Lee et al., 2018). However, to our knowledge, no study has been conducted to examine its influence on the proteins of food origin. Adopting egg and egg-derived products to thermal extremities and higher water activity improves their stability during food design, processing, and storage. This study investigated the response of Amid I secondary structural components (SSC) (antiparallel β-sheet/aggregated strands, 3_{10} helice, α-helix, unordered, β-sheet, and aggregated strands)) of egg white proteins to cold (-80 °C) and thermal (100 °C) extremities in water and deuterium oxide (D_2O) mixtures by using FT-IR, DSC, and SEM analyses.

2. Materials and methods
2.1. Materials
D_2O (99.9 atom % D) was purchased from Sigma Aldrich (Catalogue no: 151882-250G, Darmstadt, Germany), and fresh hen eggs from a market in Ansbach, Germany.

2.2. Methods
2.2.1. Sample preparation
The fresh hen egg was broken, and its white (EW) was separated in a 50 mL glass beaker by removing its chalazae. Two sampling series (T and C) were prepared separately. Each series was comprised of seven glass vials (T0, T1, T2, T3, T4, T5, T6; and C0, C1, C2, C3, C4, C5, C6) for thermal (T) and cold (C) treatments. The vials T0 and C0 contained 2 mL of fresh EW only, and T1 and C1 included 2 mL of fresh EW and distillate water (dW). In comparison, the remaining vials of both series had 2 mL of fresh EW and different concentrations of D_2O ranging over 20%, 40%, 60%, 80%, and 100% (v/v) (Table 1). The vials were closed with a cap and vortexed for 2 min.

Table 1. Composition of EW blends with/without dW and D_2O

Sample	T0/C0	T1/C1	T2/C2	T3/C3	T4/C4	T5/C5	T6/C6
V_{EW} (mL)	2	2	2	2	2	2	2
V_{dW} (mL)	0	3	2.4	1.8	1.2	0.6	0
V_{D2O} (mL)	0	0	0.6	1.2	1.8	2.4	3
V_{Total} (mL)	2	5	5	5	5	5	5

2.2.2. Thermal (T) and cold (C) treatments

After overnight storage, the samples were kept in room condition. The T-series was heated up to 100 °C in a shaking-water bath (Julabo SW22, Seelbach, Germany) for 40 min, followed by storing at + 4 °C for a few min to stop the thermal denaturation process. Similarly, the C-series was cooled to -80 °C in a cryogenic freezer (Smart-Cryo SWLF, Aachen, Germany) for 40 min and kept in a water bath at room temperature few min to stop the cold denaturation process (Rossi and Schiraldi, 1992).

2.2.3. FT-IR and curve fitting analyses

After thermal and cold treatments, the samples were freeze-dried using Epsilon 1-4 LSC plus freeze-dryer (Martin Christ, Osterode am Harz, Germany). They were initially subjected to freezing at -40 °C. Subsequently, the shelf temperature was set at −40 °C, and drying ended at 20 °C under a vacuum of 0.150 mbar for 72 h. After freeze-drying (FD) is over, the samples were kept in a desiccator over phosphorpentoxid (P_2O_5) (Merck 1.00540.1000, Darmstadt, Germany) at room temperature for several days to completely dry (Zhao et al., 2020). After the FD, about 100-µg freeze-dried sample was subjected to FT-IR analysis (Thermo Scientific Nicolet iS50 FT-IR, Dreieich, Germany). All FT-IR spectra were recorded at room temperature between 4000 and 650 cm^{-1} after 32 scans with a spectral resolution of 4 cm^{-1}. The curve fitting for the Amide I band was conducted to quantitatively investigate the changes in secondary structural components. The second derivative spectrum determined the number of bands: 1675 to 1695 cm^{-1} for antiparallel β-sheet/aggregated strands, 1660 to 1670 cm^{-1} for 3_{10} helice, 1648 to 1660 cm^{-1} for α-helix, 1640 to 1648 cm^{-1} for β-sheet, and 1610 to 1628 cm^{-1} for aggregated strands. The relative amounts of secondary structural components based on the modeled peak areas were calculated according to the report generated by the Thermo Fisher Scientific OMICS software (Jackson and Mantsch, 1995; Kong and Yu, 2007).

2.2.4. Thermal (DSC) analysis

The samples were analyzed for determining the changes in the latent heat of melting (ΔH_m, J/g), melting temperature (T_m, °C), and heat capacity (C, J/gK) using Mettler Toledo Differential Scanning Calorimetry (DSC) (Greifensee, Switzerland). An amount of 5 to 10 mg of the treated sample was measured. The temperature range was selected as 20 °C to 100 °C for the T-series, whereas it was set as 20 °C to -80 °C for the C-series with a scan rate of 5 °C/min. The Mettler Toledo STARe 17 software was used for thermal analysis (Mettler Toledo, 2022).

2.2.5. Scanning electron microscopy (SEM) analysis

The microstructure of the samples was studied using Tescan Clara GMU SEM (Bruno, Czech Republic). To improve conductivity and image contrast, all the samples were initially subjected to surface treatment at 0.30 mbar/3 min for cleaning, etching, and activating the samples by Diener Tetra 30-LF-PC (Nagold, Germany). Subsequently, they were coated with a layer of Pt/Pd in an argon atmosphere (30 mA, 0.1 mbar, 30 s) to sputter conducting layers to prevent charging effects by Cressington 108 Auto Sputter Coater (Watford, UK). The acceleration voltage used in SEM was 5 keV, the beam current was 5×10^{-9} mA, and the working distance was 6 mm. The microstructure of the samples was viewed and photographed at a magnification of 2.23 kx (Liu et al., 2015).

2.2.6. Statistical evaluation

The strength of association between Amid I secondary structural components, ΔH_m, T_m, and C values of T- and C-treated samples were tested with the Pearson correlation coefficient method using SPSS statistical package program at *p<0.01*.

3.Results and discussions

This study evaluated the response of Amide I secondary structural components of fresh EW proteins to 100 °C and -80 °C in different concentrations of dW and D_2O by FT-IR, DSC, and SEM analyses. Overall, D_2O contributed to the protein stability of EW at 100 °C and -80 °C by indirectly controlling the unfolding process.

3.1. Results of FT-IR and curve fitting analyses

The curve fitting analysis for the Amide I band was investigated for quantitative estimation of antiparallel β-sheet/aggregated strands, 3_{10} helice, α-helix, unordered, and β-sheet, and aggregated strands in T (100℃) and C (-80℃) series (Table 2, Figure 1).

3.1.1. Response of fresh EW to temperature extremities

Abrosimova et al. (2016) found the frequencies of α-helix, β-sheet, and unordered strands in the raw EW to be 33%, 38%, and 12%, respectively, whereas they were 4%, 54%, and 6% in the boiled EW. Luo et al. (2022) reported that heating makes the protein structure disordered, i.e., a decrease in α-helix content increase in β-sheet content. In our study, the reference sample T0 contained only 2 ml of fresh EW. After the heat treatment, the major strand in T0 was α-helix (98.4%), followed by antiparallel β-sheet/aggregated strands (0.6%), 3_{10} helice (0.3%), β-sheet and aggregated strands (0.2%), and unordered (0.1%), respectively. Our SSC results of T0 were different from Abrosimova et al. (2016) and Luo et al. (2022). Freezing is generally used to maintain quality and extend the shelf life of foods. Lee et al. (2022) determined that freezing at -18 °C caused the denaturation of proteins, altered the secondary structure, and increase in β-sheet content and decrease in α-helix at -60.15 °C, in line with Hu and Xie (2021) and Li et al. (2021). However, Sun et al. (2016) and Hu et al. (2021) oppositely reported a decrease in β-sheet content. In our study, the major SSCs in the reference sample C0 including 2 ml of fresh EG only -80 °C were β−sheet/aggregated (24.5%) and unordered strands (16.9%), indicating the deterioration of some functional and sensorial qualities of EW before processing. Our SSC results of C0 matched with Hu and Xie (2021) and Li et al. (2021).

3.1.2. Response of fresh EW to temperature extremities in dW

Regular water can stabilize proteins, particularly globular ones, through hydrophilic and hydrophobic interactions. The interaction of proteins with water makes the FT-IR analysis of SSCs difficult. However, its evaporation may also destabilize the helical structure of a protein, i.e., an increase in β-sheet strands and α-helix decrease (Abrosimova et al., 2016). In addition, higher water activity (a_w) makes egg and egg-derived products perishable and highly susceptible to physicochemical changes (Kocetkovs et al., 2022). In our study, sample T1 included 2 ml of fresh EW in 3 ml of dW only. The common strand in T1 was the antiparallel β-sheet/aggregated (20.5%), followed by unordered (18.4%) and α-helix (17.1%) strands. Our sample C1 contained only 2 ml of fresh EW in 3 ml of dW. For C1, the α-helix (30.1%) was the dominant strand, followed by antiparallel β-sheet/aggregated strands (25.6%) and 3_{10} helice (15.7%) strands. Overall, dW could stabilize the EW proteins at -80℃ compared to the sample T1 at 100℃. Therefore, our findings provided data about the response of the SSCs of EW proteins to 100℃ and -80℃ in the solution of regular water (dW).

3.1.3. Response of fresh EW to temperature extremities in dW and D_2O

D_2O can stick proteins with one another together by forming hydrogen bonds strong-er than in solutions of regular water (Schnauß et al., 2021). It might weaken the strength of the van der Waals attractions between EW proteins and water molecules, leading to the protection of hydrogen bonds and electrostatic interactions responsible for the stability of proteins (Luo et al., 2022). An average equivalent of 7-25% D_2O can prevent protein denaturation in vaccines, and the presence of 95% D_2O is equivalent to a 4-5 °C reduction in storage temperature relative to regular water (Sen et al., 2009). The reference samples T2 to T5 and C2 to C5 contained 2 mL of fresh EW and different concentrations of D_2O (from 20%, 40%, 60%, and 80% in ascending order), and T6 and C6 included 100% of D_2O as the diluent. The primary strand in T2, T3, T4, and T5 samples was α-helix (29.4 ± 7.1%), followed by antiparallel β-sheet/aggregated (21.3 ± 3.7%). Similarly, the frequencies of the common SSCs in C2, C3, C4, and C5 were found to be α-helix (32.3 ± 1.7%) and antiparallel β-sheet/aggregated (24.5 ± 1.2%) strands, respectively.

Table 2. FT-IR spectra and estimated area changes of Amid I secondary structural components

Sample no	λ_{peak} (cm^{-1}) *	$\sum A_{peak}$ (cm^2)	Estimated area of strands (%)						
			Antiparallel β-sheet/ aggregated strands	3_{10} helice	α-helix	Unordered	β-sheet	Aggregated strands	Indefinite
T0 (Reference)	1629.15	1.01353	0.6	0.3	98.4	0.1	0.2	0.2	0.2
C0 (Reference)	1634.54	0.02249	24.5	15.2	14.7	16.9	9.3	10.2	9.2
T1 (100% dW)	1625.96	0.02380	20.5	15.9	17.1	18.4	7.9	9.4	10.8
C1 (100% dW)	1635.42	0.02407	25.6	15.7	30.1	3.7	9.1	6.6	9.3
T2 (20% D$_2$O)	1625.35	0.01307	25.2	10.7	20.4	6.8	6.0	14.1	16.8
C2 (20% D$_2$O)	1633.47	0.03159	23.5	14.7	30.7	4.3	10.1	8.0	8.6
T3 (40% D$_2$O)	1624.11	0.01932	23.7	10.7	27.2	4.1	6.3	10.2	17.8
C3 (40% D$_2$O)	1633.85	0.03369	25.7	15.7	34.8	4.1	8.9	1.6	9.1
T4 (60% D$_2$O)	1624.10	0.03294	18.2	7.7	35.4	3.3	10.2	11.4	13.8
C4 (60% D$_2$O)	1633.98	0.02783	23.5	14.4	31.6	3.8	10.1	8.2	8.4
T5 (80% D$_2$O)	1623.80	0.03113	18.1	14.9	34.7	0	9.8	10.2	12.4
C5 (80% D$_2$O)	1633.95	0.02125	25.4	8.5	32.0	4.8	10.1	10.7	8.6
T6 (100% D$_2$O)	1622.00	0.02233	18.0	17.2	37.8	0	9.2	6.9	10.9
C6 (100% D$_2$O)	1633.35	0.02809	23.3	14.4	33.4	3.8	8.6	8.3	8.2

*Amide I spectra: 1700 to 1600 cm^{-1}
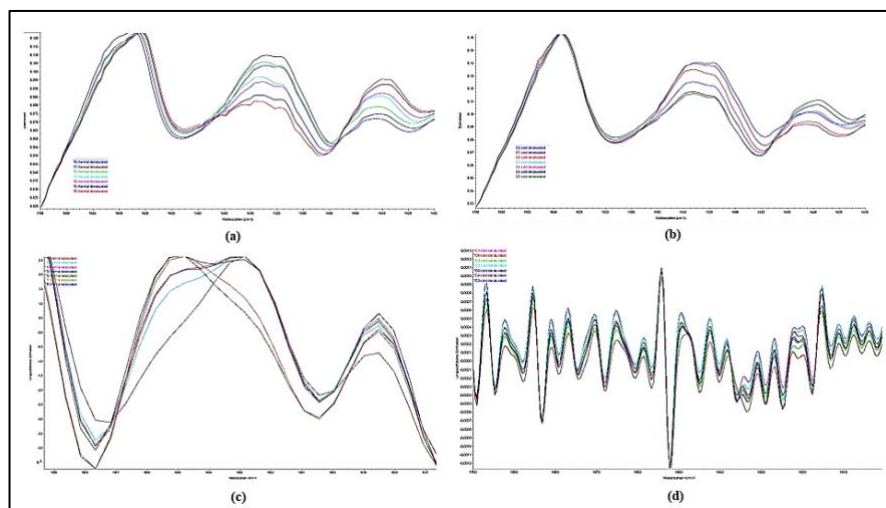
Figure 1. FT-IR spectra and second derivative of Amid I band of T and C series: (a) FT-IR spectra of T-series, (b) FT-IR spectra of C-series, (c) second derivative spectra of T-series, and (d) second derivative spectra of C-series

Besides, for the samples T6 and C6 including 100% D_2O as the diluent, the contents of the α-helix strand were detected to be 37.8% and 33.4%. Denaturation relates to the number of α-helical strands (Van Der Plancken et al., 2006). D_2O could tolerate the temperature extremities by making the protein more compact and less flexible than in dW through the hydrophobicity effect (Clark et al., 2019). For instance, hen egg lysozyme was more stable in D_2O than H_2O (Pica and Graziano, 2017). Our findings matched with Van Der Plancken et al. (2006), Sen et al. (2009), Abrosimova et al. (2016), and Pica and Graziano (2017), respectively. Accordingly, D_2O could positively moderate the transformation of α-helix strands to other unordered strands under temperature extremities. Our study, therefore, contributed to the knowledge gap by providing data over D_2O on the EW proteins for the food area.

3.2. Results of thermal (DSC) analysis

Our DSC thermograms indicated that the ΔH_m, T_m, and C values for T0, T1, and T2 to T6 were found as 118.6 J/g, 69.8 °C and 206.4 J/gK, 131.7 J/g, 68.1 °C and 220.1 J/gK, and 129.9 ± 7.0 J/g, 68.6 ± 1.3 °C, and 208.1 ± 6.0 J/gK, respectively, whereas those of C0, C1, and C2 to C6 were measured as -60.1 J/g, 17.1 °C and 70.0 J/gK, -133.4 J/g, 8.9 °C and 106.6 J/°C, and -124.3 ± 63.9 J/g, 3.3 ± 4.0 °C and 99.5 ± 11.1 J/gK, respectively. Overall, D_2O raised ΔH_m and C by 9.5% and 0.9% at 100 °C and 106.8% and 42.2% at -80 °C, whereas T_m decreased by -1.7% at 100 °C and -80.5% at -80 °C (Table 3, Figure 2).

3.2.1. Results of ΔHm measurement

The behavioral characteristics of the heat- and cold-denatured proteins remain a theoretical issue (Oshima et al., 2009). Thermal denaturation is associated with increased entropy for protein unfolding, whereas cold denaturation is driven enthalpically (Lee et al., 2022). A decrease in denaturation enthalpy indicates a partial loss of protein structure during heating (Van Der Plancken et al., 2006). For many proteins, denaturation is a two-state transition, relating T_m to the transition enthalpy, and EW is an example of a heat-setting thermo-irreversible gel (Ballauff, 2022). From this perspective, our study provided data for determining how cold-denatured EW would differ from heat-denatured.

In the literature, the denaturation enthalpy of fresh EW was reported to be 20.6 J/g (Ferreira et al., 1997) and 15.3 J/g at -19.0 ℃ (Wootton et al., 1981). In our study, ΔH_m values of T- and C-series with different concentrations of D_2O (i.e., 20%, 40%, 60%, 80%, and 100%) were measured as 129.9 ± 7.0 J/g (max 139.5 J/g for T2 with 20% D_2O), and -124.3 ± 63.9 J/g (lowest -229.7 J/g for C6 with 100% D_2O), respectively, whereas they were 118.6 J/g for T0 and -60.1 J/g for C0 (EW only), and 131.7 J/g for T1 -133.4 J/g for C1 (EW and dW only). Our findings of ΔH_m at 100 °C and -80 °C exhibited specific characteristics and various changes compared to fresh EW and dW, including samples. In our case, D_2O could tolerate energy fluctuations, increasing the heat-absorbing capacity for EW blends.

Table 3. Results of thermal (DSC) analysis: ΔH_m (J/g), T_m (°C) and C (J/gK)

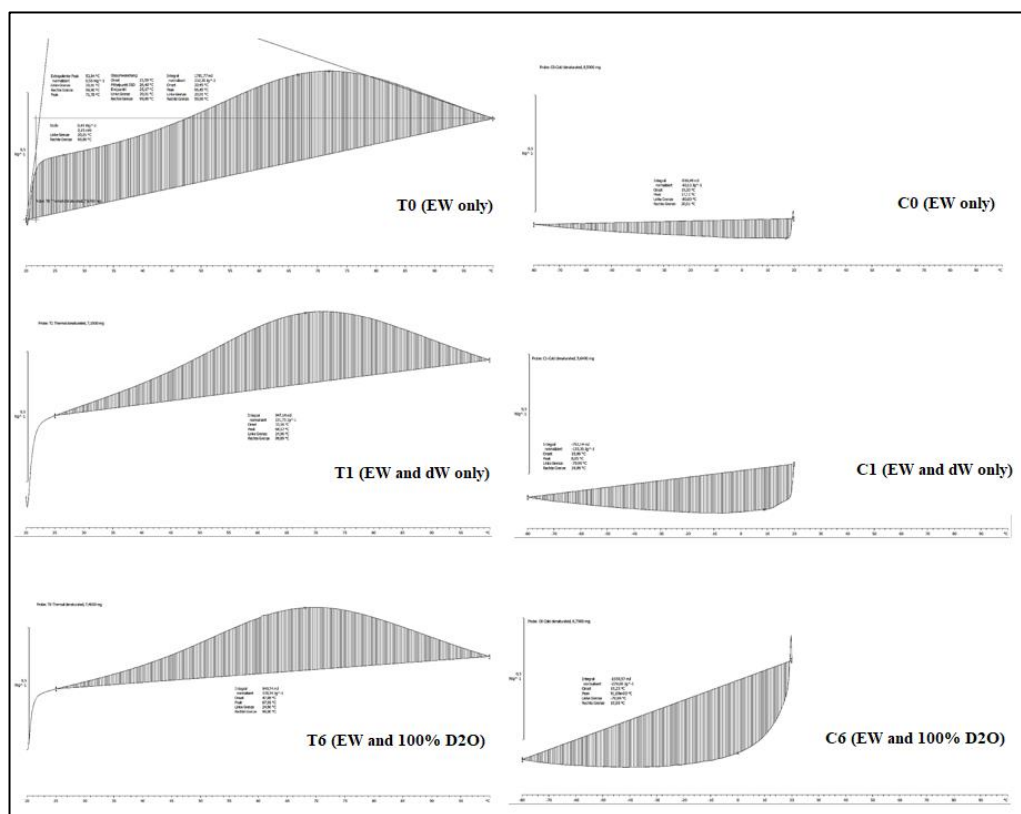| Parameter / Sample no	T0	T1	T2	T3	T4	T5	T6
Latent heat of melting (ΔH_m, J/g)	118.6	131.7	139.5	122.4	134.2	125.0	128.3
Peak (T_m, °C)	69.8	68.1	66.8	68.9	70.4	69.0	68.0
C (J/gK)	206.4	220.1	208.3	198.8	212.7	206.9	213.9
Parameter / Sample no	C0	C1	C2	C3	C4	C5	C6
Latent heat of melting (ΔH_m, J/g)	-60.1	-133.4	-126.5	-96.4	-109.7	-59.3	-229.7
Peak (T_m, °C)	17.1	8.9	5.1	1.1	0.8	9.6	0.1
C (J/gK)	70.0	106.6	108.7	86.3	107.5	106.6	88.5

Figure 2. DSC thermograms of T- and C-series

To understand this intriguing phenomenon, i.e., heat and cold denaturation, a two-state model of water structure, as proposed by Tsai et al. (2002), can be considered to explain the role of the physicochemical properties of D_2O with ice dominating at -80℃ and liquid state at 100 ℃, to figure out which folding ↔ unfolding steps are entropy-driven, and which are enthalpy driven, possibly an enthalpy-dependent process in our study. Overall, our findings point out that D_2O has significant potential to be utilized for protein stability compared to enzymatic, physical, or chemical approaches.

3.2.2. Results of T_m measurement

A common issue in protein stability is the change in the T_m of a protein in the aqueous phase. Denaturing a protein's native structure is so-called melting. However, some protein SSCs can remain after denaturing above T_m and do not necessarily melt at typical processing temperatures (Bier et al., 2014). In the literature, some works observed the different peaks of T_m of EW as 72℃ and 86 ℃ (Wootton et al., 1981), 50 °C and 65 °C (Der Plancken et al., 2006), and 60 ℃ (Ferreira et al., 1997). In our study, the

average T_m values of T- and C-series with different concentrations of D_2O (i.e., 20%, 40%, 60%, 80%, and 100%) were determined to be 68.6 ± 1.3 °C (max 70.4 °C for T4 with 60% D_2O) and 3.3 ± 4.0 °C (max 9.6 °C for C5 with 80% D_2O), respectively. At the same time, they were 69.8 °C and 17.1 °C for T0 and C0 (EW only) and 68.1 °C and 8.9 °C for T1 and C1 (EW and dW only), respectively. Our findings showed that, for D_2O containing T- and C-series samples, the T_m values changed by -1.7% at 100 °C and -80.5% at -80 ℃. Denaturation is a two-state transition, relating T_m to the transition enthalpy (Ballauff, 2022). In our study, D_2O might absorb more heat without relatively changing T_m through a temperature-independent process. On the other hand, in cold denaturation, the reduction of T_m by -80.5% might enable the EW proteins to remain after denaturing.
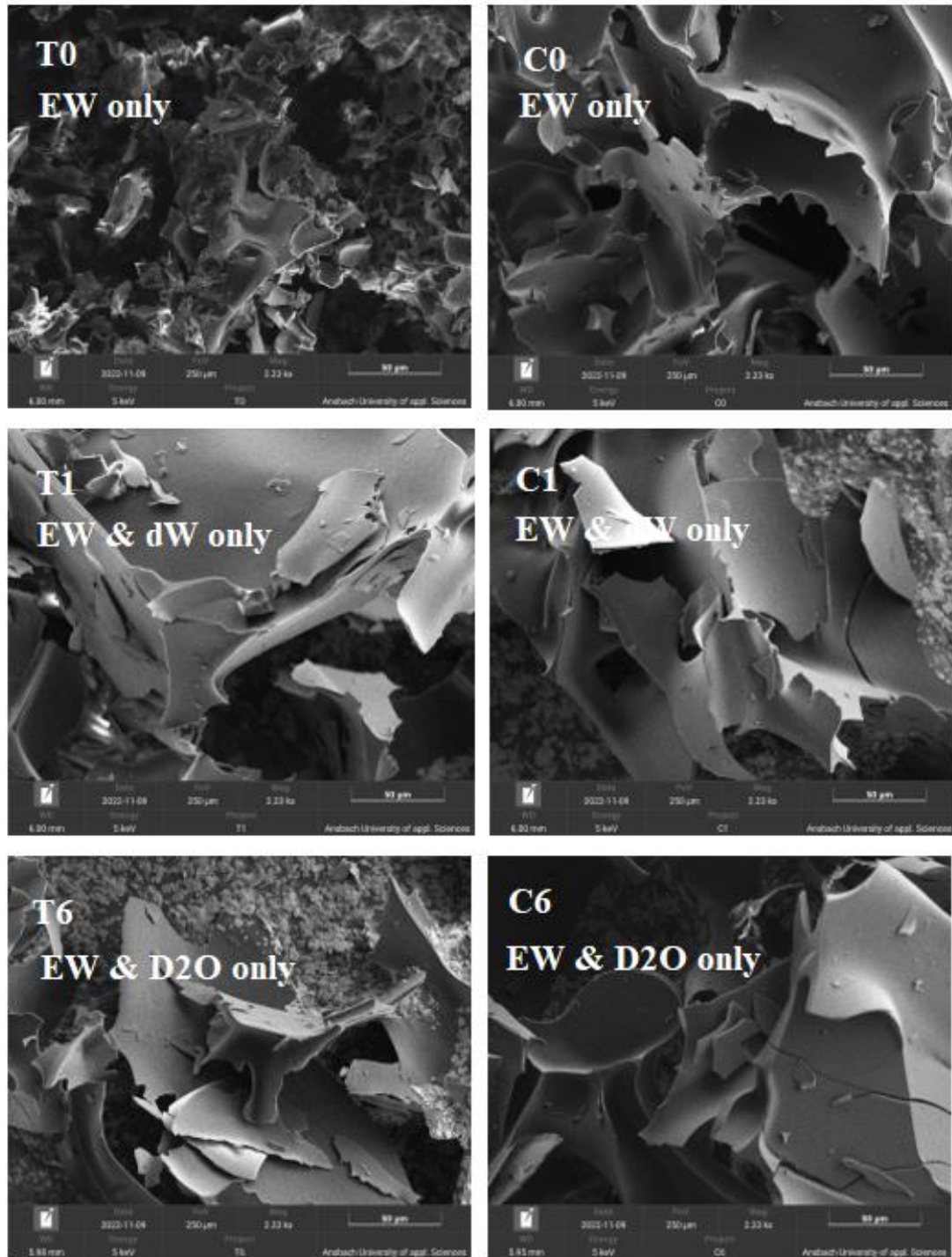
Figure 3. SEM images of T-and C-series (magnification 2.23 kx)

3.2.3. Results of C measurement

Heat capacity (C) is one of the significant thermophysical properties of foods over a broad range of T and a_w required for evaluating, designing, and modeling heat transfer processes. In the literature, some studies reported the C value of EW as 2.6 to 3.7 J/gK at temperatures ranging from 0 to 38℃ and water concentrations from 51.8 to 88.2% (Coimbra et al., 2006) and 2600 to 3.7 J/gK (Lee et al., 2016). An increase in C makes a significant contribution to the total unfolding enthalpy. The increment of C is temperature-independent between 20 to 80℃, and C of native and denatured states changes in

parallel with an increase in T, proceeding with heat absorption and, consequently, with increases in enthalpy and entropy. However, in the case of cooling, it proceeds with heat release and, thereby, with enthalpy and entropy reductions (Privalov, 1990). In our study, D_2O raised C by 0.9% at 100 °C (ave. 208.1 ± 6.0 J/gK) and 42.2% at -80 °C (ave. 99.5 ± 11.1 J/gK), whereas it was 206.4 J/gK and 70.0 J/gK for T0 and C0 (EW only), and 220.1 J/gK and 106.6 J/gK for T1 and C1 (EW and dW only), respectively. Our findings revealed that D_2O might act as an energy-absorbing buffer at 100 °C and an indirect controller of unfolding enthalpy and entropy at -80 °C.

3.3. Results of SEM analysis

Our study captured the SEM images with a magnification of 2.23 kx of the T and C series (Figure 3).

Ogawa et al. (2003) demonstrated that heated-dried EW comprised small gel structural units that formed a dense and heterogeneous network, suggesting the suppression of protein aggregation. In contrast, nonheated-dried EW comprised large protein particles that formed coarse and random networks. Preethi et al. (2021) detected that conductive hydro-dried EW had a flaky structure, while spray-dried and freeze-dried flakes exhibited spherical and porous structures. Our SEM images exhibited flaky structures for both treatment groups with different shapes, dimensions, and fissures, which can indicate the thermal behavior of proteins related to the influence of D_2O on protein stability.

3.4. Results of statistical evaluation

Pearson correlation coefficient calculator was used to measure the strength of a linear association between the T and C treatment series. All analyses were performed using SPSS statistical package program. A *p*-value less than 0.01 was considered statistically significant. The statistical evaluation indicated that there was a strong positive correlation among SSCs (*p=0.0001*), ΔHm (*p=0.00008*), and C (*p=0.00001*) changes at temperature extremities, except for Tm values (*p=0.558182*).

4. Conclusions

Understanding the stability of proteins is of great interest among food science and technology researchers, resulting in an insight into the physicochemical principles that govern the changes and reactions in food processing. The results presented in this study have significant implications for the behavior of amino acids in complex food matrices (EW) under temperature extremities: (a) D_2O influences protein stability to temperature extremities, (b) D_2O indirectly controls unfolding process, (c) D_2O possibly acts through enthalpy-dependent process, and (d) D_2O can be used to establish kinetic models for stable protein-rich foods, respectively. Therefore, it can be used as a reference solvent with its unique properties to establish kinetic models for biomacromolecules with prolonged stability, as an alternative and/or complementary substance to other methods, including enzymatic, physical, or chemical approaches for protein stability.

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Conflict of Interest

None to declare.

Authors' Contribution

İ.H. Tekiner conceptualized the study, did the project administration, and wrote the original draft of the paper. A. Knoblauch, A. Sover, P. Häfner and N. Muschler performed the investigation and executed the experiments. M. Tainsa edited the final draft of the paper and did the visualization.

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HEAT-MOISTURE TREATMENT OF FOXTAIL MILLET STARCH: EFFECT ON PASTING, TEXTURAL AND RHEOLOGICAL PROPERTIES

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ABSTRACT

The impact of heat-moisture treatment carried out at moisture content basis of 20%, 25%, and 30% on the physicochemical, textural, pasting and rheological characteristics of foxtail millet starch was evaluated. The swelling capacity and solubility index of modified starches accelerated with temperature but decreased significantly ($p \leq 0.05$) with increased moisture percent. The decreased swelling capacity and solubility could be caused by the reduced stability of granules arising due to the disentanglement of double helices in the crystalline region of the starch granules. The heat-moisture treated foxtail millet starch depicted a decrease in the birefringence intensity at the granular centre and remained unaltered at the periphery. The reorientation of molecules of heat-moisture treated starches resulted in a significant ($p \leq 0.05$) decrease in peak viscosity, breakdown, and final viscosity. The significant ($p \leq 0.05$) increase in the gel hardness was observed for HMT20 as comparison to native starch. Dynamic rheological properties of the native and modified foxtail millet starches revealed the fragile gel structure. The increase in loss tangent of paste resulted in the gummy nature of starches.

1. Introduction

Foxtail millet is receiving recognition due to its excellent nutritious content, more adaptability to the environment, and good health benefits. It belongs to family Poaceae and genus Setaria. Foxtail millet is underutilized and drought tolerant crop. The foxtail millet grain contains starch, protein, lipids, dietary fiber, and minerals. Starch is the major carbohydrate present in the millet grain and the amount of amylose and amylopectin is responsible for the production and quality of the millet-based products (Sharma and Niranjan, 2018). Some studies found that changes in the food quality i.e. texture depend on the kind of starch in preference to the quantity of starch (Da Mota *et al*., 2000).

The physicochemical and functional properties as well as liable modifications of starch depend upon the amount of amylose and amylopectin and their association within starch molecules. Despite the wide usage of starch in food and other non-food applications, native starch had some flaws which limited its commercial use (Hoover, 2010). Native starches have some poor functionality like thermal properties, unstable texture, poor stability, poor heating, and cooling stability, shear during processing. Therefore, it is necessary to modify native starch to overcome all these flaws and to intensify its properties to meet current technological advancements (Zavareze and Dias, 2011). The natural form of starch is often modified with the aid of physical, enzymatic, and chemical methods.

Heat-moisture treatment (HMT) is the physical method commonly used to enhance the physicochemical characteristics of native starch. HMT brings out the physicochemical changes in starch by disintegrating the crystalline structure

of starch and promoting the interaction between the starch chain in the amorphous and crystalline zone (Zheng *et al*., 2018). The moisture content, heating time, the temperature throughout the treatment, and arrangement of amylopectin and amylose chains in native starch molecules determine the magnitude of changes that occurs during HMT. Several studies have been found on heat moisture treated starches of various crops like potato (Colussi *et al*., 2020), wheat (Li *et al*., 2019), pearl millet (Sharma *et al*., 2015), and proso millet (Zheng *et al*., 2019). However, there is no study on the effect of HMT at different moisture levels on the physicochemical and rheological characteristics of foxtail millet starch. Hence, the goal of this study was to analyze the impact of heat-moisture treatment at different moisture levels (20-30%) on the physicochemical, textural, pasting, and rheological characteristics of foxtail millet starch. The knowledge of the influence of heat moisture treatment on the functional and rheological characteristics of foxtail millet starch can prove useful for the application of foxtail millet starch for the formulation of novel processed foods.

2. Materials and methods
2.1. Starch extraction from foxtail millet
Commercial foxtail millet grains were obtained from a local market, Haryana (India). The grains were ground to obtain flour using laboratory mill (Milcent mill). The starch from foxtail millet was extracted according to the procedure of Balasubramanian *et al*. (2014) with slight changes. The millet flour was soaked in NaOH (0.5%, 1:6 w/v) for 60 min with regular shaking and centrifuged (Sigma 3-18KS, Germany) at 3000 rpm. The supernatant was decanted; the sediment was re-dissolved in distilled water and washed again and again to obtain the white residue. Then pH of the slurry was set using HCl (0.1 N) to 7.0 and dried in an air oven (at 50 °C). The dried starch was ground, sieved from 100 µm sieve, and packed in an airtight bag till further used.

2.2. Heat-moisture treatment of foxtail millet starch
The foxtail millet starch was modified using the procedure reported by Sun *et al*. (2014). The moisture level of foxtail millet starch was equilibrated to 20, 25, and 30 %. The starch was heated for 8 hours at 110°C in an oven and dried at 40°C. The samples were then grounded and screened through a sieve of pore size 100 µm, then stored in airtight pouches till further analysis.

2.3. Chemical analysis
Native foxtail millet starch was analyzed for chemical analysis i.e., moisture, fat, protein, ash, and crude fiber using the standard AACC method (2000).

2.4. Physicochemical properties
2.4.1. Water binding, oil binding capacity, least gelation concentration and bulk density
The water binding and oil binding capacity of starch samples were measured by applying the procedure described by Sosulski *et al*. (1976). The LGC of starch samples was evaluated by using the procedure of Coffman and Garcia (1977). The bulk density of native and HMT starch samples was determined using the procedure of Owalabi *et al*. (2010).
2.4.2. Swelling capacity and solubility index
The swelling capacity and solubility of starch extracted from foxtail millet were measured by the procedure of Sosulski *et al*. (1976) with slight changes. The starch sample (0.5 g) was slightly mixed with distilled water (25 ml) and the suspension was heated at varying temperature at 55°C, 65°C, 75°C, 85°C, and 95°C for 15 min in a shaking water bath. Then removed the tubes from the water bath, centrifuged (Sigma 3-18KS, Germany) the paste for 10 min at $3000\times$ g, and then poured the supernatant in a petri plate. The weight of sediment was then recorded and the supernatant was dried for 3 h at 105° C. Swelling capacity and solubility were measured as follows:

Swelling capacity (g/g) =

$$\frac{\text{Weight of swollen granules}}{\text{Dry weight of a sample}}$$

(1)

Solubility index (g/g) =

$$\frac{\text{Weight of solubles}}{\text{Dry weight of a sample}}$$

(2)

2.4.3. Polarized microscopy

The microstructures of native and HMT starch were studied using a polarized microscope (Olympus CX 21 iLed) at a magnification of 400 x.

2.4.4. Pasting properties

The native and HMT foxtail millet starch were analysed for their pasting characteristics using the rapid visco-analyzer (RVA Starch Master TM, Newport Scientific, Australia). The aqueous starch suspension was made by dispersing 3g of starch in 25 ml of distilled water in an RVA canister. The heating cycle involved holding the starch slurry at 50°C for 1 min before being heated to 95°C for 3 min 42 s followed by retaining at 95°C for 2 min. Following the heating cycle, the cooling cycle began with a drop in temperature to 50°C in 3 min 48 s, which was maintained for 2 min at 160 rpm. The peak time, pasting temperature, peak viscosity, hot paste viscosity, final viscosity, breakdown, and setback were evaluated through the pasting graph.

2.4.5. Particle size distribution

The particle size analyzer (Mastersizer 3000, Malvern, UK) with an attached wet dispersion unit was used to analyze the size of native and HMT foxtail millet starch. The suspension of samples was added to the port within an obscursion range of 12-20% and the distribution of particles was expressed by function of diameter i.e. Dv 10, Dv 50 and Dv 90. The analysis of starch particles distribution was based on the phenomena of diffraction of light.

2.4.6. Texture analysis

The texture analyzer (TA.XT plus, Stable Micro Systems, Godalming, UK) was used to assess the textural profile of native and HMT foxtail millet starch gels (10% w/v). The 5 mm diameter cylindrical probe (P/0.5R Derlin) was punctured into the gel to the depth of 10 mm at a pre and post speed of 1 mm/s to analyze the texture of gel. The different textural attributes including hardness, springiness, cohesiveness, gumminess, chewiness and resilience were measured using the software.

2.4.7. Rheological properties

Dynamic visco-elastic and steady flow behavior of starch pastes of native and HMT foxtail millet starches were measured by dynamic rheometer (Dynamic Rheometer, Anton Paar) using the cone (1° cone angle) and plate geometry sensor (diameter 40 mm, 0.08 mm gap) (Shrivastava *et al.*, 2018). The rheological determination for dynamic-viscoelastic behavior was performed in two steps: (1) deformation sweep to measure the maximum distortion achievable by the paste within the linear viscoelastic region at a constant frequency of 10 rad/s and (2) frequency sweeps, 0.1–100 rad/s range at 0.5% strain within the linear viscoelastic zone.

The viscosity of native and modified starches was determined by measuring the steady flow characteristics of starch pastes at 25°C. The shear rate was increased in 3 min sfrom 0 to 300s^{-1} and the viscosity was determined as a function of shear rate.

2.5. Statistical analysis

The analysis of all the observations was carried out by applying one way ANOVA (SPSS 19). The significant difference among the mean values was measured at $p<0.05$.

3. Results and discussion

3.1 Physicochemical properties

The average yield of isolated foxtail millet starch was found to be 61 % (dry basis). Chemical analysis was done to assess the purity of the obtained starch. The recovered starch had 11.58% moisture, 0.68% fat, 1.36% protein, 0.75% crude fibre, and 0.3% ash content. The minimal residual crude fibre, ash and protein concentration suggested that the starch was effectively isolated with high purity. The observed values were in agreement with the previously reported results (Babu *et al.*, 2019; Bangoura *et al.*, 2012).

Table 1 shows the effects of HMT on the physicochemical parameters of foxtail millet starch. The WAC of foxtail millet starch increased significantly (p<0.05) from 131 to 157% after heat moisture treatment and was observed to be highest for HMT30. The hydrothermal treatment of starch enhanced the water-binding tendency which resulted in the increased value of WAC. The hydrophilic affinity of starches inclined with the increasing moisture levels from 20 to 30%. Adebowale *et al*. (2005) also noted that the WAC of finger millet starch increased after hydrothermal treatment which suggested the high-water holding efficiency of starch. The oil absorption capacity of foxtail millet starches ranged from 123 to 158%. The OAC of foxtail millet starch significantly expanded (p<0.05) to a great extent after HMT and was observed to be highest for HMT30. The hydrophobic affinity of starches increased with the increasing moisture levels during hydrothermal treatment. Olayinka *et al*. (2008) also reported similar results for white sorghum starches. It has been reported that the formation of the lipophilic layer after the treatment on the surface of the starch molecule could be responsible for the increase in oil absorption capacity (Abraham, 1993). The similar results regarding the increased WAC and OAC have been noted for wheat and potato starches (Kulp and Lorenz, 1981) and finger millet starch (Adebowale *et al*., 2005).

The LGC may be defined as the least amount of starch that is required to form a gel. The higher the amount of starch needed for gel formation the higher will be the LGC. The LGC of foxtail millet starch revealed that there was no change in the LGC after the hydrothermal treatment. The bulk density of foxtail millet starch increased significantly (p<0.05) after HMT and was observed to be highest for HMT30 ($0.71g/cm^3$). Owolabi *et al*. (2010) also observed that the bulk density of corn starch increased after hydrothermal treatment which is an indication of the improved flowability of starch.

Table 1. Physico-chemical properties of native and heat-moisture treated foxtail millet starch.

Sample	WAC (g/100g)	OAC (g/100g)	LGC (%)	Bulk density (g/cm³)
Native	131.00 ± 1.00^a	123.25 ± 0.75^a	8	$0.62^a\pm0.00$
HMT20	142.25 ± 0.75^b	143.00 ± 1.00^b	8	$0.66^b\pm0.00$
HMT25	148.75 ± 0.25^c	151.00 ± 1.00^c	8	$0.68^c\pm0.01$
HMT30	157.25 ± 0.75^d	158.00 ± 1.00^d	8	$0.71^d\pm0.00$

The values are expressed as the mean +SD of three independent determinations.
Where, HMT20 = Heat-moisture treatment at 20% moisture content; HMT25 = Heat-moisture treatment at 25% moisture content; HMT30 = Heat-moisture treatment at 30% moisture content; WAC = Water absorption capacity; OAC = Oil absorption capacity; LGC = Least gelation concentration
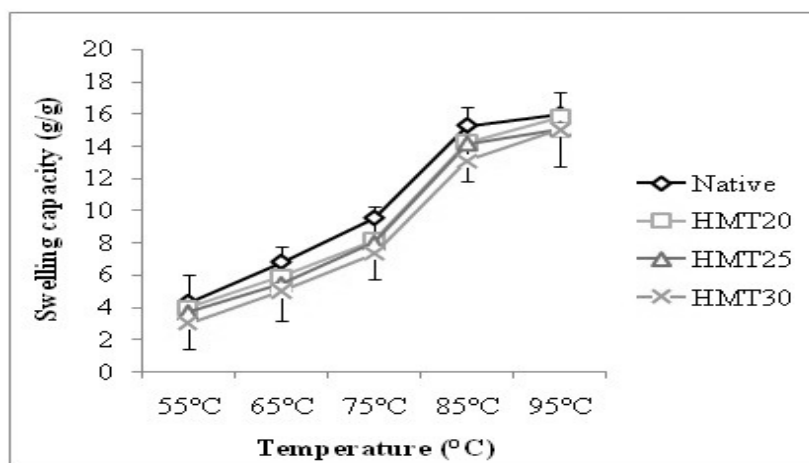
3.2. Swelling capacity (SC) and solubility index (SI)

The swelling capacity and solubility index of the foxtail millet starches were assessed within a temperature range of 55 to 95ºC and are shown in figure 1 (a) and (b), respectively. It was seen that both swelling and solubility increased with increasing temperatures. The swelling capacity and solubility index reflects the presence of strong binding forces among the starch molecule due to the interaction of the crystalline and amorphous zone. The SC of native and modified foxtail millet starch ranged from 15.0 to 16.0 g/g at 95°C. After HMT, the swelling capacity was found to be significantly reduced (p≤0.05). The higher the degree of hydrothermal treatment lower was the swelling capacity. The reduction in SC might be due to alteration in structure within the starch molecules and formation of more starch molecules interactions after HMT.

The stability of starch granules increases due to the formation of more crystallites during HMT and thus leading to the decrease in SC. The decrease in SC after HMT as reported in present study is similar as noted in previous studies on rice starch (Hormdok and Noomhorm, 2007), finger millet starch (Adebowale *et al.*, 2005), Sorghum (Olayinka *et al.*, 2008), and pearl millet starch (Sharma *et al.*, 2015). Just like swelling capacity, SI also followed the same trend and significantly reduced (p≤0.05) after the HMT modification. HMT30 showed the lowest value of solubility (14.93 g/g) while native starch showed the highest value (15.75 g/g) for solubility at 95ºC. Similar results of decreasing solubility after heat moisture treatment have been earlier noted for water chestnut (Yadav *et al.*, 2013), and pearl millet starch (Sharma *et al.*, 2015). The reduced solubility after HMT reflects the formation of stronger bonds due to more interactions between starch molecules and thus preventing the leaching of amylose (Zavareze *et al.*, 2010).

a
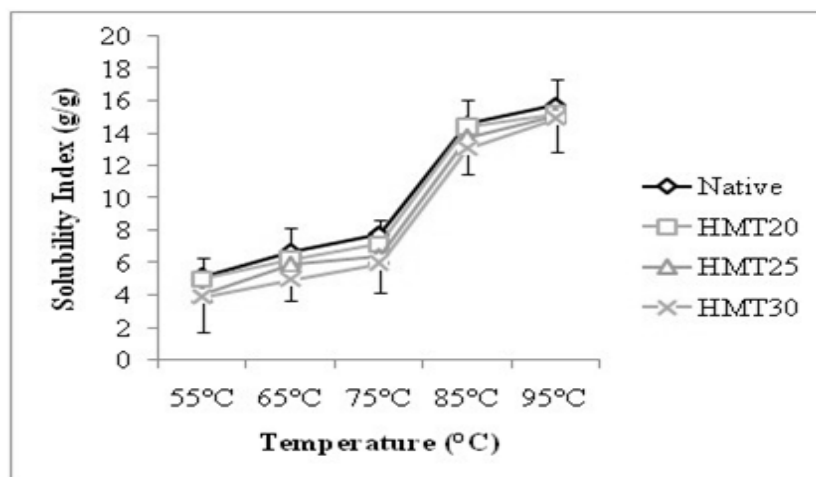
b

Figure 1. (a) Swelling capacity of native and HMT foxtail millet starch (b) Solubility index of native and HMT foxtail millet starch

The swelling and solubility index of hydrothermally treated starch samples decreased with the increase in moisture content. The leaching of typically associated amylose molecules and lipids into the continuous phase is proportional to the swelling of starch granules,

which is proportional to the pasting temperature (Yu *et al*., 2018). The inclusion of amylose and lipids in the granule reduces starch swelling, in contrast to amylopectin, which contributes mostly to water intake (Abedi *et al*,. 2019; Agi *et al.* 2019). Amylose and lipid content, as well as the structure of amylopectin, all have a role in starch granule swelling (Li *et al*., 2019). Leach *et al*. (1959) noted that the structural reorganization inside the starch granules following the hydrothermal treatment resulted in the decreased swelling and solubility index. Hoover and Vasantham (1994) reported that the

alteration in the organization of crystallites and the interactions between the starch molecules in the amorphous region could be responsible for decreased swelling and solubility index after hydrothermal treatment**.**

3.3. Polarized microscopy

The microscopic examination of native and HMT foxtail millet starch molecules was done under polarized microscope and the images are depicted in figure 2(a-h).
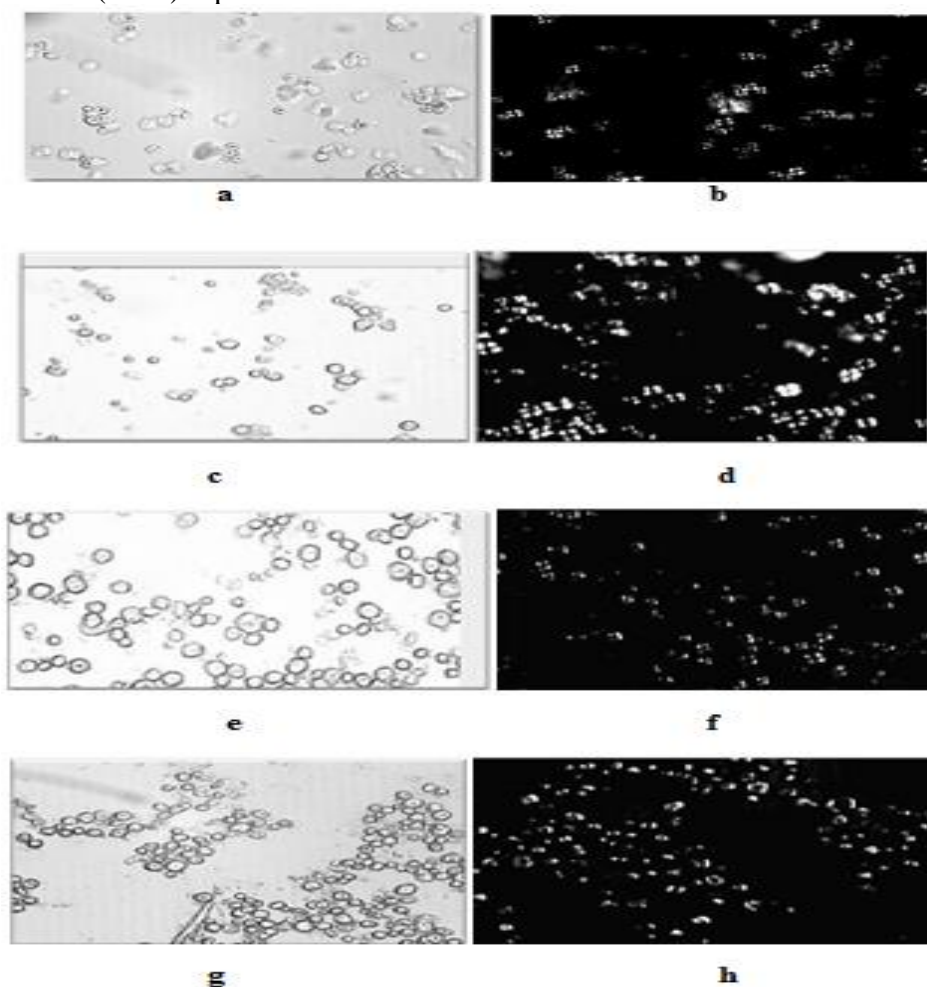

Figure 2. Photomicrographs of (a) native foxtail millet starch under ordinary light; (b) native foxtail millet starch under polarized light; (c) HMT20 foxtail millet starch under ordinary light; (d) HMT20 foxtail millet starch under polarized light; (e) HMT25 foxtail millet starch under ordinary light; (f) HMT25 foxtail millet starch under polarized light; (g) HMT30 foxtail millet starch under ordinary light; (h) HMT30 foxtail millet starch under polarized light

The foxtail millet starch molecules were small, oval to a large polygonal in shape.

Wankhede *et al*. (1979) also reported the absence of fissures, barely perceptible hilum,

and strong centric polarization crosses in native foxtail millet starch. The molecular shape and orientation regarding light beam are responsible for the intensity of birefringence. A decrease in the birefringence intensity at the granular centre was observed for HMT foxtail millet starches. The increase in mobility of starch granules due to heat treatment might have resulted in a decrease of birefringence intensity. However, the birefringence intensity of hydrothermally treated starch molecules remained unaltered at the periphery. Chung *et al.* (2010) also reported the least organization of molecules of lentil, navy bean, and pea starches at the centre as compared to the periphery and therefore suggested that these molecules were more likely to be reorganized during hydrothermal treatment. Liu *et al.* (2016) also discovered some grooves and broken granules of coix seeds starch during HMT which might have improved the water absorption, enzyme susceptibility, and adhesion qualities.

3.4. Pasting properties

The pasting behavior of isolated and treated foxtail millet starches are shown in Table 2. The pasting temperature of native starch was discovered to be 79°C and it increased significantly (p≤0.05) after HMT. The increment in pasting temperatures can be attributed to the transition from an amorphous to a hard crystalline state, as well as the formation of cross-linkages, which demands the requirement of high heat to make paste (Sharma *et al.*, 2015). The hydrothermal treatment led to a significant reduction in the hot paste viscosity (HPV), peak viscosity (PV), breakdown viscosity (BD), setback viscosity (SB), and cold paste viscosity (CPV). The PV of isolated foxtail starch was reported to be 1754 cP and the significant decrease (p≤0.05) in PV was observed for HMT starches. The capability of starch to make a viscous paste determined the cold paste viscosity. The CPV of native starch was 2140 cP which was reduced significantly (p≤0.05) to 1012 cP for HMT30 starch. The reduction in HPV, PV and CPV after HMT could be due to rearrangement within the granule of the HMT starches. The reinforcement of cross-linkages within starch chains and increase in crystallinity after HMT restricts starch swelling and amylose leaching which resulted in decreased viscosity (Adebowale *et al.*, 2005).

Table 2. Pasting properties of native and heat-moisture treated foxtail millet starch.

Sample	PV (cP)	HPV (cP)	BD (cP)	CPV (cP)	SB (cP)	Peak time (min)	PT (°C)	Stability Ratio	Setback Ratio
Native	1754±2.34[d]	1062±1.78[c]	693±5.24[d]	2140±4.43[d]	1079±4.91[c]	5.00±0.30	79.10±0.17[a]	0.82	2.02
HMT20	1139±1.15[c]	766±2.31[b]	373±3.28[c]	1208±3.75[c]	442±4.01[b]	5.03±0.15	82.18±0.20[b]	0.94	1.57
HMT25	901±2.02[b]	694±1.11[a]	208±4.01[b]	1028±2.08[b]	334±5.21[a]	5.18±0.35	86.48±0.23[c]	0.88	1.48
HMT30	855±2.33[a]	688±2.03[a]	167±3.21[a]	1012±1.76[a]	323±1.77[a]	5.48±0.30	86.48±0.23[c]	0.84	1.47

The values are expressed as the mean +SD of three independent determinations.
Where, PV= Peak viscosity, HPV= Hot paste viscosity, BD= Breakdown viscosity, CPV= Cold paste viscosity, SB= Set back viscosity, PT= pasting temperature.

The reduction in viscosity after HMT as reported in present study is similar as noted in previous studies on oat, lentil and yam starches (Hoover and Vasantham, 1994), rice starch (Hormdok and Noomhorm, 2007) and finger millet starch (Adebowale *et al.*, 2005).

Watcharatewinkul *et al.* (2009) reported that the alteration in pasting viscosity possibly because of the interconnection of the starch chains in the amorphous regime of the molecules and the altered crystallinity of starch molecules during HMT. The BD value of foxtail millet starch was reduced significantly (p≤0.05) after heat moisture treatment. The lowest value of BD was observed for HMT30 (167 cP) while the highest value was observed for native (693 cP). The lower BD value indicates the heat stable behaviour of HMT starches. Various heat-processed food products could be formed with starches having lower BD due to their thermo-stable behaviour. The ability to retrograde is measured by the setback (SB) value of the starch paste, which dropped significantly (p≤0.05) after hydrothermal treatment. Retrogradation occurs due to the rearrangement of the linear structure of amylose molecules. The decrease in SB value after HMT might be due to more interactions between amylopectin and amylose chains and low amylose leaching. Starches with a lower setback value can be employed in the production of canned and frozen foods.

3.5. Particle size distribution

The size distribution of the particles is crucial aspect which also influences the functional properties like swelling power, pasting and rheological characteristics of starch. The size distribution of the particles of isolated and treated foxtail millet starch is given in Table 3. The size of the particles in isolated foxtail millet starch ranged from 0.35-14.5 μm whereas in heat- moisture treated sample, the size of particles increased with increased moisture content. All the samples i.e. native and HMT foxtail millet starches showed bimodal distribution. The increase in the size of starch particles during HMT could be due to the agglomeration that occurred under the influence of with high moisture content during heating process (Chandla *et al.*, 2007). Zavareze *et al.* (2010) also observed the increase in size of rice starch particles treated hydrothermally for 25 min which was ascribed to their partially gelatinization at higher moisture content during heat moisture treatment.

Table 3. Particle size distribution and textural parameters of native and heat-moisture treated foxtail millet starch.

Samples	Particle size distribution			Textural parameters				
	Dv 10 (μm)	Dv 50 (μm)	Dv 90 (μm)	Hardness (g)	Adhesiveness (g/s)	Spiringiness (mm)	Cohesiveness	Gumminess (g)
Native	0.57±0.03[a]	6.25±0.03[a]	9.34±0.03[a]	84.80±1.63[a]	43.34±1.20a	0.98±0.02[c]	0.57±0.01[b]	48.07±1.00[b]
HMT20	10.37±0.15[b]	27.53±0.23[b]	113.67±1.77[b]	102.76±2.03[b]	40.72±1.31[a]	0.96±0.01[b]	0.56±0.01[a]	57.82±1.08[c]
HMT25	10.23±0.26[b]	30.30±0.15[c]	134.0±2.08[c]	82.21±1.91[a]	41.70±0.68[a]	0.95±0.02[a]	0.59±0.01[c]	49.34±1.03[b]
HMT30	10.47±0.20[b]	33.37±0.29[d]	133.0±2.30[c]	81.43±1.85[a]	41.03±0.87[a]	0.95±0.01[a]	0.61±0.01[d]	43.92±1.15[a]

The values are expressed as the mean +SD of three independent determinations.
Dv 10, Dv 50, Dv 90= 10 %, 50%, 90% particles are finer than given value.

3.6. Texture analysis

The textural data of isolated and HMT foxtail millet starch is shown in Table 3. The

bond forming ability of starch granules with water determines the gel strength. The gel hardness of foxtail millet starch increased

significantly (p≤0.05) for HMT20 (102.76 g) as comparison to that of native starch (84.80 g). The formation of more cross-linkages in amylose portion among starch molecules and development of more junction zones during HMT resulted in the increased hardness of gel (Chandla *et al.*, 2007). However, HMT25 (82.21 g) and HMT30 (81.43 g) showed the decrease in gel hardness, although it was non-significant. The decrease in gel hardness of the starch subjected to HMT for comparatively longer duration might be due to partial gelatinization of starch molecules during HMT which ultimately resulted in the rupturing of starch granules and formation of weak gel (Liu *et al.*, 2000). Hormdok and Noomhorm (2007) also reported the similar results for rice starch after HMT. No significant difference (p≤0.05) was observed between the values of adhesiveness in native and HMT foxtail millet starch. The value of cohesiveness varied from 0.56 (HMT20) to 0.61 (HMT 30). However, the impact of hydrothermal treatment was inconsistent on cohesiveness and gumminess.

3.7. Rheological properties
3.7.1. Oscillatory properties

The dynamic viscoelastic behavior of the native foxtail millet starch and HMT starch with different moisture content is shown in figure 3 (a) and (b). With an increase in frequency, both the storage modulus (G′) and the loss modulus (G″) expanded. The value of storage modulus (G′) was exceeding the value of loss modulus (G″) in all the starch samples and for the similar gels, the values of G′ and G″ did not cross over across the uniform frequency specifying a weak gel characteristic. The increase in G′ and G″ was reported with the frequency rise. The value of G′ and G″ was lower for HMT starches and it decreased more with increasing moisture content. The decrease in G′ values of HMT starches might be because of deformation of starch granules or lower continuous phase elasticity. A decrease in G′ values was observed in HMT25 and HMT30 samples which specified the softer gel formation in these two modified starch samples, in contrast, to native and

HMT20 samples. Similar observations have been noted for heat moisture treated pearl millet starch (Sharma *et al.*, 2015).

Tan δ (G″/G′) describes the visco-elastic character of starches and was found to be below than 1 for all samples, showing an elastic nature. However, the values of tan δ increased after HMT showing an increase in the viscous nature of HMT starches, and maximum values for tan δ were observed for HMT30. The alteration of the gel structure of HMT25 and HMT30 to a fluid state without a sharp broken end indicates the balanced arrangement of elastic and viscous sections. The starch gels indicated shear-thinning behavior and the decrease in rigidity of starch gel was noted with increasing moisture content during HMT. Liu *et al.* (2016) also discovered that following HMT, the G' and G" modulus of coix seeds starch suspensions reduced. HMT causes the suspensions to have a weaker structure and less gel-like behaviour as indicated by lower tan δ values for native coix starches. This effect could be owing to the breakdown of starch granules crystalline structure during HMT, which imparts them more viscous character (Sui *et al.* 2015).

3.7.2. Steady shear properties

The change in starch structure caused by shearing force is referred to as steady flow behaviour. The steady flow behavior of isolated and HMT foxtail millet starch is shown in figure 3 (c). All the pastes showed the shear thinning behavior as there was a decrease in viscosity with increasing shear rates. The viscosities of HMT starches were less in comparison to the native starch. This behavior suggested that modified starches were less stable against high shear rates and hence, HMT starches cannot be suitably used as a thickener in high shear processing conditions. Similar behavior has also been reported for heat moisture treated sago and arenga starches (Adawiyah *et al.*, 2017) and it was suggested that HMT starches could find applications in products having lower moisture content and low shear processing such as cookies. The hydrothermal treatment of arrowroot starch resulted in a lower consistency index, reflecting a decreased perceived

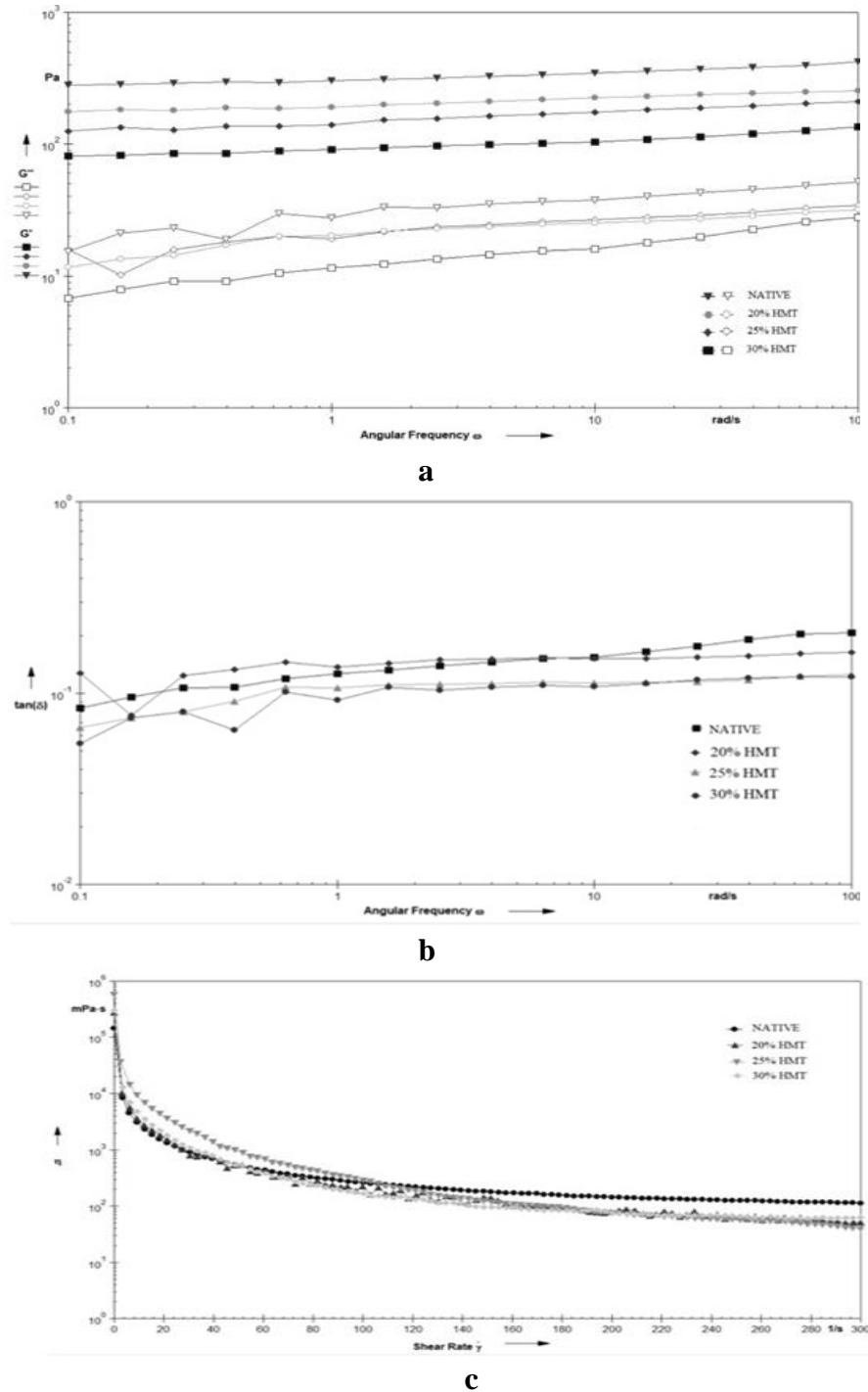viscosity. The apparent flexibility of arrowroot starches was also lowered by the HMT (Pepe *et al.*, 2015).

a

b

c

Figure 3. Dynamic mechanical spectra of (a) native and HMT foxtail millet starch (b) Dynamic mechanical loss tangent (tan ϱ) of native and HMT foxtail millet starch (8% suspension, w/w) (c) Flow curve of native and HMT foxtail millet starch (8% suspension, w/w)

4. Conclusions

In this study, the foxtail millet starch was modified by the HMT at different moisture level i.e. 20%, 25%, and 30%. The creation of cross-linkages and the conversion of the amorphous zone into a more compact crystalline zone during HMT, the SP, SI, and pasting parameters were considerably altered. The reorientation of molecules of hydrothermally treated starches resulted in a significant decrease in viscosities and increase in pasting temperature. The decrease in peak viscosity eventually resulted in lower swelling capacity and lower amylose leaching. HMT20 showed a significant increase in gel hardness however, no significant difference ($p \leq 0.05$) in gel hardness of HMT25 and HMT30 with native foxtail millet starch was observed. The rheological characteristics of native and HMT foxtail millet starches in dynamic state revealed the weak gel structure. The increase in loss tangent of paste resulted in the viscous behavior of starches. Therefore, the study concluded that the foxtail millet starch with some specific characteristics can be obtained by giving heat moisture treatment at different moisture level. This research will be helpful in the development of some new food products using hydrothermally treated foxtail millet starch.

5. References

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