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Preparation and Evaluation of Oleogels Incorporated with *Moringa oleifera* Leaves Extract in Biscuits Production

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ABSTRACT

This study investigated the physicochemical, antioxidant and sensory properties of sunflower oil-beeswax oleogels at different concentrations (SFOBW3), 5% (SFOBW5), and 7% (SFOBW7), with and without the addition of *Moringa oleifera* leaves phenolic extract (MOLE), in the context of biscuit production. It was found that increasing concentrations of beeswax expedited gelation time (8.25 min for SFOBW7), enhanced oil binding capacity (OBC) and contributed to a firmer texture and higher melting points (52.48 oC for SFOBW7), indicating a stronger oleogel network. Moreover, the addition of beeswax resulted in lighter (L) oleogels with improved color attributes (55.95 for SFOBW7) and stability upon centrifugation. The inclusion of MOLE did not significantly alter the gelation time or OBC. However, it increased the total phenolic content (TPC) (from 14.65 mg/100g for SFOBW5 to 40.26 mg/100g for SFOBW5+0.45g MOLE) and antioxidant activity (DPPH) (from 38.25% for SFOBW5 to 60.98% for SFOBW5+0.45g MOLE). Sensory evaluation revealed that oleogels with 5% beeswax exhibited the best balance of appearance, spreadability, and overall acceptability. Furthermore, biscuits prepared from oleogels with a combination of 50% oleogel, both with and without MOLE, enhanced sensory attributes compared to those prepared with 100% oleogels. The study underscored the potential of using sunflower oil-beeswax oleogels, enriched with MOLE, to produce biscuits with desirable nutritional and sensory qualities.

1. Introduction

In recent years, there has been a significant shift in the food industry towards incorporating healthier and more sustainable ingredients into food production. The widespread use of solid or semi-solid fats is crucial in the food sector due to their key role in determining the texture and taste of food items, as well as their notable resistance to oxidation (Saghafi et al., 2019a). Hence, methods such as hydrogenation and interesterification were used to transform liquid oils into solid or semi-solid fats (Doan et al., 2018). The hydrogenation process increases the levels of saturated and trans fatty acids in the oil. These fats have been conclusively linked to negative health effects (Saghafi et al., 2019b). The extensive usage of these fatty acids enhances the risk of cardiovascular diseases (Islam et al., 2019), metabolic syndrome, and type 2 diabetes (Mirmiran et al., 2019). Consequently, it is critically important to create alternative techniques for converting vegetable oils into solid or semi-solid fats that contain lower amounts of saturated and trans-fatty acids (Doan et al., 2018).

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The technique of trapping liquid oil inside a three-dimensional organogel framework with a modest amount of one or more oleogelator (organogelator) chemicals is known as oleogelation, or organogelation (Lim et al., 2017). Oleogels have emerged as a novel ingredient in the rapidly developing field of food technology, especially in baked foods. These structured oils replicate the functional characteristics of solid fats while avoiding the health risks tied to trans and saturated fats, offering a hopeful path for the development of healthier food options (Moghtadaei et al., 2018). This innovation aligns with the growing consumer demand for food products that not only satisfy taste but also contribute to a healthier diet. Liquid plant-based oils, including those from olive, corn, sunflower, and flaxseed, are frequently utilized in food preparation and the industry (Tian et al., 2023). These plant oils offer a broad range of fatty acid compositions, leading to varied applications and potential health advantages (Kumar et al., 2016). Nonetheless, liquid oils may not always be suitable for certain food applications, like bakery items, due to oil leakage during storage and handling. As a result, transforming these oils into solid-like oleogels could enhance their usability (Patel and Dewettinck 2016). Sunflower oil, known for its rich unsaturated fatty acid content, is an ideal candidate for creating oleogels (Pehlivanoğlu et al., 2018). Its light taste and nutritional profile make it a preferred choice for health-conscious consumers and food technologists alike (Bhuvaneshwari and Umamaheswari 2013). Once converted into an oleogel, sunflower oil has the potential to substitute solid fats in baked products, thereby lowering the content of harmful fats while maintaining the texture and sensory characteristics essential to the cherished consistency of bakery items. Natural plant waxes, like beeswax, are attracting attention as oleogelators due to their safe and edible properties (Yilmaz et al., 2021; Mattice and Marangoni, 2017). These waxes are composed of a unique blend of hydrocarbons, wax esters, fatty acids, and other elements. The specific composition of each wax plays a crucial role in determining the properties of the resulting oleogels (Mattice and Marangoni, 2017). Beeswax, in particular, has been identified as an effective structuring agent for oleogels (Dimakopoulou-Papazoglou et al., 2023). The process involves dissolving beeswax in liquid oil and then cooling it. During cooling, beeswax crystals reform, creating a network that traps the oil, similar to how fat crystals work in traditional bakery products (Yilmaz et al., 2021; Blake and Marangoni, 2015). This allows beeswax-based oleogels to mimic the texture and mouthfeel of conventional fats, making them a valuable tool for creating healthier bakery items like biscuits (Li et al., 2022).

Furthermore, incorporating extracts rich in antioxidants, like those from Moringa oleifera leaves, can further enhance the health benefits and shelf life of bakery products made with oleogels (Owon et al., 2021; Qu et al., 2022). These extracts not only add valuable bioactive compounds but also potentially improve the oxidative stability of the oleogel, leading to longer-lasting baked goods.

This study explored the use of oleogels as a substitute for traditional fats in bakery products, focusing specifically on biscuits. An oleogel made from sunflower oil and beeswax was developed and selected for its health benefits and accessibility. Additionally, this study was pioneering in incorporating Moringa oleifera leaves phenolic extract (MOLE) into the oleogel. By infusing the oleogel with MOLE, the aim was to enhance the antioxidant capacity of the biscuits, providing additional health benefits. The application of this novel oleogel in biscuit production represented an exploratory step towards determining its feasibility as a healthier alternative to conventional fats in bakery products.

2. Materials and Methods

Materials

The sunflower oil was obtained from Tanta Company of Oils and Soaps in Tanta City, Egypt. Moringa oleifera leaves were sourced from the Food Technology Research Institute in Sakha, Kafrelsheikh City, Egypt. Beeswax (BSW) was provided by Kahlwax Co. (Kalh GmbH & Co., Trittau, Germany). The beeswax (BSW) was provided by Kahlwax Co. (Kalh GmbH & Co., Trittau, Germany).

All standards, chemicals, and solvents used were of analytical grade and purchased from Sigma Chem. Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Methods

Extraction of Moringa oleifera leaves extract

Twenty grams of Moringa oleifera leaves were finely pulverized using an IKA mill (model A11 BS000, Germany) and then extracted with 200 mL of 70% ethanol. After sonication for 30 minutes in a bath-type sonicator, the mixture was centrifuged for 5 minutes at 3000 × g. The clear supernatant was transferred into a 500 mL flask, and the remaining solid residue was extracted two more times following the same procedure. The combined supernatants were then concentrated using a rotary evaporator (model RV 10C S93, IKA-Werke GmbH & Co. KG, Stauffen, Germany) at 40°C. The concentrated extract was frozen at −80 °C. For further drying, the material was lyophilized at −40 °C over 48 h to produce a dry MOLE, which was then stored at −20 °C until needed.

Oleogel preparation

Two types of oleogels were prepared based on the method by (Qui et al., 2021) with some modifications (Figure 1). The first type contained no Moringa oleifera leaves phenolic extract (MOLE), while the second type incorporated MOLE (Figure 1). The first type was prepared as follows: BSW was melted in a glass beaker at 80˚C for five minutes in a water bath at various concentrations (3, 5, and 7% from oil weight). Second, the appropriate amount of oil was added, using a magnetic stirrer, the mixture was heated to 80˚C for 20 minutes at 700 rpm. For the second kind, 100 mL of oil, 0.5% tween 80 surfactant, and 0.15, 0.30, and 0.45 g of MOLE were heated to 80˚C for 10 minutes at 700 rpm using a magnetic stirrer. Next, using a magnetic stirrer, the melted BSW was added to the prior mixture and stirred for 20 minutes at 800 rpm and 80 ˚C. At last, the mixtures remained.

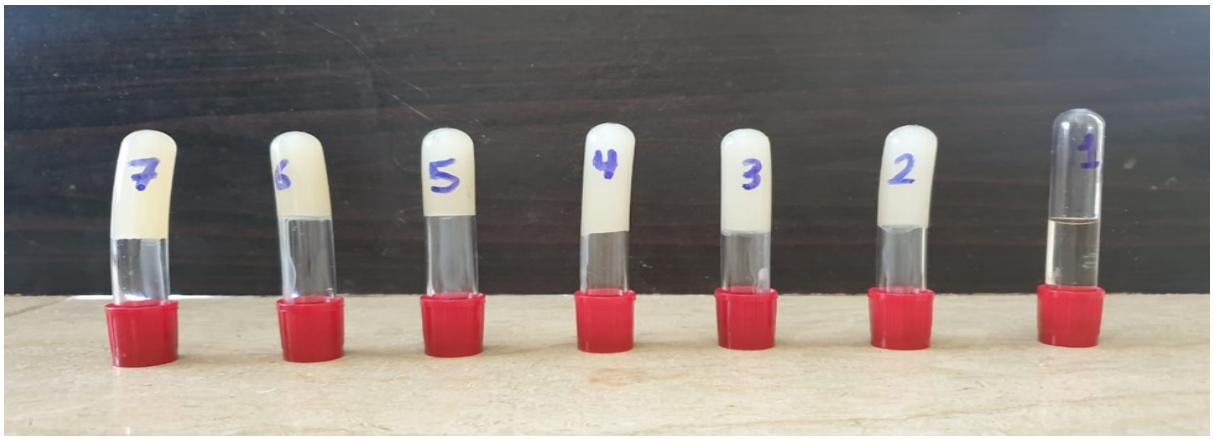

Figure 1. Different types of prepared sunflower oil and beeswax oleogel

1= sunflower oil, 2= 3% beeswax sunflower oleogel, 3= 5% beeswax sunflower oleogel, 4= 7% beeswax sunflower oleogel, 5= 5% beeswax sunflower oleogel + MOLE 0.15g, 6= 5% beeswax sunflower oleogel + MOLE 0.30g and 7= 5% beeswax sunflower oleogel + MOLE 0.45g.

Physico-chemical properties of the prepared oleogels gelation time

The organogels in the filled glass tubes were melted completely in a water bath (90°C) and kept for 2 hours for isothermal setting. The tubes were then taken out of the water bath and brought to room temperature, and in the in the meantime, a timer was started. When the tubes were turned 90° and no flow was observed, the time was recorded as the gelation time (Yılmaz et al., 2015).

Oil binding capacity (OBC)

1 ml of the melted organogel was transferred into a pre-weighed Eppendorf tube (a) and then placed in a refrigerator at 4 °C for 1 hour. After refrigeration, the tube was weighed again (b). The tube was then centrifuged at 9,167g for 15 minutes at room temperature (25 °C). Following centrifugation, the tube was inverted onto a paper cloth to drain any

excess liquid oil. After the drainage process, the tube was weighed once more (c) (Yılmaz et al., 2015). That follows:

$$C = Amax * V * D * 10^{4} / A^{1\%} 1cm * W$$

Total Phenolic Content (TPC)

The total phenolic content was determined by the Folin- Ciocalteu method according to (Naeem et al., 2019). The absorbance was measured at 765 nm after 30 min. the results were expressed as mg of Gallic acid equivalent (GAE) per 100 g of oil sample.

Total Flavonoids content (TFC)

The aluminum chloride method was used for the determination of the total flavonoid content according to (Khatiwora et al., 2010). Absorbance at 415 nm was recorded after 30 minutes of incubation. The concentrations of flavonoids were calculated as mg quercetin equivalent /100g of sample.

Physio-chemical parameters of crude and enriched oil samples

Refractive index, Peroxide value and Iodine number were determined according to (AOAC, 2016). And acid value was determined according to (PN-ISO 660:2020).

Conjugated constituents

Values of specific extinctions at 232 nm（K232 and 270 nm（K270）for conjugated dienes and trienes, were determined according to **(**PN-EN ISO 3656:2011).

Antioxidant activity of oil samples by DPPH

The antioxidant activities of the oil samples were determined using 1,1-diphenyl-2-picry lhydrazyl (DPPH) as reported by (Zahran and Najafi 2020). The absorbance of the mixture was recorded at 517 nm, and the percentage inhibition was calculated from the following equation:

%DPPH = [(Abs. control - Abs. sample) / Abs. control] ×100

IC_{50} values of the plant extracts were calculated from the inhibition percent against concentration plot. The IC_{50} value indicates the concentration in µg/mL of the extract, which is required to scavenge 50% of DPPH free radicals.

Oxidative stability of oil samples by Rancimat method

Oxidative stability of oils was evaluated according to **(**Salta et al., 2007) using Rancimat 679 apparatus, a sample weighs 5g set at constant temperature 100°C with an air flow of 20 L/h and measures the induction period (by hours).

Fatty acids composition: were converted into methyl ester and determined by GC according to (PN-ISO 12966-2:2017).

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Preparation of salad dressing

Mustard-vinaigrette salad dressing were prepared with an oil: vinegar ratio of 3:1 according to methods of **(**Gendreau and Ruiz, 2019). The other ingredients include: 5% mustard, 4% garlic powder, 1% black paper powder, 5% salt, 10% sugar. All ingredients were gentle stirred and mixed at 60 rpm with a homogenizer at room temperature for 10 minutes. All samples were kept at 5°C for7 days.

Sensory evaluation

Samples were evaluated using a 9-point hedonic scale (9- extremely liked; 1- extremely disliked), when to their general appreciation. A total of ten panelists from food technology research institute were asked to make their evaluations on the basis of appearance, taste, aroma, texture and overall acceptability, (Mihov et al., 2012).

Statistical analysis

The results were expressed as mean ± SD and the statistical analysis performed using one-way analysis of variance. The obtained data were exposed to proper statistical analysis according to the SPSS software (SPSS Inc., Chicago, IL, USA) (Gieraldin et al., 2022).

3. Results and discussions

Fruits and vegetable waste are exclusive due to their capacity for human consumption and the high value of bioactive components such as chlorophyll, carotenoids, polyphenols, flavonoids, and other antioxidants. The data in Table 1. show some of

these compounds determined in the dried powder of

Physico-chemical properties of the prepared oleogels gelation time

The organogels in filled glass tubes were completely melted in a water bath at 90°C and held for isothermal setting for 2 hours. The tubes were then removed from the water bath and allowed to cool to room temperature, at which point a timer was started. When the tubes were tilted 90° and no flow was observed, the time was recorded as the gelation time (Yılmaz et al., 2015).

Oil binding capacity (OBC)

One milliliter of melted organogel was transferred into a pre-weighed Eppendorf tube and weighed (recorded as weight a). The tube was then placed in a refrigerator at 4°C for 1 hour. After refrigeration, the tube was weighed again (recorded as weight b). Subsequently, the tube was centrifuged at 9,167 g for 15 minutes at room temperature (25°C). Following centrifugation, the tube was inverted onto a paper cloth to drain any excess liquid oil. After drainage, the tube was weighed once more (recorded as weight c) (Yılmaz et al., 2015). The OBC values were then calculated using the specified equation:

$$\%\ Released\ oil = \frac{(b-a)-(c-a)}{(b-a)} \times 100$$

% OBC =100 -% Released oil

Colors of the oleogel samples

The color of the samples was measured according to CIE standards using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Osaka, Japan). L, a*, and b* values were recorded (Yılmaz et al., 2015).

The centrifuge stability test

The centrifuge stability test was conducted by applying 1300× g centrifugation force for 15 min at room temperature to the 5g oleogel sample placed in tubes, and then determining the stability of the gel by visual inspection (Yilmaz and Demirci, 2021).

Melting point

The melting point of the oleogels was determined determined following (Zampouni et al., 2022). Briefly, 7 g aliquots of molten oleogels were transferred into 10 mm diameter glass tubes. Following the same storage protocol as other analyses, the tubes were placed in a water bath at 38 °C and heated at a constant rate of 1 °C/min until completely melted. Two temperature readings were recorded during heating: the softening point (when the sample began to soften) and the clearing point (when the sample became entirely clear). The melting point was reported as the average of these two temperatures.

Firmness

The firmness of the oleogel samples was measured using a texture analyzer (CT3 4500, Brookfield, USA) with a 12.7-mm-diameter cylindrical probe at ambient temperature (~28°C). The oleogel samples (30g) were placed in a 50-mL glass beaker and stored in a fridge at 5 ºC for 24 h., then were directly taken one by one and penetrated at a probe speed of 0.2 mm/s and a penetration depth of 5 mm. The firmness was calculated as the maximum penetration force. All measurements were performed in triplicate (Ögütcü and Yilmaz, 2014).

Fatty acid composition

Fatty acid profiling was conducted by analyzing their corresponding methyl esters through gas chromatography. A CPWax 52CB column, measuring 30 meters in length with an inner diameter of 0.25 millimeters and a film thickness of 0.25 µm, was employed for this purpose. Helium served as the carrier gas, flowing at a rate of 1 mL per minute. The temperature settings for the oven, injector, and detector were established at 170°C, 200°C, and 230 °C, respectively. Each analysis involved the injection of 1µL of the sample in a split mode with a split ratio of 1:50 (Besbes et al., 2004).

Phenolic extraction from oleogel

Extraction of phenols from oleogel: (Steel et al., 2005) technique was used to process 50g of oleogel via a glass column filled with silica gel (60-Å pore diameter). To prepare the column for sample introduction, it is first conditioned with a combination of hexane and methanol (1:1 V:V) and then washed with hexane and ethyl acetate (9:1 V:V). The phenolic fraction is extracted into

methanol by inserting the oil sample, which has been dissolved in hexane, into the column. To obtain a concentrated extract, the extract is collected after going down the column and the solvent is extracted under vacuum at 40 °C. To avoid oxidation, these extracts are flushed with nitrogen and then stored at -20 °C.

Total phenolic content (TPC)

100 µL of samples and standards (gallic acid) were added to 750 µL of Folin's reagent and 750 µL of 7% Na_2CO_3 and incubated for 45 min at room temperature in the dark. The absorbance was then read at 765 nm using a spectrophotometer (Analytik Jena Specord 250). The concentration was measured using gallic acid as a standard, and the results were expressed as mL gallic acid equivalents (GAE)/g sample (Attard, 2013).

Antioxidant activity using the DPPH assay

0.1mL of samples were mixed with a 0.2 mM DPPH solution (2mL). A control was also performed simultaneously. In the dark, the samples were kept for 1/2 h. After that, at 517 nm, the absorbance was measured using a spectrophotometer (Analytik Jena Specord 250) (Boly et al., 2016). The DPPH radical-scavenging activity was determined using the provided equation.

DPPH antioxidant activity (%) = (control ab-sample ab)/(control ab) ×100

Where control ab represented the absorbance of the DPPH working solution without the sample, and sample ab corresponded to the absorbance of the DPPH working solution when mixed with samples.

Induction period (INP) by Rancimat

The induction period is a test designed to assess the relative stability of an oil sample. In this analysis, the Metrohm® 743 Rancimat apparatus was employed. Initially, 3600 mg of oil was weighed and placed in a block (110°C). A continuous flow of air, at a rate of 20 liters per hour, was passed through the sample (Salama et al., 2020).

Acid and peroxide values

The acid value (AV) and peroxide value (PV) were determined according to the methods Cd 3d-63 and Cd 8b-90, respectively (AOCS 2017).

Sensory evaluation

The sensory evaluation of the oleogel and biscuit samples was done by 20 panelists from the Staff of the Food Technology Research Institute, Sakha Station, Kafrelsheikh, Egypt (Yılmaz and Öğütcü 2015).

Biscuit preparation

The method of (Adeyemo et al., 2022) was employed with moderate modification. 80 g of fat (margarine and oleogels (Table A) were added to the flour and 120 mL of water was used to mix all the ingredients to form a smooth dough. The dough was rolled out by hand on a tray (5 mm in height) and cut with the aid of a rectangle-shaped biscuit cutter (4.9 cm in diameter). The dough pieces neatly arranged on a baking tray laced with buttered aluminum foil were baked (160±2°C for 30 min) in a preheated Unox steam convection oven and cooled to room temperature for 30 min. Biscuits were packed in airtight, thick, zip-seal food bags, placed in low-density polyethylene bags, and preserved at 4±2°C.

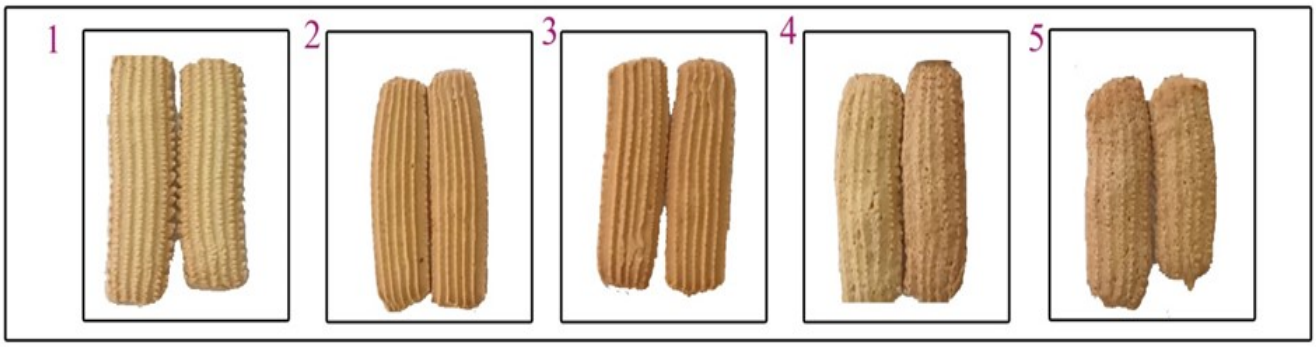

Figure 2. Prepared biscuits from different types of prepared sunflower oil and beeswax oleogel

1= control biscuits, 2= 5% beeswax sunflower oleogel (50%), 3= 5% beeswax sunflower oleogel (100%), 4= 5% beeswax sunflower oleogel+0.30g MOLE (50%), 5= 5% beeswax sunflower oleogel + MOLE 0.30g MOLE (100%).

Table A. Fat type and its amount used in prepared biscuits

Sample	Fat type
Control	100% margarine
Biscuit 1 (SFOBW5 50%)	50% margarine + 50% SFOBW5
Biscuit 2 (SFOBW5 100%)	100% SFOBW5
Biscuit 3 (SFOBW5 50% + MOLE 0.30 g)	50% margarine + 50% SFOBW5
Biscuit 4 (SFOBW5 100% + MOLE 0.30 g)	100% SFOBW5

SFOBW5= Sunflower oil + beeswax 5%

3. Results and discussion

Physico-chemical and antioxidant properties of sunflower oil-beeswax oleogels

Table 1. summarizes the measured physicochemical properties of the oleogels. Gelation time, defined as the total time required for a melted oleogel to solidify under specific conditions, decreased with increasing beeswax concentration. SFOBW7 exhibited the fastest gelation time (8.25 min) compared to SFOBW3 (20.34 min). This aligns with previous research by (Mattice and Marangoni 2017) and (Scharfe and Flöter 2020), who reported that gelation time is influenced by organogelator type and concentration, oil type, melting and cooling conditions, and external factors like shear and ultrasound. The observed decrease in gelation time suggests that higher beeswax concentrations promote faster formation of the oleogel structure, likely due to the presence of more nucleation sites for gelation by the beeswax. Similar results were obtained by (Yılmaz and Öğütcü 2014) who reported gelation times of 18.50, 9.70, and 7.00 min for hazelnut oil with 3%, 7%, and 10% beeswax, respectively. Oil binding capacity (OBC) increased with increasing beeswax content (Table 1), indicating that oleogels with more beeswax can retain more oil within their structure. This can be attributed to the formation of a more robust network by the higher beeswax concentration, effectively entrapping the oil.

This finding is consistent with the work of (Ögütcü and Yilmaz 2014), who observed an increase in OBC from 40.82% to 93.41% when carnauba wax content was raised from 3% to 10%. As a measure of oil entrapment ability, OBC can significantly impact the perceived texture, spreadability, and stability of the oleogels (Co and Marangoni, 2012). All oleogel samples displayed stability upon centrifugation (Table 1), indicating a strong resistance to disruption under stress (Yilmaz and Demirci, 2021). This is a valuable property for food applications where mechanical stability is crucial.

Textural Properties

In many food products, the textural properties of the solid fat are paramount for quality. They contribute to shortening, aeration, texture, flakiness, lubrication, moisture retention, shelf-life extension, and flavor (O'brien, 2008). As expected, firmness (N) increased with increasing beeswax concentration (Table 1). SFOBW3 exhibited a firmness of 1.50 N, which progressively increased to 2.45 N for SFOBW7. This is because a higher beeswax content translates to a stronger gel network, resulting in a firmer texture. Similar observations of increasing firmness with increasing oleogelator content were reported by (Yılmaz and Öğütcü 2014). The melting point data in Table 1. also demonstrates a rise with increasing beeswax content. This aligns with the formation of a more stable gel network that requires higher temperatures to melt. The color of an oleogel is the result of the ingredients used. The increase in L value (40.58, 44.39, and 55.95 for SFOBW3, SFOBW5, and SFOBW7, respectively) with higher beeswax content indicated that the oleogel became lighter (Ögütcü and Yilmaz 2014). Changes in b values suggested alterations in yellow color components, which could be due to the inherent color of beeswax. It could be quite possible to modify the color of the organogels by adding oil-soluble colorants to the stock oil. Depending on where and how the products will be used, a color selection is possible (Table 1). Acid (AV) and peroxide values (PV) are indicators of oil quality (Salama et al., 2020). No significant differences were reported in AV

among all samples. The slight increase in PV (4.89 mEq. O_2/kg) with higher beeswax content (7%) might indicate a minimal increase in acid values and peroxides, but values were still within acceptable ranges for edible oils. Adding beeswax to sunflower oil caused an increase in total phenolic content (TPC) (14.07, 14.65, and 14.51 mg GAE/100g for SFOBW3, SFOBW5, and SFOBW7, respectively) in comparison with sunflower oil without beeswax (12.05 mg GAE/100g). The same results were reported for antioxidant activity (DPPH). It increased with the presence of beeswax content in comparison with sunflower oil alone. It reached about 37.69, 38.25, and 38.68% for SFOBW3, SFOBW5, and SFOBW7 (Table 1).

Table 1. Physico-chemical and antioxidant properties of sunflower oil-beeswax oleogel

Characteristics	Sunflower oil	SFOBW3	SFOBW5	SFOBW7
Gelation time (min)	-	20.34±0.25^a	11.56±0.12^b	8.25±0.05^c
Oil binding capacity (%)	-	80.54±1.35^b	89.89±1.05^a	91.35±0.97^a
Centrifuge stability	-	Stable	Stable	Stable
Firmness (N)	-	1.50±0.21^c	2.06±0.15^b	2.45±0.10^a
Melting point	-	44.68±1.22^c	48.72±0.75^b	52.48±1.96^a
Color L	-	40.58±0.57^c	44.39±0.86^b	55.95±0.94^a
Color a	-	-2.68±0.09^a	-1.57±0.10^b	-0.55±0.15^c
Color b	-	2.15±0.12^c	3.56±0.54^b	5.48±0.21^a
AV (mg KOH/g sample)	0.31±0.05^a	0.34±0.04^a	0.39±0.10^a	0.33±0.08^a
PV (mEq.O_2/kg sample)	4.32±0.23^a	4.33±0.19^a	4.59±0.24^a	4.89±0.10^a
TPC (mg GAE/100g)	12.05±0.24^b	14.07±0.34^a	14.65±0.33^a	14.51±0.15^a
DPPH (%)	30.28±1.25^b	37.69±1.78^a	38.25±1.11^a	38.68±1.94^a
Rancimate (hr)	9.56±0.58^a	10.05±0.11^a	10.00±0.23^a	9.88±0.10^a

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW3= Sunflower oil + beeswax 3%, SFOBW5= Sunflower oil + beeswax 5%, SFOBW7= Sunflower oil + beeswax 7%.

Fatty acid composition of sunflower oil-beeswax oleogels

The palmitic and behenic acid contents were relatively stable across all samples, suggesting that the addition of beeswax did not significantly alter the palmitic acid content in the oleogel. There was a significant increase in stearic acid content with higher beeswax concentrations. It reached about 4.20% for SFOBW7, while it was 3.77% for sunflower oil without beeswax (Table 2). Stable amounts of oleic, linoleic, and linolenic acids were reported when using different amounts of beeswax. These results were in line with (Yılmaz et al., 2015), who found that sunflower and beeswax oleogels were shown to be quite stable against oxidation during storage.

Sensory evaluation of sunflower oil-beeswax oleogels

It has been stated that the bottom line in the market success of any new food is its consumer acceptance (Stone et al., 2020). The oleogel with 3 and 5% beeswax showed the highest score for appearance without any significant differences between them. However, the appearance score decreased significantly for the 7% beeswax oleogel. This suggested that higher beeswax content may negatively impact the visual appeal of the oleogel. The 5% beeswax oleogel scored the highest in spread ability and was significantly better than the other two samples. This indicated that the consistency and ease of spreading improve at an intermediate beeswax concentration but may deteriorate at higher levels. There was a significant difference in taste across the samples. The oleogel with 3% beeswax was rated the highest (8.69), followed by the 5% (8.05) and the 7% (7.55) beeswax oleogels. This trend suggests that increasing the beeswax content may negatively impact the taste of the oleogel. All oleogel samples scored similarly in terms of odor, indicating that the beeswax

concentration did not significantly affect the odor of the oleogels. The oleogel with 5% beeswax was rated the highest (8.99) in overall acceptability, suggesting it was the most preferred. The acceptability decreased significantly for the 7% beeswax sample (8.11) and the 3% beeswax sample (8.65. In general, the sensory evaluation suggests that the oleogel with 5% beeswax appears to offer the best balance sample (Table 3).

Table 2. Fatty acids composition (%) of sunflower oil-beeswax oleogel

Fatty acid (%)	Sunflower oil	SFOBW3	SFOBW5	SFOBW7
Palmitic	6.48±0.25^{a}	6.40±0.10^{a}	6.59±0.09^{a}	6.77±0.11^{a}
Stearic	3.77±0.10^{b}	3.88±0.10^{b}	4.08±0.13^{a}	4.20±0.08^{a}
Behenic	0.86±0.03^{a}	0.95±0.05^{a}	0.89±0.08^{a}	0.85±0.07^{a}
Oleic	28.16±0.21^{a}	28.09±0.09^{a}	27.88±0.12^{b}	27.89±0.26^{ab}
Linoleic	56.32±0.75^{a}	54.78±0.43^{b}	53.49±0.87^{b}	53.22±1.58^{b}
Linolenic	0.35±0.01^{a}	0.31±0.02^{a}	0.33±0.01^{a}	0.34±0.00^{a}
Palmetoleic	0.06±0.00^{a}	0.05±0.00^{a}	0.06±0.00^{a}	0.04±0.01^{a}
Saturated fatty acids	11.11	11.23	11.56	11.82
Unsaturated fatty acids	84.89	83.23	81.76	81.49
Total fatty acids	96.00	94.46	93.32	93.31

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW3= Sunflower oil + beeswax 3%, SFOBW5= Sunflower oil + beeswax 5%, SFOBW7= Sunflower oil + beeswax 7%.

Table 3. Sensory evaluation of sunflower oil-beeswax oleogel

Sensory attributes	SFOBW3	SFOBW5	SFOBW7
Appearance	8.46±0.14^{a}	8.55±0.21^{a}	8.01±0.09^{b}
Spreadability	7.68±0.24^{b}	8.69±0.22^{a}	7.88±0.38^{b}
Taste	8.69±0.24^{a}	8.05±0.31^{b}	7.55±0.22^{c}
Odor	8.77±0.21^{a}	8.69±0.11^{a}	8.64±0.19^{a}
Acceptability	8.65±0.15^{b}	8.99±0.11^{a}	8.11±0.13^{c}

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW3= Sunflower oil + beeswax 3%, SFOBW5= Sunflower oil + beeswax 5%, SFOBW7= Sunflower oil + beeswax 7%.

Physico-chemical and antioxidant properties of sunflower oil-beeswax oleogels with MOLE

The addition of *Moringa oleifera* leaves extract (MOLE) did not significantly affect the gelation time of the oleogel. This suggested that the extract, in the given concentrations, did not impact the speed of oleogel formation. Oil binding capacity (OBC) remained significant across all samples, indicating that the addition of MOLE did not significantly influence the oleogel's ability to retain oil. Also, all oleogel samples, regardless of the extract concentration, showed stability under centrifugation, suggesting good mechanical integrity of the oleogel matrix (Table 4). Firmness was reported as constant across all samples, which suggests that MOLE did not affect the physical hardness of the oleogel. The melting point slightly decreased with the addition of MOLE in comparison with the control. This suggested MOLE had a minimal effect on the thermal properties of the oleogel (Table 4). The L value (lightness) significantly increased with higher concentrations of the extract (66.12 for 0.45g), indicating a lighter oleogel with more extract. The a value (red-green spectrum) decreased significantly with increasing MOLE concentration (-7.72 for 0.45g). The b value (yellow-blue spectrum) remained relatively constant between samples with MOLE (4.12, 4.09, and 4.10 for 0.15, 0.30, and 0.45g, respectively) and higher than samples without MOLE (3.56), indicating minimal impact on yellow-blue coloration. AV did not show any significant differences between samples with or without MOLE. On the other hand, PV significantly

decreased as more MOLE was added (4.09, 3.58, and 3.61 for 0.15, 0.30, and 0.45g, respectively), indicating improved oxidative stability with higher MOLE concentrations. This was in line with the results reported by (Martinović et al., 2020), who indicated that an oleogel system with phenolic compounds could inhibit lipid peroxidation more than an emulsion or oil with the same amount of phenolic compounds. TPC significantly increased with higher concentrations of MOLE (20.95, 29.48, and 40.26 mg/100g with 0.15, 0.30, and 0.45g of MOLE, respectively) (Table 4). This was expected since the MOLE was known for its high phenolic content (Owon et al., 2021). Antioxidant activity (DPPH) significantly increased with the concentration of MOLE, reaching its highest value with 0.45 g (60.98%). This aligned with the increase in TPC, as phenolic compounds were known for their antioxidant properties. The induction period (INP) increased significantly with the addition of MOLE, especially at the highest concentration (0.45g), which reached about 20.54 h. This suggested that the extract significantly enhanced the oxidative stability of the oleogel, which could be attributed to the antioxidant properties of the phenolic compounds present in the extract (Table 4).

Table 4. Physico-chemical and antioxidant properties of sunflower oil-beeswax oleogel with *Moringa oleifera* leaves extract (MOLE)

Characteristics	SFOBW5	SFOBW5 + MOLE 0.15g	SFOBW5 + MOLE 0.30g	SFOBW5 + MOLE 0.45g
Gelation time	11.56±0.12^{a}	11.88±0.25^{a}	11.65±0.36^{a}	11.99±0.46^{a}
Oil binding capacity	89.89±1.05^{a}	90.57±2.06^{a}	90.68±1.75^{a}	89.88±0.69^{a}
Centrifuge stability	Stable	Stable	Stable	Stable
Firmness (N)	2.06±0.15^{a}	2.16±0.02^{a}	2.09±0.04^{a}	2.11±0.09^{a}
Milting point	48.72±0.75^{a}	47.36±0.55^{b}	46.58±0.22^{c}	47.36±0.48^{b}
Color L	44.39±0.86^{c}	46.58±1.25^{c}	58.69±0.75^{b}	66.12±1.02^{a}
Color a	-1.57±0.10^{a}	-5.26±0.11^{b}	-7.65±0.19^{c}	-7.72±0.22^{c}
Color b	3.56±0.54^{b}	4.12±0.36^{a}	4.09±0.19^{a}	4.10±0.35^{a}
AV (mg KOH/g sample)	0.39±0.10^{a}	0.33±0.02^{b}	0.38±0.02^{a}	0.35±0.03^{a}
PV (mEq.O_2/kg sample)	4.59±0.24^{a}	4.09±022^{b}	3.58±0.15^{c}	3.61±0.19^{c}
TPC (mg GAE/100g)	14.65±0.33^{d}	20.95±0.55^{c}	29.48±1.01^{b}	40.26±0.94^{a}
DPPH (%)	38.25±1.11^{d}	45.21±1.48^{c}	51.69±1.04^{b}	60.98±0.79^{a}
Rancimate (hr)	10.00±0.23^{d}	11.06±0.58^{c}	13.68±0.74^{b}	20.54±1.2^{a}

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW5= Sunflower oil + beeswax 5%, MOLE= *Moringa oleifera* leaves extract.

Fatty acid composition of sunflower oil-beeswax oleogels with MOLE

In general, all fatty acids (except behenic acid) did not show significant differences across all samples. This indicated that the addition of MOLE did not significantly alter the fatty acid profile of the oleogel. The values for SFA, USFA, and TSFA remained relatively consistent across all samples, suggesting that the addition of MOLE did not significantly impact the overall saturation level of the fatty acids in the oleogel (Table 5).

Sensory evaluation of sunflower oil-beeswax oleogels with MOLE

Table 6. shows the impact of adding different concentrations of MOLE to oleogel made from sunflower oil and 5% beeswax. The sensory attributes evaluated were appearance, spread ability, taste, odor, and overall acceptability. The oleogel with 0.30g of MOLE scored the highest in appearance, suggesting it was visually more appealing than the others. The lowest score was observed for the oleogel with 0.45g of MOLE, indicating that too much extract might negatively impact the visual appeal. The highest score for spread ability was observed in

the oleogel containing 0.45 g of MOLE had the highest spread ability score, suggesting that adding more extract enhanced the spread ability. Taste ratings were highest for the oleogel with 0.30 g of MOLE; nevertheless, there was no statistically significant difference between it and the other two lower doses (control and 0.15 g). However, there was a significant decrease in taste quality with the highest extract concentration (0.45 g), suggesting that too much extract might adversely affect the taste. There were no significant differences in the odor ratings across all samples. For overall acceptability, the oleogel with 0.30 g of MOLE was rated the highest. The lowest acceptability is for the 0.45 g extract concentration, while the other two are rated in between.

Table 5. Fatty acids composition (%) of sunflower oil-beeswax oleogel with MOLE

Fatty acid	SFOBW5	SFOBW5 + MOLE 0.15 g	SFOBW5 + MOLE 0.30 g	SFOBW5 + MOLE 0.45 g
Palmitic	6.59±0.09^{a}	6.58±0.25^{a}	6.50±0.33^{a}	6.60±0.18^{a}
Stearic	4.08±0.13^{a}	4.10±0.09^{a}	4.15±0.08^{a}	4.12±0.10^{a}
Behenic	0.89±0.08^{ab}	0.77±0.06^{b}	0.95±0.08^{a}	1.02±0.07^{a}
Oleic	27.88±0.12^{a}	27.99±1.65^{a}	27.79±1.45^{a}	27.80±1.28^{a}
Linoleic	53.49±0.87^{a}	53.40±1.58^{a}	53.55±1.78^{a}	53.70±1.99^{a}
Linolenic	0.33±0.01^{a}	0.30±0.02^{a}	0.29±0.01^{a}	0.34±0.03^{a}
Palmetoleic	0.06±0.00^{a}	0.05±0.00^{a}	0.04±0.02^{a}	0.06±0.01^{a}
SFA	11.56	11.45	11.60	11.74
USFA	81.76	81.74	81.67	81.90
TFA	93.32	93.19	93.27	93.64

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW5= Sunflower oil + beeswax 5%, MOLE= *Moringa oleifera* leaves extract.

Table 6. Sensory evaluation of sunflower oil-beeswax oleogel with MOLE

Sensory attributes	SFOBW5	SFOBW5 + MOLE 0.15 g	SFOBW5 + MOLE 0.30 g	SFOBW5 + MOLE 0.45 g
Appearance	8.55±0.43^{ab}	8.69±0.45^{ab}	9.13±0.32^{a}	8.22±0.52^{d}
Spreadability	8.69±0.54^{a}	8.22±0.48^{b}	8.43±0.49^{ab}	8.69±0.34^{a}
Taste	8.05±0.22^{ab}	8.20±0.30^{a}	8.56±0.28^{a}	7.16±0.24^{b}
Odor	8.69±0.09^{a}	8.25±0.12^{b}	8.09±0.11^{b}	8.12±0.09^{b}
Acceptability	8.99±0.21^{b}	8.77±0.18^{b}	9.11±0.09^{a}	8.50±0.15^{c}

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW5= Sunflower oil + beeswax 5%, MOLE= *Moringa oleifera* leaves extract.

Sensory evaluation of biscuits prepared from sunflower oil-beeswax oleogels with and without MOLE

Table 7. shows the results of a sensory evaluation of various formulations of oleogels, including combinations of sunflower oil, beeswax, and MOLE extract. The evaluations were based on different sensory attributes: taste, color, odor, texture, mouthfeel, and overall acceptability. The control product, presumably a standard formulation without any oleogel or extract, scored highly in taste, had decent scores in color and odor, but showed relatively lower scores in texture, mouthfeel, and overall acceptability. Adding beeswax oleogel (50%) seemed to enhance the sensory properties, particularly improving the mouthfeel and acceptability. Using the beeswax oleogel (100%) significantly lowered the scores, especially in color, texture, mouthfeel, and overall acceptability. The same effect was reported with oleogel produced by adding MOLE. Biscuits prepared with 50% oleogel showed better sensory evaluation results than biscuits prepared with 100% oleogel. Similar results were obtained by (Frolova et al., 2021), who prepared cookies from beeswax and its fractions and sunflower oil.

Table 7. Sensory evaluation of biscuit prepared from sunflower oil-beeswax oleogel with and without MOLE

Sample	Taste	Color	Odor	Texture	Mouthfeel	Overall acceptability
Control	9.67±0.58^{a}	9.00±1.00^{ab}	8.17±1.89^{a}	8.00±1.73^{abc}	7.67±1.08^{ab}	7.67±1.22^{abc}
Biscuit 1 (SFOBW5 50%)	10.00±0.00^{a}	9.67±0.58^{a}	9.00±1.73^{a}	8.67±0.58^{ab}	9.33±0.58^{a}	9.50±0.50^{a}
Biscuit 2 (SFOBW5 100%)	8.30±0.58^{b}	6.00±0.00^{c}	8.00±1.00^{a}	7.50±0.05^{c}	6.33±0.58^{b}	6.00±0.00^{c}
Biscuit 3 (SFOBW5 50% + MOLE 0.30g)	9.83±0.29^{a}	9.33±0.29^{ab}	8.00±1.73^{a}	9.33±0.58^{a}	9.33±0.58^{a}	9.33±0.58^{ab}
Biscuit 4 (SFOBW5 100% + MOLE 0.30g)	8.00±1.00^{b}	7.33±1.53^{bc}	8.67±1.15^{a}	7.50±0.05^{bc}	6.33±0.58^{b}	6.67±0.58^{bc}

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW5= Sunflower oil + beeswax 5%, MOLE= *Moringa oleifera* leaves extract.

Color properties and antioxidant properties of biscuits prepared from sunflower oil-beeswax oleogel with and without MOLE

From the data in Table 8., shows that the highest L value was observed in biscuits prepared from a 100% oleogel + MOLE formulation, this suggests that the extract might contribute to a lighter appearance. There were no significant differences in the a* value between all samples except for biscuits 1 and 2, indicating that the red-green color balance remained fairly consistent between the formulations. The b* value, indicating yellowness, increased significantly with the addition of MOLE, reaching the highest value in the 50 and 100% oleogel + MOLE formulations at about 50.32 and 51.25, respectively. This suggested that the extract might contribute to a more yellow appearance. The addition of MOLE significantly enhanced the biscuits' antioxidant properties. This was evident by the increased total phenolic content (TPC), with the highest value (30.68 mg/100g) observed in biscuits formulated with 100% oleogel and MOLE. Higher TPC is associated with potential health benefits due to the antioxidant properties of phenolic compounds. Similarly, the DPPH assay revealed a significant increase in antioxidant activity with MOLE. The 100% oleogel + MOLE formulation again displayed the strongest antioxidant activity (51.09%). These findings suggest that the phenolic compounds in MOLE effectively scavenge free radicals, thereby elevating the overall antioxidant capacity of the

Table 8. Color and antioxidant properties of biscuit prepared from sunflower oil-beeswax oleogel with and without MOLE

Sample	L*	a*	b*	TPC (mg GAE/100g)	DPPH (%)
Control	66.54±0.54^{d}	8.02±1.02a	35.64±1.10d	17.35±0.54c	36.84±1.12d
Biscuit 1 (SFOBW5 50%)	68.39±0.48^{c}	7.87±0.45ab	42.36±0.69c	18.11±0.25c	39.58±1.20c
Biscuit 2 (SFOBW5 100%)	68.99±0.69^{c}	7.45±0.41^{b}	46.32±1.04^{b}	18.49±0.85^{c}	40.12±1.09^{c}
Biscuit 3 (SFOBW5 50% + MOLE 0.30 g)	70.36±0.25^{b}	8.14±0.39^{a}	50.32±0.88^{a}	25.63±0.77^{b}	45.69±0.87^{b}
Biscuit 4 (SFOBW5 100% + MOLE 0.30 g)	72.89±0.36^{a}	8.12±0.58^{a}	51.25±1.36^{a}	30.68±0.84^{a}	51.09±1.54^{a}

Values are means ± SD; means having the different case letter(s) within a row are significantly different at P≤0.05. SFOBW5= Sunflower oil + beeswax 5%, MOLE= *Moringa oleifera* leaves extract.

4. Conclusion

The incorporation of beeswax into sunflower oil to create oleogels markedly enhanced the physical and functional properties of the oleogels, making them suitable for biscuit production. The addition of MOLE further improved the antioxidant properties of the biscuits. Oleogels with a 5% beeswax concentration appeared to offer an optimal balance of textural and sensory attributes, making them an excellent fat substitute in bakery products. The study highlighted the viability of oleogels as a healthier alternative to traditional fats in food applications, providing a pathway towards the development of nutritionally enhanced and consumer-acceptable bakery products.

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