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Original Article

Fabrication of High-Fat Content Products Using Beeswax Oleo Gel to Reduce Total Energy Content and Trans Fats: Developing Healthier Alternatives

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ABSTRACT

Commercial products with high-fat content have been seeking to create healthier versions. However, the process remains challenging in terms of nutritional value, sensory characteristics, and physicochemical properties. Oleo gel has already shown promising results in addressing these issues and can compete with standard commercial versions. This study aimed to produce commercial margarine and mayonnaise using various formulations of beeswax (BW) oleo gel (OLEO), with up to 25% BW incorporated. The objective was to create healthier product versions by fully replacing traditional fats. This approach can enhance the commercial product by eliminating trans fats and significantly reducing energy content while retaining physicochemical and sensory properties comparable to those of their conventional counterparts. The BW oleo gel samples were compared to commercial versions in terms of texture, viscosity, oxidative and storage stability, melting point, sensory properties, and total energy content. Results, such as oil binding capacity (OBC), indicated that both fabricated products exhibit robust storage stability and acceptable oxidative stability for a specific storage duration. Texture analysis revealed a similar texture profile, although samples made with higher concentrations of BW oleo gel exhibited a harder texture. The calculation of energy differences showed a significant reduction in total energy content of up to 65%. Additionally, the fabricated samples recorded higher sensory and non-sensory properties, even with BW oleo gel versions with a melting point exceeding 45°C. The formulation technique and quenching process helped ensure that the mouthfeel of the fabricated products was comparable to that of commercial versions. Overall, the formulation using this effective fat replacement and energy reduction presents a promising opportunity to offer healthier versions of high-fat products and may contribute to reducing food-related chronic diseases.

Keywords: : Product fabrication, Health, Calorie reduction, Beeswax Oleo gel.

1 Introduction

Many high-fat, high-calorie foods are staples in our daily diets. The fast pace of life often makes these options more convenient. Foods rich in saturated fatty acids and trans fatty acids, or simply high in calories, are particularly common. Margarine and mayonnaise are two widely consumed items. Both are calorie-dense due to their lipid content. Margarine, in particular, is high in calories and contains trans fatty acids due to the hydrogenation process [1]. This process is important for converting the oil into a form similar to shortening, enhancing its physicochemical and sensory attributes [2]. It is worth noting that the final product is enhanced in a way that generally has a lower content of unhealthy components compared to traditional shortening, including saturated fatty acids and cholesterol. However, hydrogenation can produce trans fats, which have been proven to contribute to major health issues [3]. Mayonnaise is preferable to margarine because it contains vegetable oil rather than hydrogenated oil. However, due to its high oil content (around 80% oil phase), it is classified as a calorie-dense food. Long-term consumption of both products may be related to chronic diseases such as cardiovascular diseases, diabetes, hypertension, and stroke [4].

Research has shown that our eating habits and the consumption of a balanced diet play a crucial role in maintaining overall health and stability within the body, known as homeostasis. To optimize nutritional benefits, it is advisable to aim for a ratio of polyunsaturated fatty acids to saturated fatty acids that exceeds 4. This balance can help promote better heart health and reduce the risk of chronic diseases [5]. Although reducing or replacing fats can be one approach to achieving desired results, the extensive use of unsaturated vegetable oils does not deliver the essential physiochemical and sensory attributes necessary for optimal product quality. These attributes include crucial factors like

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visual appeal, textural integrity, and mouthfeel, all vital at room temperature for an enjoyable and satisfying food experience [6].

For decades, various approaches have been developed to achieve the goal of enhancing food products, including the incorporation of oil in its pre-emulsified state [7], encapsulation [8], and enzymatic interesterification [9]. One of the newest approaches is organogelation, which allows oil to create products with characteristics similar to traditional fats or shortenings. Over the past two decades, different organogelators and techniques have been used to replicate specific products with sensory and physicochemical characteristics that meet customer demand [10-12]. This innovative method can enable high-fat-content foods to reduce overall fat content while improving the fatty acid profile [9], thereby minimizing food-related health implications [13].

Waxes have demonstrated their potential as effective organogelators for producing stable oleo gels. Researchers have explored various waxes to create targeted products, including sunflower wax [14], rice bran wax [15], and candelilla wax[16]. Among these, BW has been recognized as a suitable stabilizing agent and a mid-range emulsifier, capable of forming stable and reliable oleo gels [6]. This type of wax has successfully undergone a rigorous approval process and has been certified by the Food and Drug Administration (FDA) under regulation 21 CFR 84 184.1973. It is also recognized within the European Union as E 901, a designation established in 1995 [14].

The literature indicates that BW-based oleo gels have promising applications in the production of high-fat content foods such as margarine, mayonnaise, cookies, and meat products [6, 17-21]. However, the oil phase replacement in these formulations can be either partial or complete. In cases of complete replacement, the concentration of BW used in the oleo gel does not exceed 10%.

This limitation means that only 10% of BW oleo gels are employed to replace the oil phase in the aforementioned products. While authors of these studies have reported promising results in reproducing products with similar physicochemical and sensory properties, the maximum concentration of BW in the oleo gels remains constrained to 10%. Consequently, this limitation restricts the potential reduction of saturated fats, trans fats, and total calorie intake in the resulting products.

In this study, we aimed to push the limits of BW OLEO by attempting full oil phase replacement with higher concentrations of BW. We used oleo gel with 10%, 17%, 20%, and 25% BW concentration to create two of the most popular high-fat content foods. The goal was to significantly reduce saturated fat, trans fat, and total energy intake while maintaining similar or at least closely comparable physicochemical, textural, and sensory characteristics to commercial products or products made with 10% BW oleo gel.

2 Materials and Methods

BW was donated by a university honey production project and is considered 100% natural. Sunflower oil and seasonings were purchased from the local market. Soya lethicine powder was purchased from Lecilite Ingredients Pvt. Ltd. Company with a phosphatidate concentration of 97% (USP-NF grade). Whey protein isolate (Hilmar™ 1090 instantized whey protein isolate) was purchased from Hilmar Ingredients company with a purity of 92% protein (dry basis).

2. 1 Beeswax purification

BW was obtained from a trusted beekeeper at the university campus to ensure the highest quality of BW, as it directly impacts the quality of the products being produced. It is then purified to obtain the highest quality. Purification is essential since the temperature change directly affects its quality [22]. The purification process was as follows: First, the rounded bottle containing the small pieces of beeswax was placed into a water bath set at 65°C. The water bath provides indirect heating to protect the quality of the beeswax. Once the beeswax was melted entirely, it was filtered into a silicone container. A fine sieve with double filtration was used to obtain the purest beeswax. After that, it was placed at room temperature until it is hard enough and put into a refrigerator until the oleo gelation process.

2. 2 Oleo gelation process

The following method was used to produce a range of oleo gels with different concentrations of BW (10%, 17%, 20%, 25%), and they marked as 10% OLEO, 17% OLEO, 20% OLEO, and 25% OLEO. Firstly, a specific amount of previously purified BW was placed in a beaker on a hotplate set to 65ºC. Once the BW was completely melted, it was stirred using a blender (high-speed laboratory digital electric mixer) at 1200 rpm, and a gradual addition of sunflower oil was crucial to ensure proper mixing with the BW. This process allowed the sunflower oil to be trapped inside the crystalline structure of the BW [17]. The mixture was stirred at 65ºC and a speed of 1200 rpm for 30 minutes. Finally, the mixture was covered to prevent air absorption in a dark room to prevent light penetration. Allowing the mixture to crystallize slowly as the temperature dropped below 65ºC. Crystallization was initiated at the bottom and proceeded into the core of the mix until it was entirely hardened (Figure 1). The oleo gel was finally stored in the refrigerator until use. Oleo gels with different concentrations of beeswax as an oleo-gelator were then used to fabricate margarine and mayonnaise.

Figure 1: The crystallization of BW during the oleo gelation process.

2. 3 Product formulation

The formulation process involves multiple attempts to develop a product by adjusting the inclusion, exclusion, or quantity of one or more ingredients at a time. This approach is essential for identifying and differentiating the most promising recipe from others. Consequently, the ones marked as "PROMISING" can be treated against the original recipe (control). Table 1 and Table 2 present the formulation attempts to determine the optimal concentration of each BW oleo gel for margarine and mayonnaise fabrication. For example, 25g of a 17%-BW OLEO demonstrated superior performance to 13g of the same formulation as a preferred model of margarine to be produced and compared to the control.

Table 1: Formulation aims to identify the most promising version of fabricated margarine for testing and comparison with commercial margarine.

Formulation								
BW	**oleo-gel (g) (BW%)**	**Skim milk (g)**	**iso protein (g)**	**lecithine (%)**	**Full fat milk (g)**	**water (g)**	**Researcher's remarks**	**Acceptability**
50g	-	-	-	-	20	30	emulsified well, hard, sandy taste	not acceptable
-	50 (50%)	-	-	-	20	40	hard texture, sandy taste	not acceptable
-	35 (50%)	-	-	-	15	50	hard texture, sandy taste	not acceptable
-	20 (25%)	-	-	-	25	50	As good as 2, but the milk separated a little after storage	not acceptable
-	25 (25%)	25	-	3	-	47	good color, texture, smooth, little palate stickiness	not acceptable
-	30 (25%)	-	-	-	25	50	good color, and texture, smoother than 3&4, little palate stickiness	poorly acceptable
-	25g (20%)	-	-	-	25	50	good color, texture, smooth, no palate stickiness	PROMISING
-	30g (20%)	25	-	3	-	47	good color, texture, smooth, little palate stickiness	not acceptable
-	25g (17%)	-	-	-	25	50	good colour, texture, smooth, no palate stickiness	PROMISING
-	13g (17%)	-	12	-	-	25	separated, sandy taste, iso not dissolved	not acceptable
-	13g (17%)	-	12	-	-	50	iso dissolved, smooth texture, but separated	not acceptable
-	20g (17%)	25	-	3	-	47	good colour, texture, smooth, little palate stickiness	not acceptable
-	25g (17%)	-	-	3	25	47	good colour, texture, smooth, no palate sickness	PROMISING
-	40g (10%)	-	-	-	20	40	good colour, texture, smooth, no palate stickiness	PROMISING

Note: formulation marked with "PROMISING" has been chosen in this study to compare with the commercial version.

Table 2: Formulation aims to identify the most promising version of fabricated mayonnaise for testing and comparison with commercial mayonnaise.

Note: formulation marked with "PROMISING" has been chosen in this study to compare with the commercial version.

Attempts	Bw	Oleo gel (g)	Egg yolk	Sugar (g)	Salt (g)	White vinegar (g)	Starch (g)	Red pepper (g)	Mustard (g)	Lethicin (g)	Water (g)	Remarks
1st	240	-	2	3	2	20	5	1	2	-	5	very viscose, sandy taste, separated
2nd	120	-	2	3	2	20	5	1	2	-	5	viscose, sandy taste, separated
3rd	-	25 of 25% oleo	1	3	2	20	5	1	2	-	5	separated
4th	-	25 of 25% oleo	1	3	2	20	10	1	2	-	5	weak system, light consistency
5th	-	25 of 25% oleo	2	3	2	20	15	1	2	1	5	not desired viscosity & texture
6th		11 of 25% oleo	3	3	2	20	5	1	2	-	-	PROMISING
7th	-	25 of 20% oleo	2	3	2	20	5	1	2	1	5	not standard consistency
8th	-	29 of 20% oleo	2	2	1	20	2.5	1	2	-	-	Poorly acceptable
9th		23 of 20% oleo	3	3	2	20	5	1	2	-	-	PROMISING
10th	-	29 of 17% oleo	2	2	1	20	2.5	1	2	-	-	Poorly acceptable
11th		22 of 17% oleo	3	3	2	20	5	1	2	-	-	PROMISING
12th	-	45 of 10% oleo	1	2	1	20	2.5	1	2	-	-	waxy & oily taste, not the desired texture
13th	-	45 of 10% oleo	2	2	1	20	5	1	2	1	-	Poorly acceptable
14th		45 of 10% oleo	3	3	2	20	5	1	2	-	-	PROMISING

2. 4 Product Preparation

Fabricated margarine was prepared according to the previous studies with a bit of modification [6, 23]. Four different types of margarine were developed with varying BW oleo gel content as follows: (10% OLEO, 17% OLEO, 20% OLEO, 25% OLEO), and marked MARG-10% OLEO, MARG-17% OLEO, MARG-20% OLEO, MARG-25% OLEO respectively. The oil and water phase's ingredients were determined by their ability to dissolve in each other. The oil phase consisted of oleo gel and lecithin, while the water phase consisted of full-fat milk, salt, and water (Table 1). The oil phase was placed in a beaker in a water bath set at 65°C. The Ultraturax was adjusted to homogenize the two phases while the water phase was gradually added. The blending process lasted for 5 minutes at 10,000 rpm. After this time, the mixture became smooth, and the process was stopped. The resulting margarine was cooled in icy water to quench the product (fabricated margarine) [19].

Mayonnaise was prepared using a particular recipe. A previous study mentioned a mixing technique, which has been modified slightly [24]. The ingredients in percentage are noted in Table 3. First, egg yolks were used instead of whole eggs blended with mustard for one minute. And then, starch was added and blended for another minute. While they were blinding, these materials were mixed together since egg yolk and mustard are strong emulsifiers, and starch is an excellent thickening agent. These three things help the final product have good stability. BW Oleo gel was added very slowly at the beginning while the blinder was operating, and then the rest of it was added. Slow addition helps the oleo gel hold the dispersed phase and continuous face together, create the emulsion system, and observe when the mixture reaches the desired consistency. Finally, seasonings were added to complete and provide a final taste and appearance. After adding all the ingredients, the blender mixed the mixture for 5 minutes to obtain the final texture. Four different fabricated mayonnaises were developed and marked as MAYO-10% OLEO, MAYO-17% OLEO, MAYO-20% OLEO & MAYO-25% OLEO.

Table 3: Ingredients of mayonnaise-based oleo gels and standard mayonnaise in percentage.

Samples	oil phase (as oil) w/w%	oil phase (as oleo gel) w/w%	egg yolk w/w%	sugar w/w%	salt w/w%	white vinegar w/w%	starch w/w%	red pepper w/w%	mustard w/w%
MAYO-10% OLEO	-	27.4	32.9	3.7	2.4	24.4	6.1	0.6	2.4
MAYO-17% OLEO	-	20.2	36.4	4	2.7	26.9	6.7	0.3	2.7
MAYO-20% OLEO	-	21.6	35.7	4	21.6	26.5	6.6	0.3	2.6
MAYO-25% OLEO	-	11.7	40.2	4.5	3	29.8	7.4	0.4	3
C.MAYO	80	-	7	0.5	1	9	1.5	0.2	1

2. 5 Oleo gel and product characterization

2. 5. 1 Structural observation

The digital microscope (Motic DM0405) was used to analyze the microstructure of different types of BW oleo gels and fabricated margarine and mayonnaise samples. This test aimed to investigate the intricate microstructure. A high-powered microscope fitted with a digital camera captured detailed microstructure images at three distinct magnifications: 4X, 10X, and 40X. This methodology was designed to provide deeper insights into how varying concentrations of beeswax in the oleo gel formulations influence the microstructural characteristics across different treatments. Examining these samples under different magnification levels has been aimed at uncovering the relationships between the composition of the BW oleo gels and their structural properties, ultimately enhancing our understanding of their functional performance.

2. 5. 2 Texture analysis

The CT3 texture analyzer model (AMETX BROOKFEIL), texture profile analysis (TPA) test dual cycle for both BW oleo gel and fabricated product samples was used to test their hardness, cohesiveness, springiness, and spreadability. For the first three properties, the TA15/1000 Cone, 30mm D, 45º probe was used, while for the spreadability test, the TA53 Cutting Wire, 33mm D, 40mm L probe was used. All samples were tested at refrigerator temperature. The sample containers used during the measurement were glass with an internal diameter of 5.5 cm and a depth of 3.4 cm. These standards were taken from previous studies with a bit of modifications. [25, 26].

2. 5. 3 Viscosity

The viscosity of all mayonnaise-based BW oleo gel samples was analyzed using a Brookfield DV-E Viscometer fitted with a suitable spindle (RV7) to ensure accurate measurements. Before conducting the test, the samples were allowed to warm up to room temperature, ensuring that the results would reflect the consistent characteristics of the mayonnaise. The test was performed at a designated shear rate (20 rpm), providing precise data on the flow behavior of each sample [27].

2. 5. 4 Oil binding capacity (OBC)

This test assesses the stability of BW oleo gel and fabricated products. It evaluates the ability of the system to maintain its structure within the crystalline network, detecting any phase separation, which is critical in colloidal systems, and this test is capable of identifying such occurrences.
BW oleo gels have been tested for their OBC following the previous studies [26, 28]. First, 5 grams of melted BW oleo gels were put into a plastic test tube and weighed (a). The samples were then placed in the refrigerator for one hour until they completely solidified and were weighed again (b). After refrigeration, the samples were centrifuged at 1300 rpm for 15 minutes. Then, the samples were turned over onto filter paper with their caps opened to drain the separated oil for another 15 minutes. After draining, the samples were weighed again (c). The percentage of %OBC was calculated using the following equation [29]:

$$\%\text{released oil} = [(b-a) - (c-a)] / (b-a) * 100$$

$$\%\text{OBC} = 100 - \%\text{ released oil}$$

Regarding OBC for mayonnaise and margarine-based BW oleo gel samples, the test was done as follows [29]: Polyethylene centrifuge tubes were used. The empty weight of each tube was recorded as (W0). A specified amount of each sample was then placed in a tube and labeled as (W1). The samples were centrifuged at 10000 rpm for 30 minutes. After centrifugation, the caps of the tubes were removed, and the tubes were positioned upside down on filter paper for an additional 30 minutes to allow the separated oil to drain. After drainage, the tubes with the caps were weighed again and marked as (W2). The following equation was used to calculate %OBC:

$$OBC = \left(1 - \frac{\mathrm{W1} - \mathrm{W2}}{(w1 - w0) * oil\ proportion}\right) * 100\%$$

2. 5. 5 Oxidative stability

Margarine and mayonnaise have a high-fat content, making it essential to test their stability against rancidity, even when modified. Conducting tests such as peroxide value, acid value, and free fatty acid levels on oleo gels, representing the product's main oil content. These tests were performed at the time of production (zero time) and after four weeks of storage to assess any rancidity development.

2. 5. 6 Peroxide value (PV)

The measurement was conducted using the AOAC method No. 965.33 (AOAC, 1995) [30]. This method is based on the reaction between saturated potassium iodide, oil in BW oleo gel samples, and the iodine formed is then titrated against sodium thiosulfate with starch as an endpoint indicator. The results are expressed as the sample's equivalent of peroxide oxygen per kilogram (mEq/kg):

PV (mEqO_2/kg oleo gel) = [(S – B) * Normality] * 1000 / sample weight (g)

Where S=titration of test sample (mL)

B=titration of blank (mL)

2. 5. 7 Acid value (AV) and Free Fatty Acid (FFA)

AOAC method 940.28 was used to perform this test. It involved mixing a specific amount of the oleo gel with ethanol and ether and then titrating the free fatty acid present with an ethanolic solution of sodium hydroxide [31].

The acid value and the percentage of fatty acid were calculated as follows:

$$\%FFA = \frac{mL\ NaOH * Normality}{sample\ weight\ (g)} * 100$$

Acid value (mgKOH / g oleo gel) = FFA * 1.99

2. 5. 8 Storage stability

Both margarine and mayonnaise-based BW oleo gel samples were stored in the refrigerator for 60 days to observe any phase separation. This test aimed to determine whether the crystalline structure of those products could effectively hold and emulsify the oil phase, as phase separation is common in such colloidal systems containing immiscible phases.

For margarine-based BW oleo gel, the samples were placed in a glass container with a diameter of 5 cm and a depth of 3 cm, positioned stably. They were checked for phase separation at regular intervals, specifically once a week, looking for any separation of oil and liquid milk, as milk is a major product component. Additionally, the margarine structure was disturbed using a spatula to see if any liquid escaped into the crystalline structure. Observations were made with the naked eye.

The same inspection procedure was followed for the mayonnaise-based BW oleo gel samples. However, a vertical centrifuge Falcon tube was used since the mayonnaise was less firm. Any oil that floated to the top was recorded in the event of separation.

2. 5. 9 Sensory Evaluation.

To evaluate the sensory quality of both margarine and mayonnaise-based BW oleo gel samples, a team of 15 recipients was assembled. Although they are academic staff from the Food Sciences and Quality Control Department and possess significant experience, they received a two-hour lecture explaining the sensory evaluation parameters necessary for obtaining accurate results (Table 4). The group of no more than three recipients took the test at a time to perform the test as accurately as possible. The sensory evaluation test consisted of two parts: the first part was the sensory and non-sensory evaluation (texture, flavour, palate stickiness, overall acceptability, and body and creaminess, appearance) based on five attributes within 5-point hedonic test scaling from 1-dislike a lot, to 5- like a lot. This approach was taken from the previous studies with a little modification [32, 33].

The second part involved a factor known as "purchase intention and still purchase." Purchase intention is a measure designed to gauge how strongly recipients wanted to buy each sample evaluated in the first part of the sensory evaluation. This was scaled from "definitely purchase" to "probably purchase," "not sure," "probably not purchase," and "definitely not purchase." Meanwhile, "Still purchase" was another factor, where recipients were selected from the same scale after being informed about the total energy content of the samples, allowing for comparison with commercial mayonnaise. The energy content was calculated based on theoretical energy values: one gram of fat provides nine Kcal, while one gram of protein and one gram of carbohydrate each provide four Kcal. This indicates a significantly reduced energy availability, particularly from the fat component.

Table 4: Sensory and non-sensory parameters of 5-point hedonic test with their description.

Taste & Odor	The intensity of the bittersweet flavor & cocoa aroma
Texture	Consistency, homogeneity, mouthfeel
Mouthfeel	stickiness on the palate surface, amalgamation, and smoothness.
Appearance	Glossiness, broken surface, and roughness,

Overall acceptability	Acceptable or not acceptable
Purchase intention	The extent of the desire to purchase

2.5.10 Oleo gel melting point determination.

The capillary method characterised different BW oleo gel samples for their melting points. The time to melt and melting temperature range were recorded by placing the samples in narrow glass capillary tubes, which allowed them to form thin layers and facilitated faster heat flow.

Initially, the samples were melted at 65°C, after which, using a syringe, they were transferred into the capillary tubes. Both ends of the capillary tubes were sealed with a flame to prevent the samples from escaping. Following this, the tubes were placed in the refrigerator to allow the samples to solidify completely. Once the full solidification was achieved, the samples were transferred to a water bath, starting at a temperature of 37°C. The temperature increased steadily, enabling both partial and complete melting points to be observed. Changes in the sample's colour served as indicators of melting.

3 Statical analysis

Statistical analysis was conducted using the 2022 XLSTAT one-way analysis of variance (ANOVA) with a 95% confidence interval to differentiate between samples and effects. Each sample includes three replicates.

4 Results and Discussion

In this study, BW oleo gel samples were produced with varying concentrations of BW, reaching up to 25%. These oleo gels were then used to fabricate margarine and mayonnaise, both high in lipid content and often considered staples. The formulation process allowed the selection of the most promising variants for comparison with commercial products. As shown in Tables 1 and 2, these selected variants were marked as "PROMISING," indicating their potential to be tested for acceptability regarding physicochemical and sensory characteristics.

4. 1 Appearance and Microstructural observation.

4. 1. 1 Oleo gel microstructure and appearance

As shown in Figure 2, the appearance and microstructure of the BW oleo gel samples were influenced by the concentration of BW. As its concentration increased from 10% to 25%, the appearance changed from soft, oily, and bright yellow to matte yellowish, hard-like structure. This transformation may be attributed to the rigid physical properties of BW at room temperature [34, 35].

Regarding microstructure, BW can form a crystalline network that traps the oil inside. This capability arises from the close physicochemical properties shared by BW and vegetable oil [6]. Furthermore, BW contains very long hydrocarbon chains with a needle-like shape, acting as an emulsifier. This allows BW to effectively adsorb at the oil-water interface, reducing surface tension and creating oil pockets in the oleo gels [36].

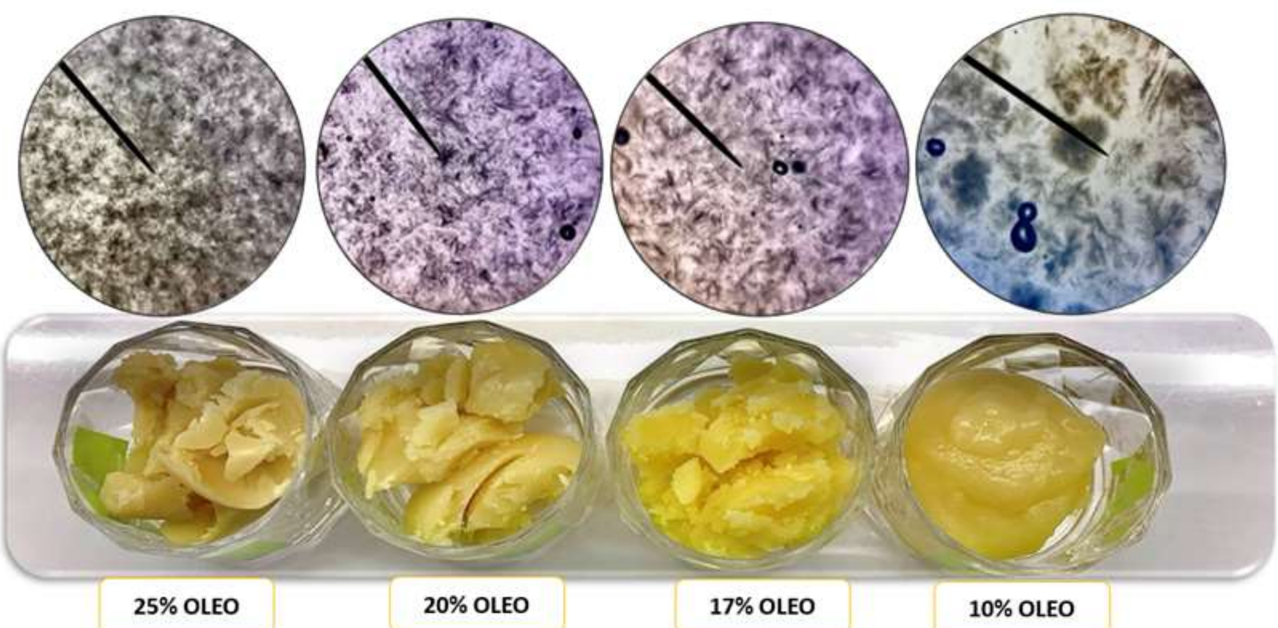

Figure 2: The appearance and microstructural of BW oleo gel samples.

4. 1. 2 Margarine and mayonnaise-based BW oleo gel microstructure and appearance.

The microstructure of both fabricated products and commercial margarine at 20 °C is depicted in Figure 3A, using polarized light digital microscopy at 40X magnification. Similar to the BW oleo gel samples, the concentration of BW directly affects the microstructure. A lower BW content results in a finer microstructure, as observed in MARG-10% OLEO. This indicates less droplet aggregation and a more organized, crystal-like network structure. Conversely, droplet aggregation and a more complex crystallized network structure are denser as the BW content increases in the oleo gels. This change is particularly noticeable in MARG-17% OLEO and higher. A similar result was observed in the previous study [6]. This event was attributed to the physicochemical properties of the BW needle-like structure at the water-oil interface, which stabilized the droplets and the continuous phase. Furthermore, similar to fabricated margarine, fabricated mayonnaise samples were observed under different magnifications, and similar results were observed when it came to BW concentration (Figure 3B).

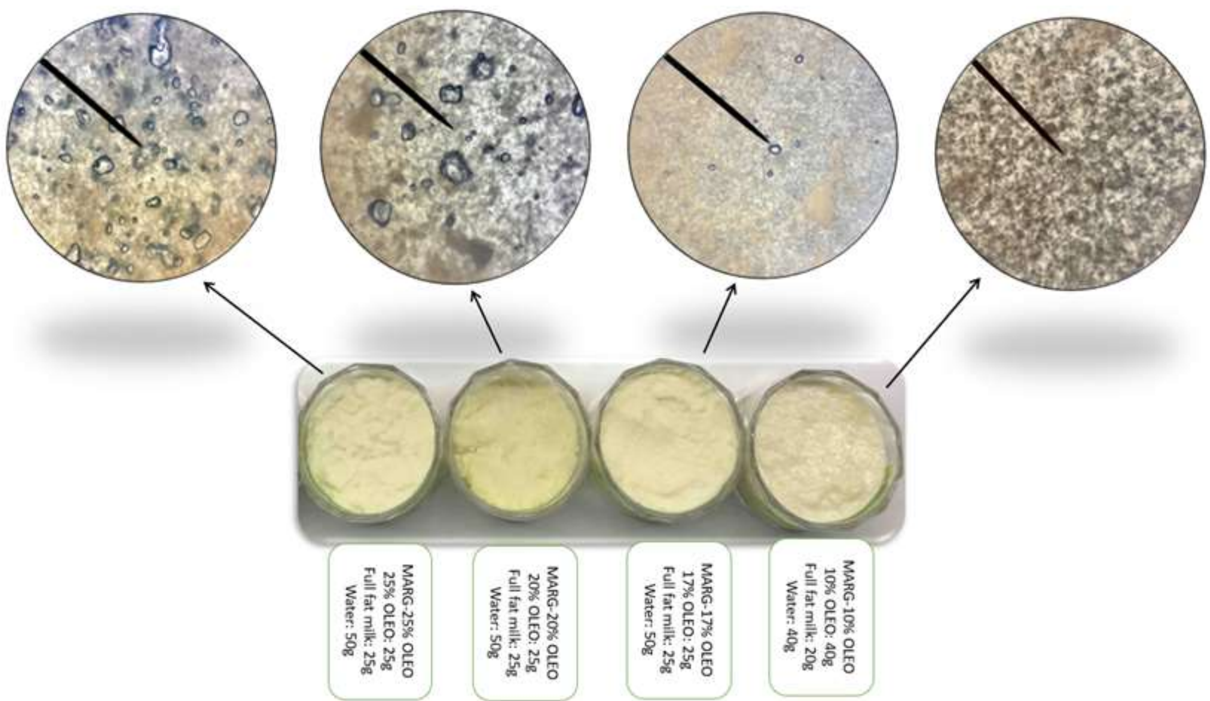

Figure 3. A: Margarine-based different BW oleo gel samples under 40X zoom.

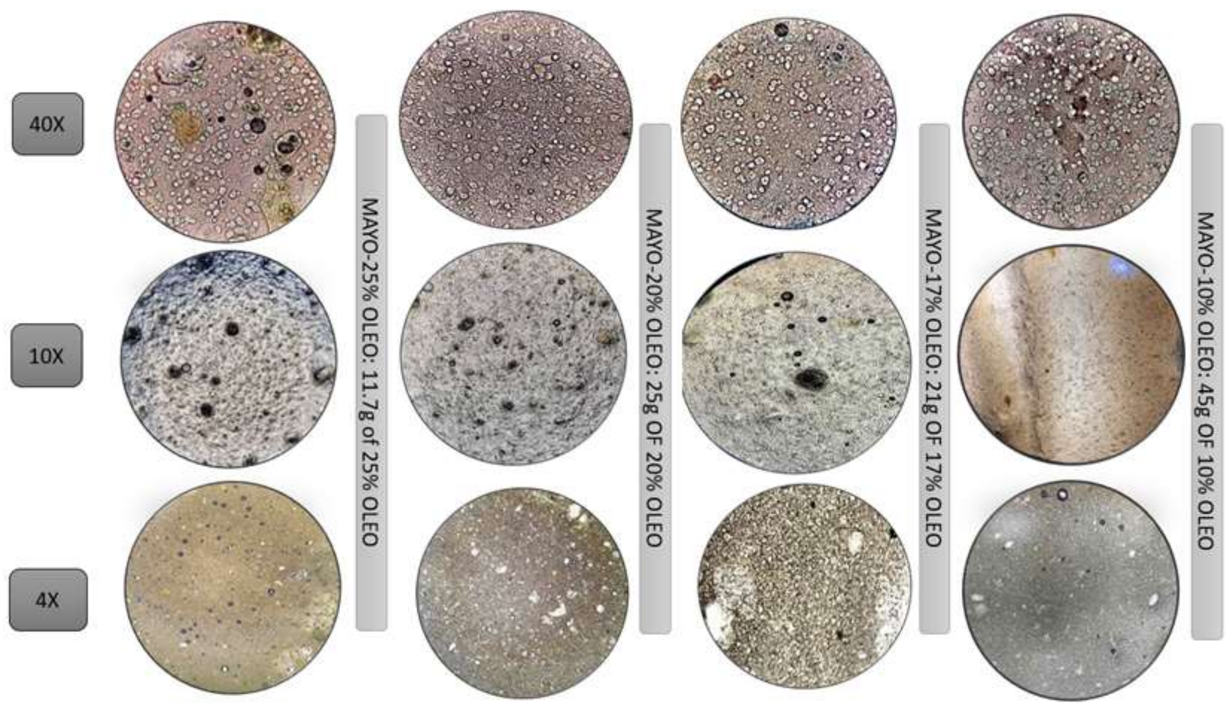

Figure 3. B: Mayonnaise-based different BW oleo gel samples under three different magnifications.

4. 2 Texture analysis

4. 2. 1 Hardness, cohesiveness, and adhesiveness (TA15 cone)

The harness plays a crucial role in the customer experience with margarine, as shown in Figure 4A. The higher content of BW in various oleo gels significantly increases hardness in fabricated margarine samples (p>0.05). For instance, MARG-10% OLEO exhibits a hardness of 41 gm/cm², whereas MARG-25% OLEO shows a hardness of 217 gm/cm². All samples fall within the acceptable range when compared to commercial margarine (up to 200 gm/cm²) [37]. Additionally, cohesiveness and adhesiveness, which also influence hardness properties, had different values due to BW content but exhibited values similar to those of commercial margarine. This means that margarine-based BW oleo gel samples are acceptable regarding texture, specifically harness, even the ones with high BW oleo gel (MARG-25% OLEO).

Naturally, BW has a high hardness value due to its rigid crystalline structure and high melting point (65°C) [38]. This characteristic contributes to a hard texture when used in fabrication. However, the hardness can be reduced by adjusting the liquid-to-solid fat content ratio, which directly affects lowering hardness [39]. In this study, the fabricated margarine samples were produced using oleo gel with entirely sunflower oil, resulting in a liquid-to-solid ratio significantly greater than that of commercial margarine. As a result, the rigid crystalline structure may be softened due to the incorporation of oil, creating oil pockets.

Additionally, this incorporation can lower the melting point, as sunflower oil has a very low melting point of -17°C. These two factors contribute to a marked reduction in hardness in the fabricated samples. Furthermore, since solid fat content is not the sole determinant of hardness, the optimal hardness of the fabricated margarine may also be influenced by polymorphic behavior and crystal size formation [40].

Previous studies have tried to increase BW content in oleo gel for fat replacement. Studies used 3%, 4%, and 5% to maintain the overall sensory characteristics [41-43]. So far, the highest level of BW used to produce oleo gels for the proposed fabrication has reached 10% [17]. Although BW oleo gels and fabricated products in these studies were promising and even better than the original products in terms of hardness and texture properties. In this study, the BW concentration was increased to 25% and still had a similar textural result, which around 200g/cm hardness as an applied force [42]. This means the BW is up to 15% higher for the same harness value. The hardness of beeswax can be modified by adding different substances, such as oils, emulsified water, and emulsifiers. The crystalline structure of beeswax is changed, leading to a lower hardness [44].

Furthermore, the concentration of the beeswax oleo gel is essential for this adjustment, as it directly affects the thermal characteristics of the composite material [45]. For example, MARG-25% OLEO is supposed to have a higher hardness than MARG-10% OLEO. However, the former still had an acceptable harness due to the requirement for a lesser 25% BW oleo gel to achieve the desired consistency. In other words, those samples have a different formulation yet close BW content. This achievement serves to reduce total calorie content and trans fat. Thereby, significant health benefits resulted.

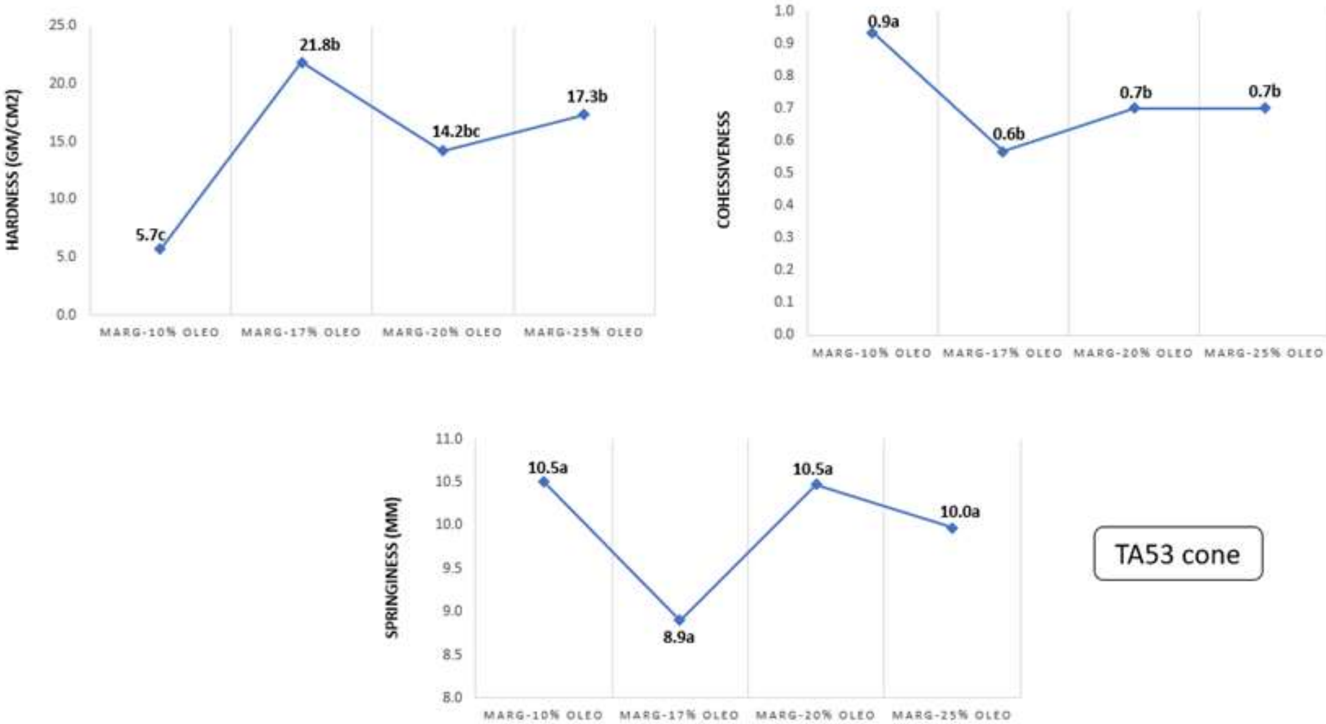

Figure 4. A: Shows the physical properties (hardness, cohesiveness, and springiness) of various types of margarine-based oleo gels at a certain amount of 10% OLEO, 17% OLEO, 20% OLEO, and 25% OLEO. Values sharing the same superscript letter within the same row for each parameter indicate no statistically significant difference ($p < 0.05$).

4. 2. 2 Spreadability (TA53 cone)

Figure 4B shows that hardness, cohesiveness, and adhesiveness indicate the overall spreadability when using a cutting weir cone (TA53). There is a significant difference ($p<0.05$) between margarine-based BW oleo gel samples. Harness increased significantly when the BW concentration in oleo gel increased from 10% to 17% and higher, cohesiveness decreased by a factor for a quarter, while springiness did not significantly change, although there was a fluctuation. This means that increasing the concentration of BW has dominantly affected the harness. This can be translated to less spreadable on the toast, for example. Moreover, margarine-based oleo gels in this study showed a very close texture property, especially MARG, OLEO 10%, that produced a similar result compared to commercial margarine at around 5-15g/cm [42].

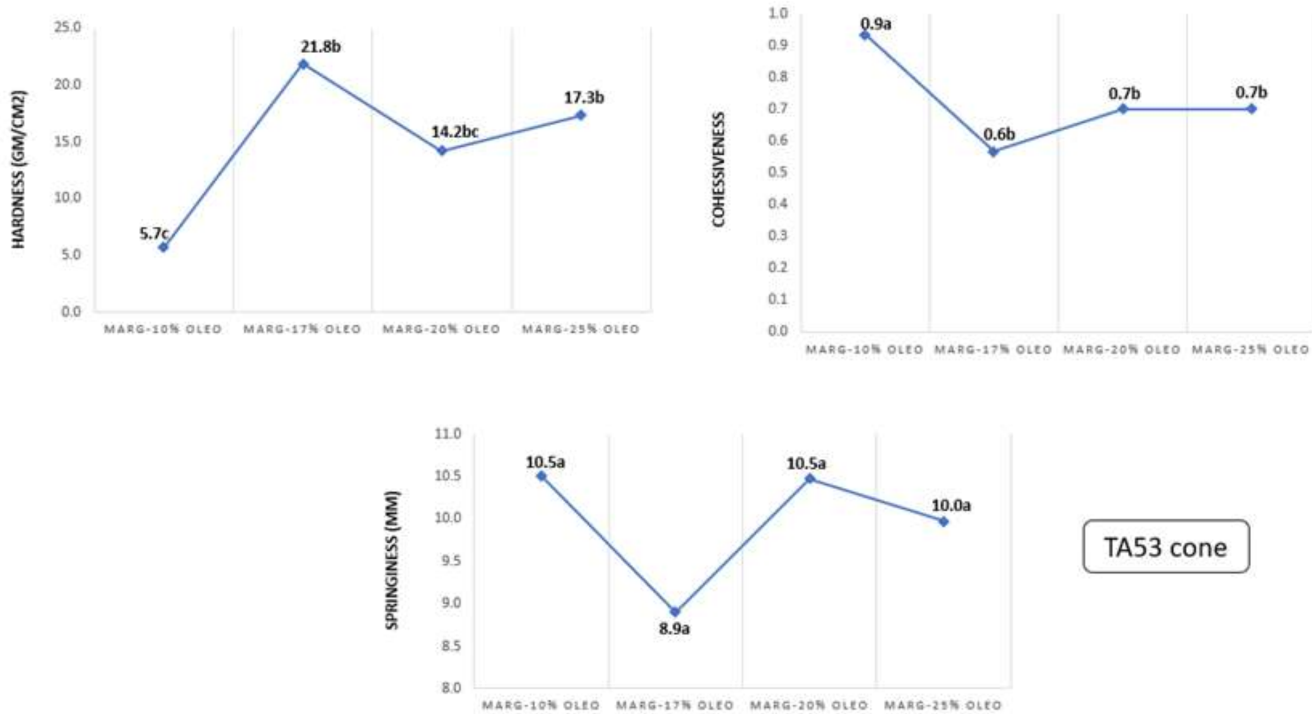

Figure 4. B: Shows the cutting properties (spreadability) by measuring the hardness, cohesiveness, and springiness of various margarine-based BW oleo gels at a certain amount of 10% OLEO, 17% OLEO, 20% OLEO, and 25% OLEO. Values sharing the same superscript letter within the same row for each parameter indicate no statistically significant difference ($p < 0.05$).

4. 2. 3 Viscosity

Since mayonnaise has a less viscose texture, a viscometer has been used to measure the mayonnaise-based oleo sample's physical texture instead of a texture analyzer. This test determined the viscosity measurement between different mayonnaise-based BW oleo gels (MAYO-10% OLEO, MAYO-17% OLEO, MAYO-20% OLEO, and MAYO-25% OLEO) and then compared to commercial mayonnaise (C. mayonnaise). Figure 5 depicts all sample's viscosity within the range of 19000-23000 centipoises. These values are very close to a standard absolute viscosity of mayonnaise (20,000cp) [40]. However, statistical analysis shows a significant difference between them, in which C.mayonnaise has a lower viscosity than mayo-10% oleo and mayo-25% oleo ($p<0.05$). However, there is no significant difference between the other two treatments. This means that increasing the BW concentration in oleo gels has the potential of having the same viscosity, which is one of the crucial properties when considering customer experience.

Furthermore, although there is a significant difference between fabricated mayonnaise samples and C. mayonnaise, they all fall within a very close range. The reason might be the quantity of oleo gels based on different BW concentrations. For example, a higher quantity of OLEO 10% was used, while a smaller quantity of OLEO 25% was used to produce the same consistency in different fabricated mayonnaise samples (Table 3). In other words, this is because the BW concentration in different oleo gels makes different samples accept a certain amount of oleo gel to reach the desired consistency. That is what makes mayonnaise-based oleo gel samples have a close viscosity value.

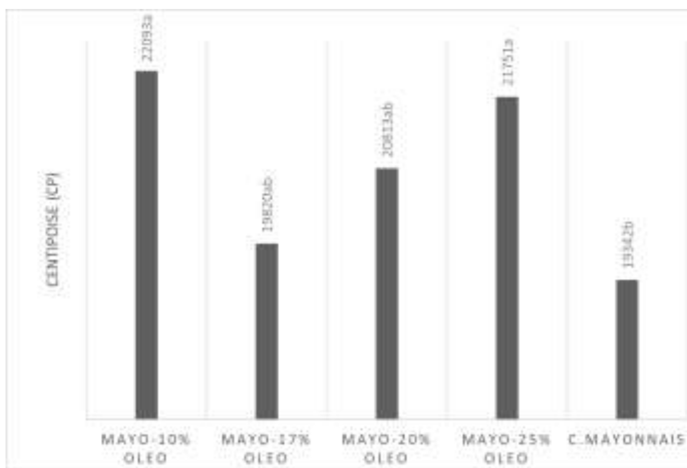

Figure 5: The viscosity measurement for both margarine-based oleo gel samples and commercial mayonnaise. Values sharing the same superscript letter within the same column for each parameter indicate no statistically significant difference ($p < 0.05$).

4. 2. 4 Oil Binding Capacity (OBC)

The OBC test is crucial to assess the durability of a system that can hold together two immiscible liquids. Figure 6 presents the OBC results for all samples, including BW oleo gels, margarine-based BW oleo gels, and mayonnaise-based BW oleo gels. All samples demonstrated a robust OBC of at least 97%, except for the Mayo-25% OLEO sample, which achieved 95% capacity. Despite the significant difference from other samples, 97% is enough to prevent phase inversion and separation [20]. Moreover, the storage time of over 60 days has proven the integrity of all samples.

The mechanism behind this high OBC value can be explained by beeswax being considered a good structuring agent in a colloidal system. It can stabilize large oil droplets to compact the entire system [46]. Another reason is that beeswax is considered a mild-range emulsifier. It contains fatty acids, esters, and hydrocarbons, making it amphiphilic. These substances can reduce the surface tension at the oil-water interface, a common occurrence in fabricated margarine and mayonnaise samples [44]. However, in the case of BW oleo gel samples, the system consists solely of oil and beeswax. In this scenario, the beeswax produced a crystalline network that trapped the oil inside, reducing the oil phase's motion and increasing the viscosity [21, 47].

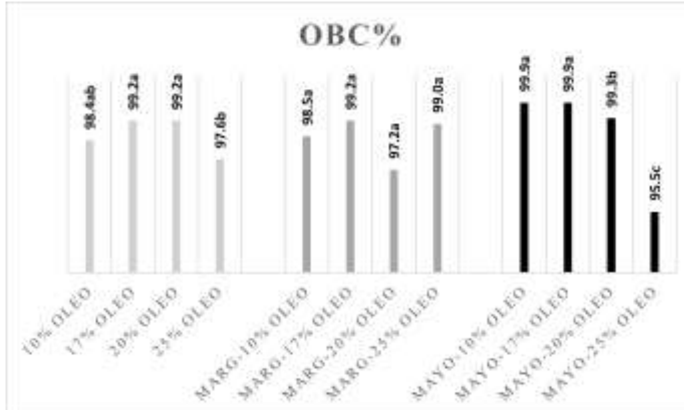

Figure 6: The oil binding capacity of all BW oleo gels, margarin & mayo-based BW oleo gels. Values sharing the same superscript letter within the same row for each parameter indicate no statistically significant difference (p > 0.05).

4. 3 Oxidative stability

The oxidative stability parameters (PV, FFA, AV) of different BW oleo gel samples are shown in Figure 7. The four-week storage period has been used to observe any change in those values regarding primary oxidation, especially PV, which is considered a main factor of primary oxidation [48].

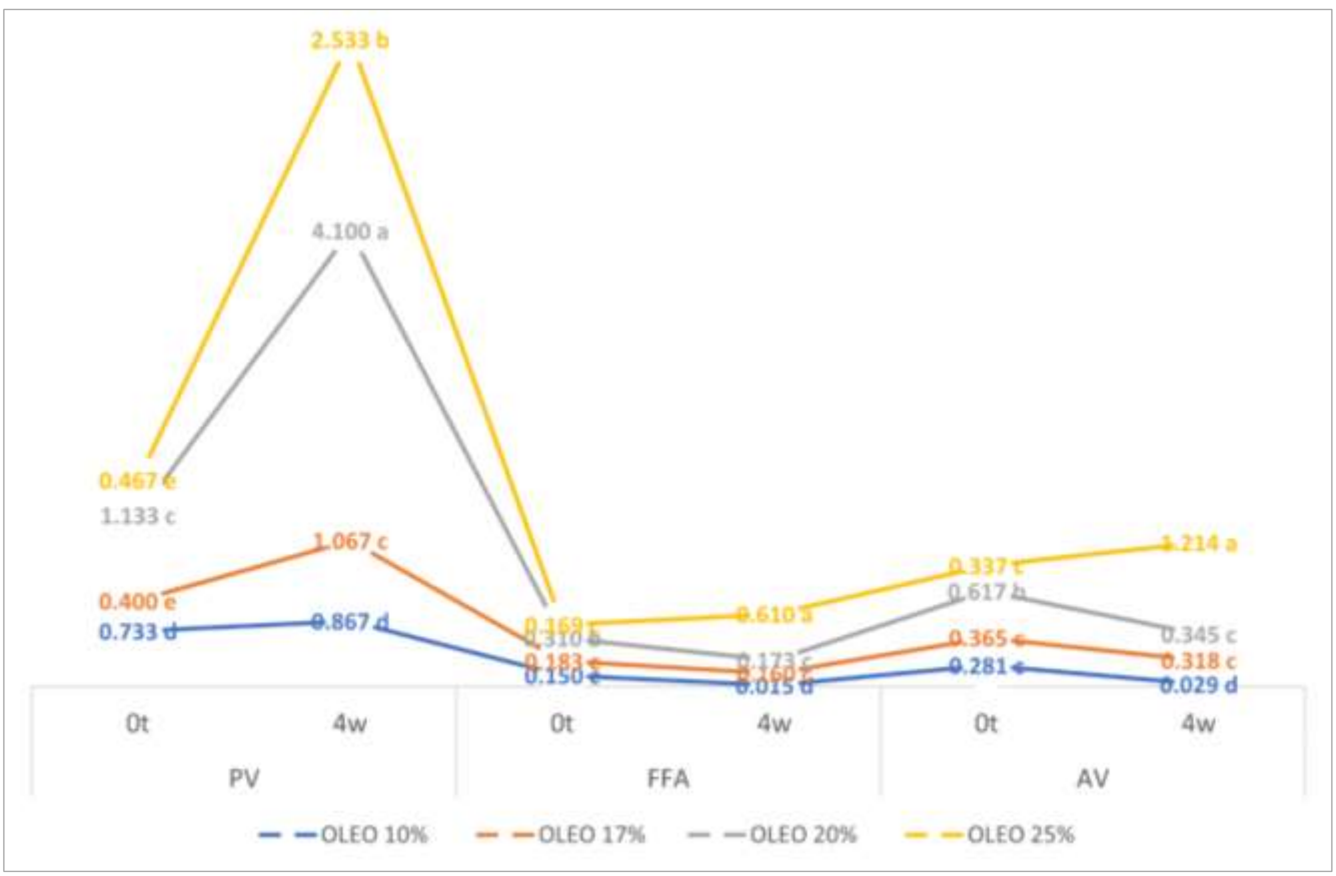

Figure 7: Shows the rancidity tests for all BW oleo gel samples. Values sharing the same superscript letter within the same line for each parameter indicate no statistically significant difference (p > 0.05).

BW oleo gels samples, such as 10% OLEO, 17% OLEO, and 25% OLEO, recorded a low PV value at 0.7, 0.4, and 0.46 mEq/kg, respectively. Meanwhile, 20% of OLEO had a higher PV of just above 1.0 mEq/kg. It can be said that all samples recorded a low level of primary oxidation as fresh samples (zero time). After four weeks of storage, the PV of 10% OLEO and 17% OLEO samples did not rise significantly. However, the other two samples, 20% OLEO and 25% OLEO, increased significantly to 4.0 and 2.5 mEq/kg, respectively. However, they are still below an acceptable range, as determined in the literature, as 0-5 is a low, 5-10 is medium, and more than ten is considered high PV [49]. Other parameters, such as FFA and AV, recorded values below 1.0 mgKOH/g of BW oleo gels over four weeks of storage. It means that BW oleo gel samples may have the potential to prevent the oxidation process.

This test has been applied only on BW oleo gel samples, not fabricated products. This is because the oleo gel is considered a main oil component in the products and can be a main factor that indicates any change in oxidative stability. Moreover, both fabricated product samples tested for PV after an accelerated oxidation rate test. Figure 8 shows the values of both products-based different BW oleo gel samples after storing for 2 weeks under unsuitable environments that can help accelerate the primary oxidation process, such as increased temperature, humidity, increased oxygen, and light exposure [50]. As can be seen from the graph, all samples had a PV lower than 1.0 mg KOH. In this study, fabricated samples do not contain preservatives (tocopherol content) that protect against oxidation. This means BW as an oleo gelator might potentially protect physiochemical and sensory properties. The literature also stated that certain product-based oleo gels recorded the same PV values after 14 days of storage within γ- and δ-tocopherols content.

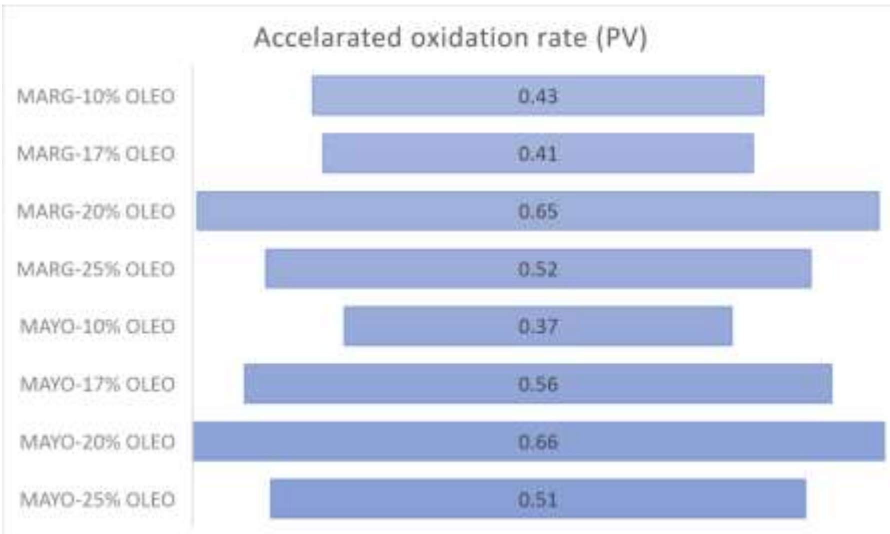

Figure 8: PV values for both of fabricated products after acceleration oxidative rate.

4. 4 Total Energy Content

The primary goal of fabrication or fat replacement is to reduce energy content or to substitute oil with healthier alternatives. Oleo gels uniquely provide the opportunity to achieve both objectives. In this study, the results showed that employing various oleo gel samples to fabricate margarine and mayonnaise using healthier oil options significantly reduced total energy content. As illustrated in Figure 9A, the margarine-based BW oleo gels exhibit a total energy content of up to 68% lower than conventional margarine, with the minimum difference recorded at 48% for MARG-10% OLEO. This magnitude of energy reduction is nearly two times lower than the previous studies that used 10% BW oleo gel with an acceptable sensory evaluation (Figure 10A) [6]. It might be related to the formulation process that required the oil phase to have significantly less BW oleo gel to obtain the appropriate consistency. Otherwise, it might be sticky on the palate. This substantial reduction can substantially impact an individual's overall daily calorie intake while maintaining similar sensory and physicochemical properties. The notable decrease in energy content is due to the high levels of water and full-fat milk, which account for 50% and 25%, respectively (Table 1).

Concerning the fabricated mayonnaise (Figure 9B), the mayonnaise-based BW oleo gel samples demonstrate a significant energy difference compared to a conventional mayonnaise, with a minimum variation of 35% observed in MAYO-17% OLEO and MAYO-20% OLEO, followed by MAYO-25% OLEO and MAYO-10% OLEO, which show differences in total energy content of 44% and 50%, respectively. Since mayonnaise is a widely consumed confection with a high oil content, reducing energy can notably impact daily energy intake. This model of mayonnaise contains even less energy than light mayonnaise, in which the oil phase is reduced to less than 50% [32]. However, this reduction is only valuable if the physicochemical and sensory characteristics are maintained; otherwise, the product may be deemed unacceptable from a customer experience perspective. Therefore, sensory evaluation is an essential technique for assessing this aspect.

It is essential to highlight that the oil phase, which can include up to 25% BW, may lead to an energy reduction greater than what has been previously indicated in terms of bioavailability. Research suggests that BW comprises long-chain fatty acids and hydrocarbons that render it completely indigestible [51]. Therefore, the incorporation of BW oleo gels may have a dual impact on energy reduction: it can both decrease the quantity of the oil phase and include the undigested BW within this phase.

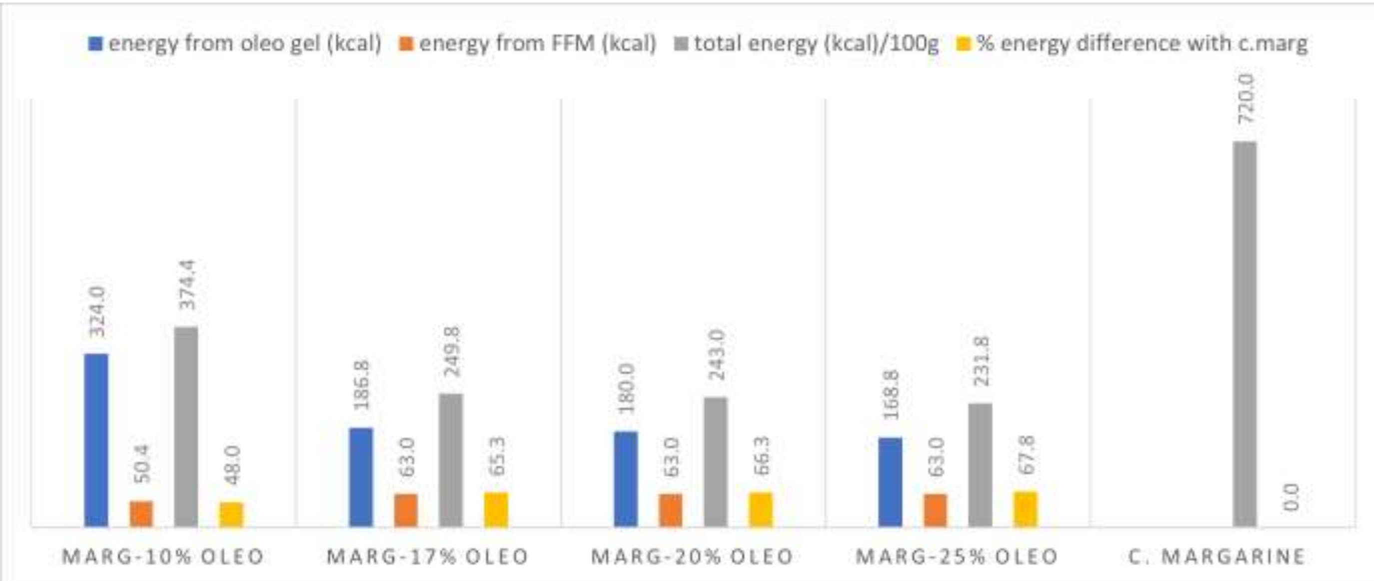

Figure 9. A: Percent calorie content (kcal) of margarine-based different oleo gels.

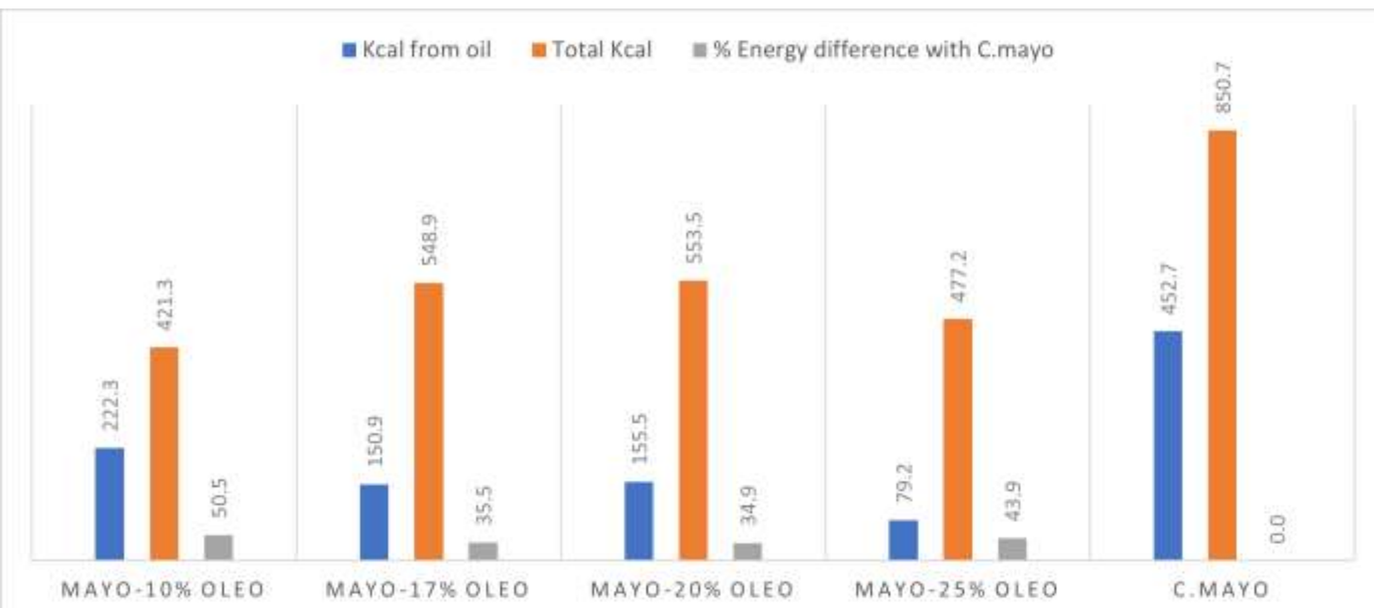

Figure 9. B: Percent calorie content of mayonnaise-based different oleo gels.

4. 5 Sensory evaluation

This test is essential for evaluating the physiochemical and sensory attributes of newly developed products. It helps determine whether the fabricated products meet commercial acceptability standards. Both fabricated products were compared with commercially available ones, as illustrated in Figure 10A and Figure 10B. The results from the five-point hedonic test highlight five essential properties. Notably, all the fabricated samples outperformed the commercial products. This difference is particularly evident in the MARG-17% OLEO, MAYO-20% OLEO, and MAYO-25% OLEO samples, indicating that these formulations were preferred over the other treatments. It appears that substituting the oil phase with 17% BW oleo gel yielded the most promising results in terms of both sensory and non-sensory attributes for both products, closely followed by the 25% oleo gel substitution in mayonnaise.

The results suggest that participants could not differentiate the control sample, which received the lowest scores. Palate stickiness is a key parameter in both fabricated samples. Given that BW has a higher melting point. It influences the overall melting characteristics of the final product, particularly in terms of mouthfeel. This elevated melting point corresponds to an increased level of palate stickiness. In this study, both fabricated samples—specifically, MARG-25% OLEO and MAYO-25% OLEO—exhibited an excellent score for plate stickiness as the highest BW content formulations. It suggests that participants did not experience any stickiness on the palate. Such results reflect the promising fabrication of both products. In essence, achieving energy reduction to the specified level is feasible without sacrificing either sensory or non-sensory properties.

The sensory evaluation test incorporated an additional component: “purchase intention.” In this section, participants indicated their likelihood of purchasing each sample based on the sensory evaluation results and their intention to make a purchase afterwards, named “still purchase”. This aspect highlights whether participants changed their purchase intention upon learning about the total calorie reduction for each sample (Figure 11). In the context of fabricated margarine, MARG-25% OLEO demonstrated the highest purchase intention, with 33% of participants indicating they would definitely buy it. This was closely followed by MARG-17% OLEO at 31% and MARG-10 OLEO at 25%. However, once participants were informed about the total reduced energy content, 62% opted to change their preference to MARG-17% OLEO. In contrast, C.margarine experienced a decline in purchase intention of up to 15% after the total calorie content was disclosed.

Concerning mayonnaise-based BW oleo gel samples (Figure 11), the MAYO-17% OLEO achieved a purchase intention of 73%, with a significant 20% of respondents indicating they would "definitely purchase" it. This made it the most preferred option, comparable to C.mayonnaise. However, upon revealing the details of the calorie reduction, the fabricated samples showed a remarkable increase in purchase intention, surpassing the C.mayonnaise sample by up to 79%, while C.mayonnaise lost its appeal entirely. This outcome demonstrates that participants are highly attracted to the healthier mayonnaise alternative. Notably, maintaining both sensory and non-sensory properties directly contributed to the success of the fabricated samples in achieving this favourable score.

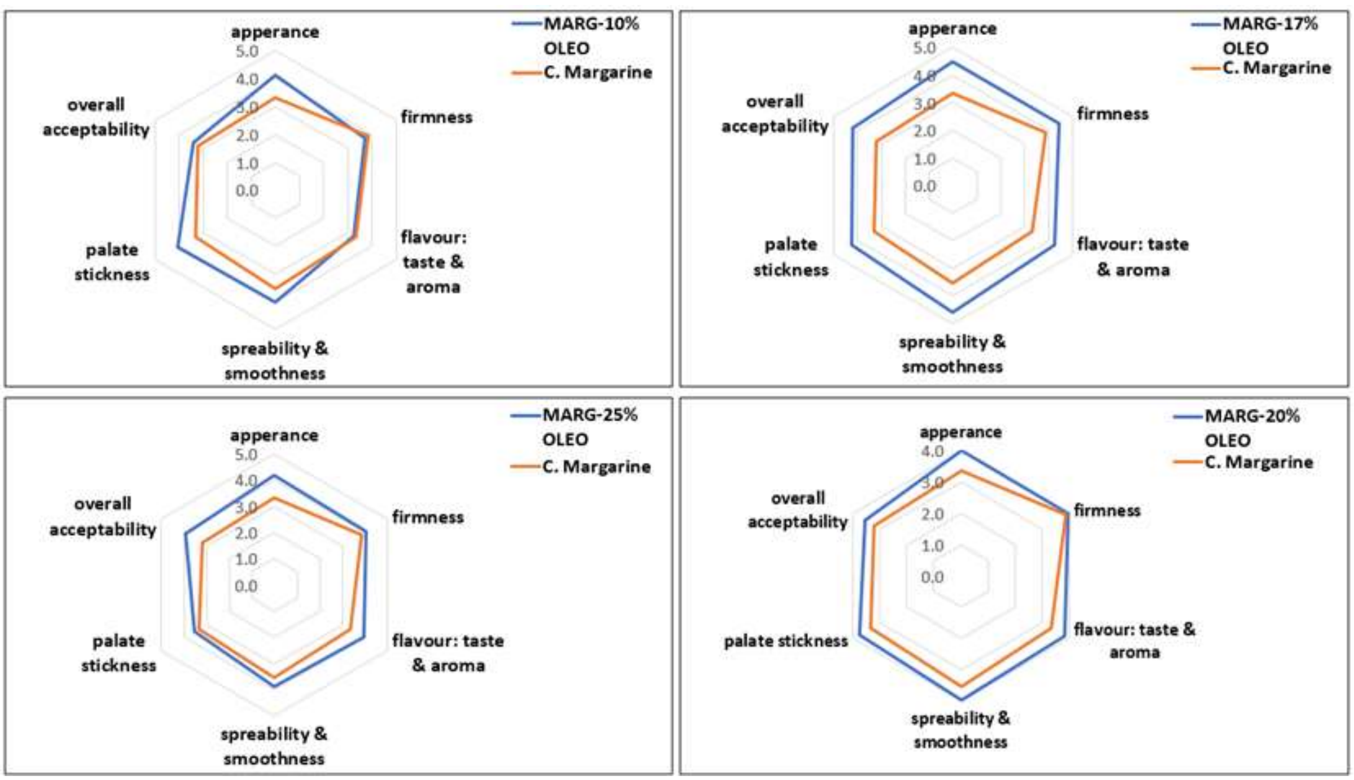

Figure 10. A: Five-point hedonic test sensory evaluation of commercial and fabricated margarine samples.

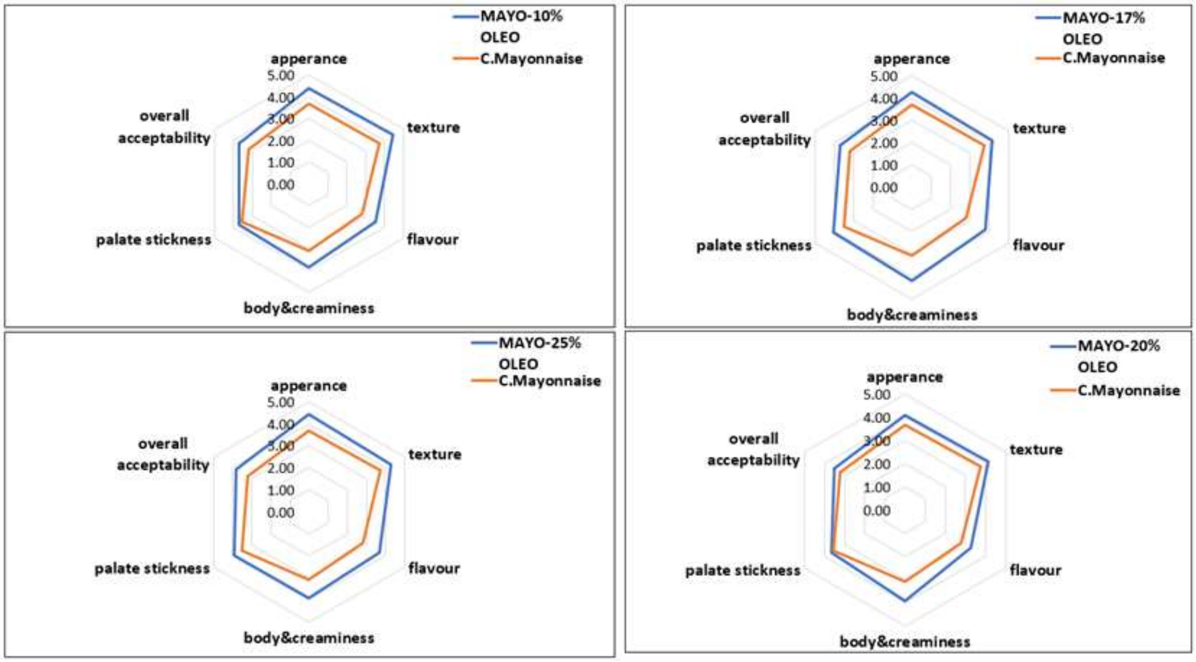

Figure 10. B: Five-point hedonic test sensory evaluation of commercial and fabricated mayonnaise samples.

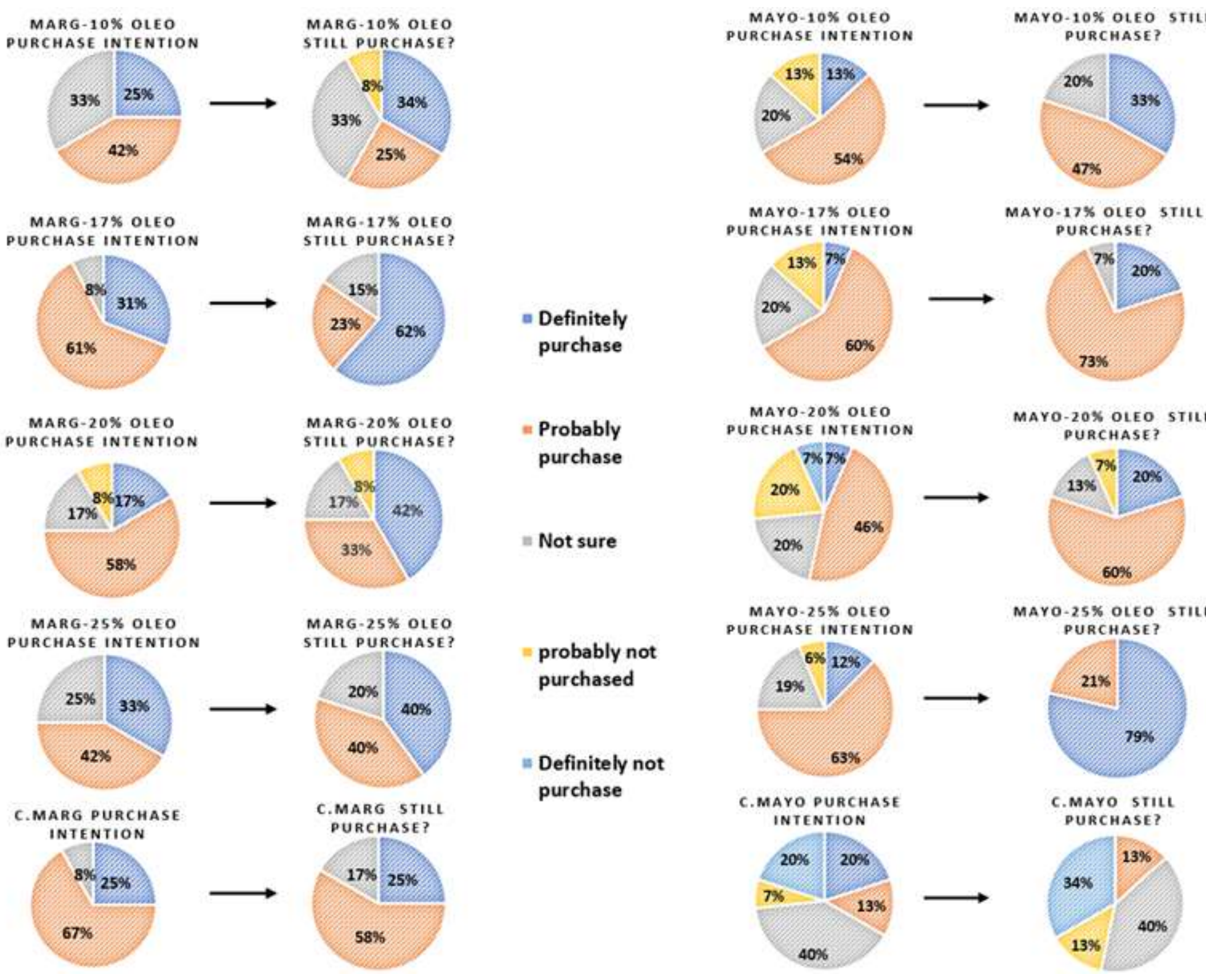

Figure 11: Purchase intention for both different fabricated margarine and mayonnaise samples.

4. 6 The impact of BW oleo gel on the melting point and other sensory and physicochemical properties of various fabricated samples.

Margarine and mayonnaise-based BW oleo gels are emulsions of tiny droplets of water dispersed within oil, combined with other ingredients with very low melting points, especially in the oil phase. This unique composition provides these commercial products with a smooth mouthfeel and prevents any stickiness on the palate. In contrast, beeswax as an oleo gel is a complex mixture of esters, fatty acids, and alcohols, characterized by a relatively high melting point that typically ranges from 62 to 64°C [38]. This high melting point may result in a desirable mouthfeel but can also lead to some stickiness on the plate in a final product (Table 1).

The high melting point of BW oleo gels can be adjusted by incorporating various additives through formulations. The introduction of fats, oils, emulsified water, and emulsifiers alters the beeswax's crystalline structure, decreasing its melting point. Additionally, the concentration of the beeswax oleo gel plays a critical role in this modulation, as it directly influences the thermal properties of the composite material [44].

Different oil's viscosity and saturation levels can significantly influence BW oleo gel melting point. Oils with lower viscosity and higher levels of unsaturated fatty acids can lower the melting point of BW. For instance, sunflower oil, which has one of the lowest melting points at -17°C and a relatively low saturation level [52, 53], can substantially reduce the melting point of the BW oleo gel. The physicochemical properties and structure of the sunflower oil are the mechanisms underlying this. Incorporating an oil with higher unsaturation into beeswax disrupts its crystalline structure. This interference inhibits the close packing of molecules, leading to a reduced melting point [36]. The introduction of the more fluid oil disrupts the inherent solid structure of beeswax. In other words, the greater the number of double bonds in the oil, the more likely it is to lower the melting point [48] significantly. This occurs because unsaturated oils are more fluid, effectively softening BW's crystalline structure when blended. For instance, sunflower oil, known for its high unsaturation, will reduce the melting point of beeswax more than coconut oil, which is predominantly saturated.

Emulsified water can also lower the melting point of beeswax by disrupting its molecular structure. Beeswax is composed of long-chain molecules that form crystalline structures. When water is emulsified, it interferes with these formations. The small water droplets create disruptions that lead to a less stable arrangement, resulting in the wax melting at a lower temperature. Furthermore, water acts as a plasticizer, softening the wax and enhancing its flexibility, which further reduces its melting point compared to pure beeswax [54].

Beeswax is composed of long-chain molecules that crystallize into a structured arrangement, which contributes to its high melting point and firm texture. Emulsifiers, such as egg yolk, powdered lecithin, and vinegar, can disrupt this crystallization process due to their amphiphilic property, allowing for interaction with both water and oil phases [55]. When an emulsifier is incorporated into beeswax, it can position itself among the wax molecules during the cooling process, preventing the wax from forming a regular, tightly packed structure. This disruption results in a more amorphous or disordered crystal formation, ultimately reducing the overall rigidity of the wax.

Table 5: The melting point of different BW oleo gels.

	10% oleo partially melted	fully melted	17% oleo partially melted	fully melted	20% oleo partially melted	fully melted	25% oleo partially melted	fully melted
Temp (⁰C)	48	50	50	52	53	54	55	57
Duration (sec)	0-5	10	10	20	0-5	15	0-5	10

The concentration of BW in the oleo gel also significantly affects its melting point. Higher concentrations tend to elevate the melting point, whereas lower concentrations result in a decrease. This behavior is attributed to the oleo gel's more crystalline and compact structure [45]. This phenomenon is illustrated in Figure 2. Consequently, the products developed in this study are expected to exhibit a harder structure. For example, margarine and mayonnaise-based formulations with 25%-BW OLEO tend to be firmer than those containing 10%-BW OLEO. However, sensory evaluation revealed that both formulations exhibited similar levels of firmness and palate stickiness. This can be attributed to the fact that the samples formulated with a former required less oleo gel amount to achieve the desired consistency. In terms of BW concentration, these instances correspond to samples created with lower concentrations of oleo gel, such as the formulations with 10% OLEO AND 17% OLEO. As a result, different fabricated product samples can demonstrate similar sensory and physicochemical characteristics.

These factors outlined earlier play a significant role in lowering the melting point of BW from its initial 65°C down to under 50°C when employed as an oleo gelator (Table 5). Interestingly, even further reductions in melting point can be observed when the oleo gel is used to produce two different products that might remained below 40°C. Participants in the study reported a pleasantly surprising absence of any undesirable mouthfeel or the sensation of stickiness on the palate. This suggests that the final products may exhibit desirable qualities that could make them more appealing than their commercial counterparts.

5 Conclusion & Recommendations

Two popular high-calorie products have been developed using oleo gel, specifically utilizing BW as the oleo gelator. We crafted and compared various margarine and mayonnaise-based BW oleo gels against their commercial counterparts. The results indicated that the oleo gel formulated with BW resulted in a stable emulsion system without any separation, effectively protected the products from rancidity, and offered an appealing texture profile. Sensory evaluations revealed that these products were at least comparable to or even superior to the commercial versions. Most notably, the total energy content was significantly lower and excluded saturated and trans fats compared to the commercial products. This achievement has the potential to help mitigate food-related diseases significantly.

BW oleo gel can be integrated into various high-fat, high-calorie foods like chocolate, ice cream, and soft cheese. This formulation might enable these products to match commercial alternatives' physicochemical, textural, and sensory properties, providing consumers with healthier food options.

6 Limitations

It is widely recognised in the literature that oils possess a healthier fatty acid profile in comparison to fats and hydrogenated oils. However, gas chromatography analysis can provide valuable insights into the distinctions in healthfulness between commercially available and fabricated products.

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