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Utilisation of hazelnut-derived oleosomes in liquid margarine formulation: An investigation into stability and functional enhancement

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ABSTRACT

This study investigates the potential of utilising oleosomes extracted from hazelnuts in the formulation of liquid margarines. Aqueous extraction methods were employed to isolate oleosomes from hazelnuts, revealing approximately 83.07% fat and 2.48% protein content in hazelnut oleosomes. The stability of oleosomes at various pH levels (3–10) was examined, showing stability at pH 7 but instability at extreme pH values. Evaluation at pH 7 indicated small particle size ($D_{3,2} \approx 3.58\,\mu m$) and a ζ-potential of approximately −33.8 mV for isolated oleosomes. Subsequently, double emulsions were formulated by substituting traditional oil with varying oleosome concentrations (0–30%) in liquid margarine. Rheological and oxidative analyses of these margarines demonstrated decreased elastic and viscous moduli, hardness, and spreadability, alongside enhanced oxidative stability with increasing oleosome concentration. These findings suggest hazelnut-derived oleosomes offer significant stability advantages over conventional liquid margarine, presenting a promising avenue for functionally enhanced food products in the food industry.

KEYWORDS

oleosome, liquid margarine, rheology, oxidation

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1. INTRODUCTION

Oleosomes are micron-sized natural lipid storage structures commonly found in oilseeds. These structures are present in almonds, peanuts, pumpkin seeds, rice bran, soybeans, and hazelnuts (Iwanaga et al., 2007; Abdullah et al., 2020).

Oleosomes are composed of three primary components including a protein layer surrounding the triacylglycerol (TAG) core composed of phospholipids and specific proteins such as oleosins, caleosins, and steroleosins (Abdullah et al., 2020). The arrangement of these proteins at the oleosome's interface renders them hydrophilic and thus facilitates extraction using water (Nikiforidis and Kiosseoglou, 2009). Furthermore, these protein coatings serve to shield oleosomes from environmental stresses (Iwanaga et al., 2007; Abdullah et al., 2020). Indeed, in the conducted studies, the integration of oleosomes into the production of various milk beverages, soy milk, and chocolate resulted in products with significantly increased stability (Gallier et al., 2012; Shakerardekani et al., 2013; Nikiforidis et al., 2014; Mantzouridou et al., 2019).

The remarkable properties of oleosomes, such as their high emulsifying capacity, low toxicity potential, biocompatibility, and cost-effectiveness, have garnered significant attention in the context of their application as natural emulsifiers (Abdullah et al., 2020; Ntone et al., 2023). While numerous studies have explored the diverse applications of oleosomes, their utilisation in the context of liquid margarines has not been previously documented. In this investigation, oleosomes obtained from raw hazelnuts *via* an aqueous extraction method were employed as a key ingredient in the formulation of liquid margarine. The primary objectives of this study were to assess the rheological properties and oxidative stability of the resulting liquid margarine, thereby contributing to the current body of knowledge regarding the multifaceted utility of oleosomes in food science.

2. MATERIALS AND METHODS

2.1. Materials

Natural raw hazelnuts (*Corylus avellana* L.) were procured from the Giresun province of Turkey and kept unshelled in a dark room at 5 °C until analyses were carried out. Glycerol monostearate (40–55), used as an emulsifier, and other chemicals were purchased from Sigma Aldrich (Munich, Germany).

2.2. Preparation of oleosome

First, the hazelnut sample (100 g) was soaked in distilled water (at a ratio of 1:4) at 4 °C for 16 h, followed by grinding using a meat-grinding machine (a hole diameter of 3 mm). The resulting slurry was then filtered through two layers of cheesecloth to obtain raw hazelnut milk. For oleosome purification, the liquid sample was centrifuged for at least 15 min at 3,000 *g* and 4 °C, facilitating the separation of the oleosome layer. This uppermost layer was isolated, suspended in a 1:4 ratio of distilled water, and then subjected to centrifugation at 10,000 *g* for 30 min (three times) (Romero-Guzmán et al., 2020).

2.3. Oleosome analyses

2.3.1. Lipid, protein and fatty acid composition analyses. The lipid (AOAC 995.19) and protein contents (AOAC 993.13) of hazelnuts and oleosomes were determined employing standardised methods (AOAC, 2019). Fatty acid composition analysis involved extracting hazelnut and oleosome oils using a chloroform/methanol solvent system (Kapchie et al., 2013), followed by the preparation of fatty acid methyl esters as outlined by Hartman and Lago (1973). Fatty acid compositions were analysed using a Shimadzu GC-2010plus gas chromatograph with a DB23 capillary column (60 m × 0.25 mm × 0.25 µm) and a flame ionisation detector. Helium was the carrier gas at a flow rate of 1 mL min^{-1} with a split ratio of 1:80. The injector, column, and detector temperatures were maintained at 230, 190, and 240 °C, respectively.

2.3.2. Creaming index measurement. The creaming index of oleosomes was assessed following the protocol outlined by Iwanaga et al. (2007). Initially, solutions were prepared by mixing deionised water with 0.5 g mL^{-1} oleosomes. The pH of each solution was adjusted to a range of 3–10 before the final volume adjustment. Ten grams of the oleosome solution were homogenised at approximately 5,000 r.p.m. for 1 min using an Ultra-Turrax and then stored at room temperature for 14 days. Measurements were taken in triplicate for the total height of the oleosome suspension (H_t), the height of the serum layer (H_{SL}), and the height of the cream layer (H_{CL}) to calculate the creaming index.

$$\text{Creaming index for the serum layer } CI_{SL} = 100 \times H_{SL}/H_t$$

$$\text{Creaming index for the cream layer } CI_{CL} = 100 \times H_{CL}/H_t$$

2.3.3. Particle size and ζ-potential analysis. Particle size and ζ-potential analyses were conducted at pH 7 for optimal creaming stability. The oleosome was diluted to 0.1% (v/v) in deionised water at pH 7 for particle size measurement using a static multi-angle light scattering device (Mastersizer 2000, Malvern Instruments Co., Ltd, UK). Refractive index values of 1.47 and 1.33 were set for the dispersed and continuous phases, respectively. Particle size was determined by volume-weighted mean diameter ($D_{3,2}$ and $D_{4,3}$).

For ζ-potential measurements, the oleosomes were diluted to a concentration of 0.001% (v/v) using deionised water at pH 7. The diluted suspensions were mixed and placed into a conductive cell of the Malvern Zetasizer Nano Z potential (Malvern Instruments, Worcestershire, UK). The ζ-potential measurement was reported as the average, with the standard deviation calculated from three readings taken for each of the two freshly prepared samples.

2.3.4. FT-IR analysis. The FT-IR measurements of the fresh oleosome, without undergoing any drying process, were conducted using an IR Affinity-1 Spectrometer (Shimadzu, Japan). The spectral analysis covered a range of wavenumbers from 4,000 to 400 cm^{-1}.

2.4. Liquid margarine production

For the water phase, 1 g of salt and 1.5 g of skim milk powder were dissolved in 24 g of water. The oil phase was prepared at 80 °C, combining 0.75 g of glycerol monostearate with a mixture

of 24 g of palm stearin and 96 g of hazelnut oil. These phases were then mixed using an Ultra-Turrax to form an emulsion. The emulsion was crystallised using a soft ice cream maker at +6 °C as the control sample.

In the production of oleosome-containing samples, salt and skim milk powder were dissolved in water, followed by the addition of varying amounts of oleosome. This aqueous phase was then combined with an oil phase containing glycerol monostearate and the oil mixture. The resulting emulsion underwent crystallisation using a soft ice cream maker operating at +6 °C, following the specifications outlined in Table 1. The production process was repeated three times for liquid margarine, while analyses were performed twice.

2.5. Liquid margarine analyses

2.5.1. Monitoring of emulsion droplet. Observations of the emulsion droplets were conducted using a light microscope (Leica DM5500 B, Leica Microsystems, Germany) at a magnification of 20×.

2.5.2. Physical measurements and oxidation analyses. The viscoelastic properties of liquid margarines were assessed using a rheometer (TA.AR2000 EX, TA Instruments, DE) across a frequency range of 0–100 rad s^{-1} at 25 °C, with a 20 mm diameter acrylic probe.

Textural characteristics were evaluated using the 'Measure Force in Compression' method with specific settings: Test speed: 3.0 mm s^{-1}, Post-Test speed: 10 mm s^{-1}, and Distance: 16 mm, employing the TTC Spreadability Rig HDP/SR hardware.

Stability was determined by storing liquid margarines in centrifuge tubes at room temperature (+25 °C) for 28 days.

Peroxide value (PV) (Cd 8b-90) and thiobarbituric acid (TBA) value (Cd19-90) of the samples were measured according to AOCS (1998).

2.6. Statistical analysis

Analysis of variance (ANOVA) was conducted using the SPSS software package, and the significance of differences between means was assessed using Duncan's multiple range test at the significance level of 0.05.

Table 1. Liquid margarine formulations

Samples	Oil phase		Water phase			
	Oil mix (%)	GMS (g)	SMP (g)	Salt (g)	Oleosome (g)	Water (g)
Control	100	0.75	1.5	1	–	24
10%	90	0.75	1.5	1	14.458	21.542
20%	80	0.75	1.5	1	28.915	19.085
20%	70	0.75	1.5	1	43.370	16.630

GMS: Glycerol monostearate; SMP: Skimmed milk powder.

3. RESULTS AND DISCUSSION

3.1. Oleosome analyses

3.1.1. Lipid and protein content. The lipid and protein contents of hazelnuts were approximately 50.03 ± 1.42% and 14.95 ± 0.16%, respectively. On the other hand, the lipid and protein contents of oleosomes were determined to be 83.07 ± 2.28% and 2.48 ± 0.07%, respectively. In a study on oleosome extraction from raw hazelnuts, the oleosomes contained 77.5% oil and 1.2% protein (Capuano et al., 2018). This study observed higher oil content and lower protein content compared to previous findings.

3.1.2. Fatty acid composition. Hazelnut and oleosome-derived oils shared similar fatty acid profiles, with oleic acid being predominant at 84%. Palm stearin, on the other hand, had a significant palmitic acid content of 64%. In the oil mix for liquid margarine, composed mainly of palm stearin and hazelnut oil, oleic acid accounted for 72.14%, while palmitic acid was at 17.35%. Remarkably, replacing 10%–30% of the oil mix with oleosomes increased oleic acid content to 75.67% and decreased palmitic acid content to 13.87% (Table 2).

3.1.3. Creaming index measurement. The impact of pH on the stability of oleosome suspensions stored at room temperature for 14 days was examined through the creaming index, a commonly used parameter for evaluating suspension stability. This index offers indirect insights into the degree of droplet aggregation within oleosome suspensions, with higher values indicating greater particle aggregation.

Figure 1 demonstrates that oleosomes extracted from hazelnuts exhibited full separation into cream and serum phases within the pH range of 3–4, resulting in suspension destabilisation. This observation is consistent with the findings of Qi et al. (2017), who noted a similar tendency of oleosome suspensions to form serum at acidic pH levels. At pH 8, although no serum layer was observed, the presence of a cream layer indicated instability in the suspension. However, oleosomes demonstrated complete stability at pH 7. Yet, at pH 5–6 and pH 9–10, the oleosomes separated into three distinct phases. Consequently, oleosome characterisation analyses were conducted at pH 7. Consistently, Iwanaga et al. (2007) found optimal stability for soybean oleosomes within the pH range of 7–8. Additionally, Qi et al. (2017) noted that the most stable soybean oleosome suspension occurred at pH 9.

Table 2. Fatty acid compositions

Samples	14:0	16:0	18:0	18:1	18:2
Hazelnut oil	0.02 ± 0.0	5.59 ± 0.12	2.48 ± 0.13	84.23 ± 1.12	7.67 ± 0.20
Oleosome	0.01 ± 0.00	5.74 ± 0.10	2.66 ± 0.14	83.90 ± 1.02	7.68 ± 0.20
Palm stearin	2.40 ± 0.10	64.41 ± 0.10	4.52 ± 0.11	23.82 ± 0.12	4.91 ± 0.08
Oil mixture	0.50 ± 0.10	17.35 ± 0.10	2.88 ± 0.10	72.14 ± 0.10	7.12 ± 0.15
Liquid margarines containing oleosome					
10%	0.45 ± 0.10	16.19 ± 0.10	2.86 ± 0.10	73.32 ± 0.19	7.17 ± 0.14
20%	0.40 ± 0.10	15.03 ± 0.13	2.84 ± 0.10	74.50 ± 0.18	7.23 ± 0.10
30%	0.35 ± 0.10	13.87 ± 0.12	2.82 ± 0.12	75.67 ± 0.21	7.29 ± 0.10

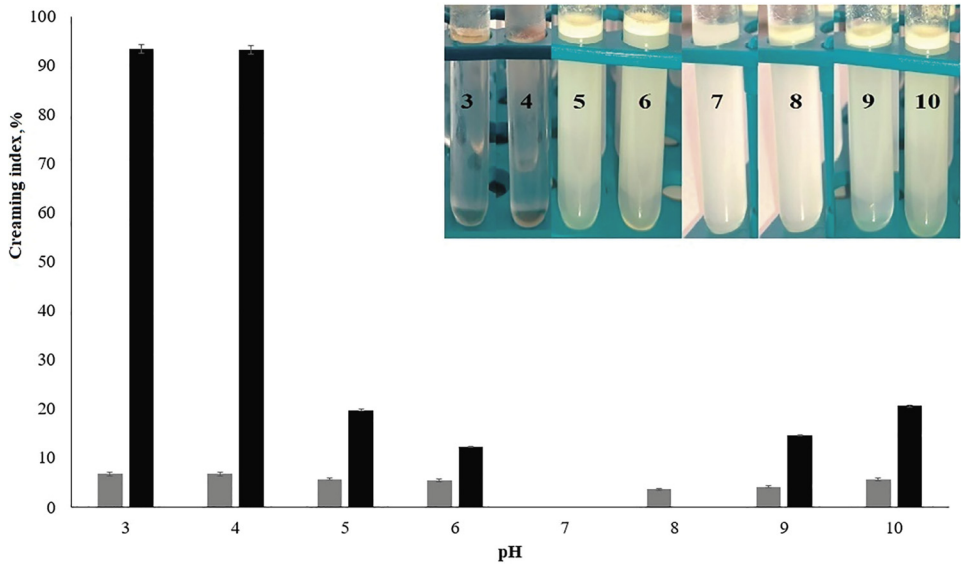

Fig. 1. Creaming index of oleosome suspensions at different pH values ($CI_{SL;}$ Creaming index for the serum layer; $CI_{CL;}$ Creaming index for the cream layer)

3.1.4. Particle size and ζ-potential. Particle size and ζ-potential analyses are effective methods for assessing emulsion stability. Prior research consistently indicates that suspensions or emulsions with higher absolute ζ-potential values and smaller particle sizes tend to have better stability (Ding et al., 2020).

In our study, the oleosomes displayed a volume mean diameter, $D_{4,3}$, of 10.22 ± 0.06 μm and a surface area mean diameter, $D_{3,2}$, of 3.58 ± 0.02 μm (Fig. 2). Previous investigations have reported volume mean diameters, $D_{4,3}$, for oleosomes derived from various sources within the range of 0.5–26.56 μm (De Chirico et al., 2017; Qi et al., 2017; Capuano et al., 2018; Ding et al., 2020; Ntone et al., 2020; Kara et al., 2024).

The surface charge of oleosomes significantly affects suspension stability. Higher absolute ζ-potential values generally correspond to greater stability. In our study, the ζ-potential of hazelnut oleosomes at pH 7 was approximately −33.8 ± 1.61 mV (Fig. 2). Previous studies have reported a range for the maximum absolute ζ-potential values of oleosomes from various plant sources from 19.3 to 41 mV (Qi et al., 2017; Capuano et al., 2018; Ding et al., 2020; Zambrano and Vilgis 2023; Kara et al., 2024). These results indicate variations in the particle size and ζ-potential of oleosomes based on their source and extraction method.

3.1.5. FT-IR. FT-IR results provide information about the chemical composition and structural characteristics of a substance. Figure 3 presents the FT-IR spectrum of hazelnut oleosomes, revealing the presence of several distinctive functional groups. The peaks at 2,922 and 2,854 cm^{-1} can be attributed to the stretching vibrations of $–CH_2$ groups. Additionally, a prominent peak at 1,745 cm^{-1} corresponds to the carbonyl group of triglycerides within the oil component. Furthermore, the peak at 1,462 cm^{-1} is associated with the N–H bending vibration of protein amides (oleosome

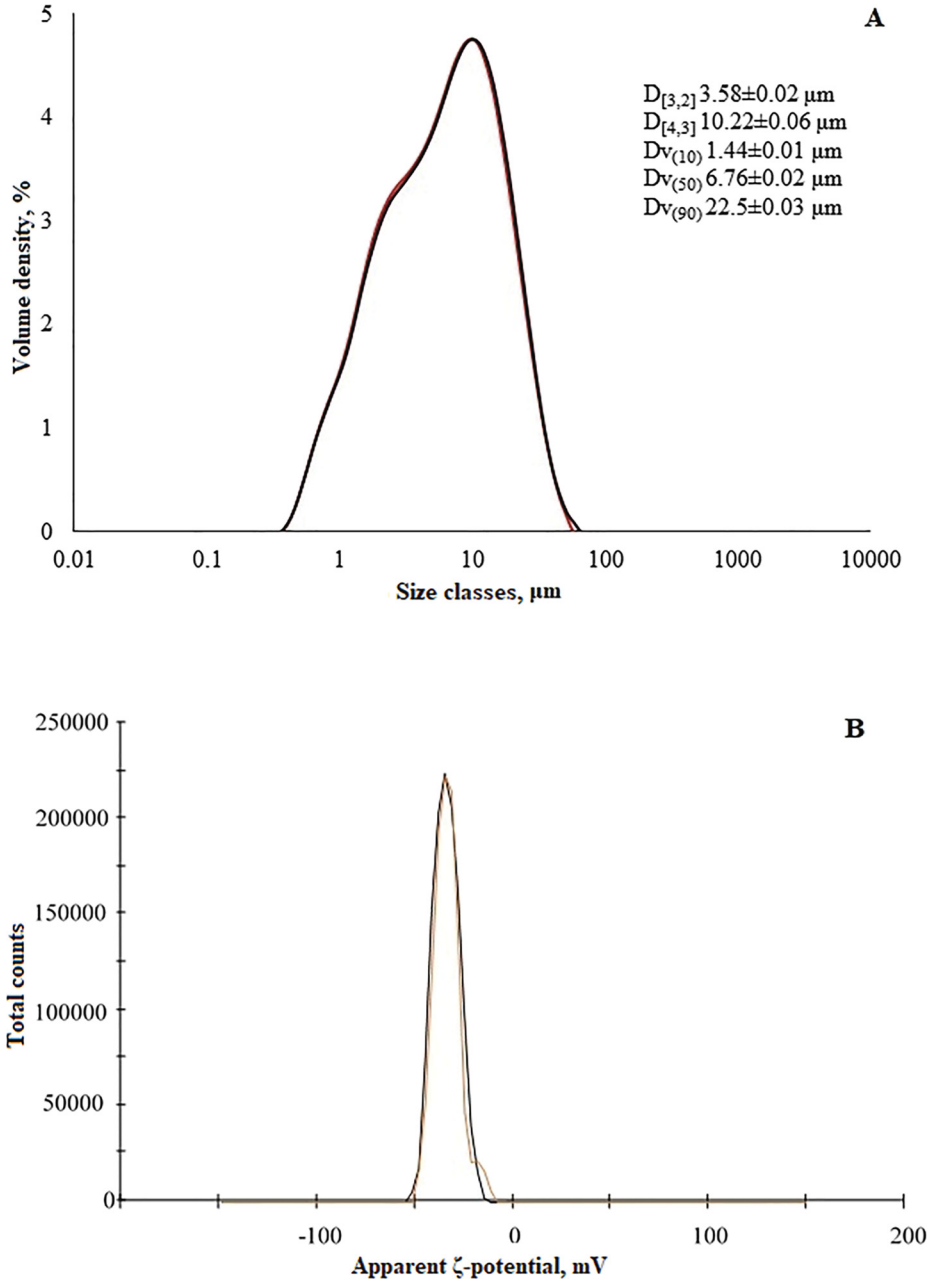

Fig. 2. Particle size distribution (A) and ζ-potential (B) of the oleosome at pH 7

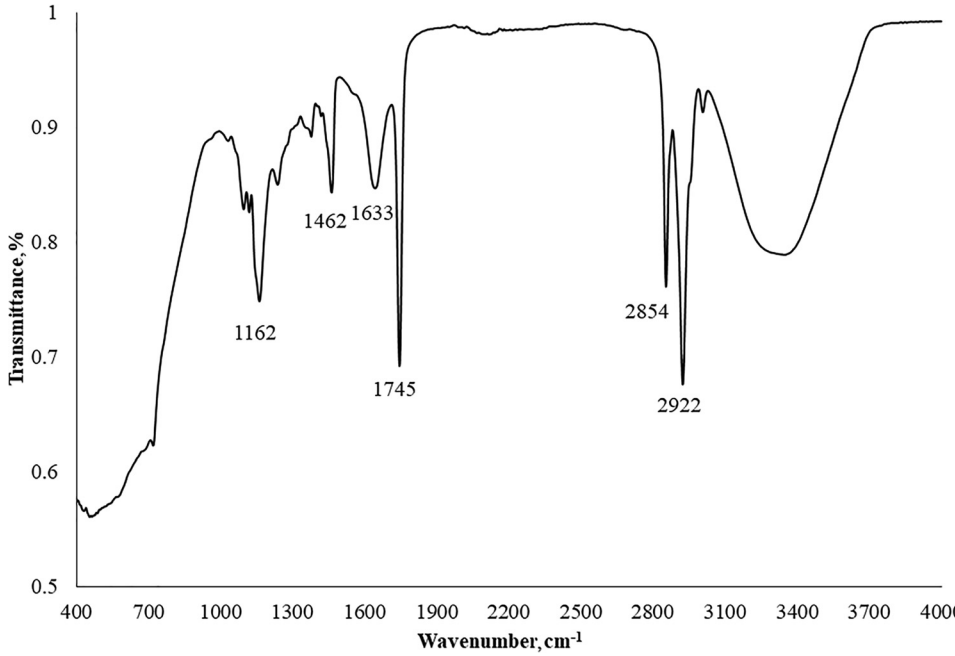

Fig. 3. The FT-IR spectrum of the hazelnut oleosome

proteins), while the peak at 1,162 cm^{-1} primarily arises from C–O stretching vibrations (Matsakidou et al., 2019). In the hazelnut oleosome spectrum, a singular β-sheet structure peak was observed at 1,633 cm^{-1}.

3.2. Liquid margarine analyses

3.2.1. Observation of droplet. As illustrated in Fig. 4A, the control sample exhibited the formation of a single emulsion. In contrast, when oleosomes were introduced (Fig. 4B), a clear depiction of double emulsion formation was observed.

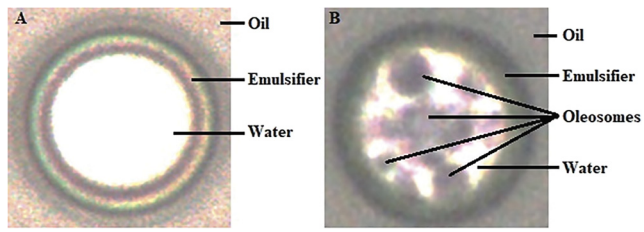

Fig. 4. Microscopic images of the liquid margarines. Control (A) and liquid margarine including oleosome (B)

3.2.2. Physical and oxidative quality. The quality of margarine relies on its physical properties, encompassing textural and rheological characteristics. Elastic and viscous moduli (G′ and G″), spreadability, and firmness are pivotal indicators directly impacting mouthfeel, meltability, and overall quality. G′ and G″ of liquid margarines, plotted against frequency (rad s^{-1}), are depicted in Fig. 5. G′ consistently surpasses G″, indicating a semi-solid structural nature. With increasing oleosome proportions (from 10% to 30%) in liquid margarines, G′, G″, firmness, and spreadability values decrease (Table 3). This observed phenomenon is attributed to oleosome

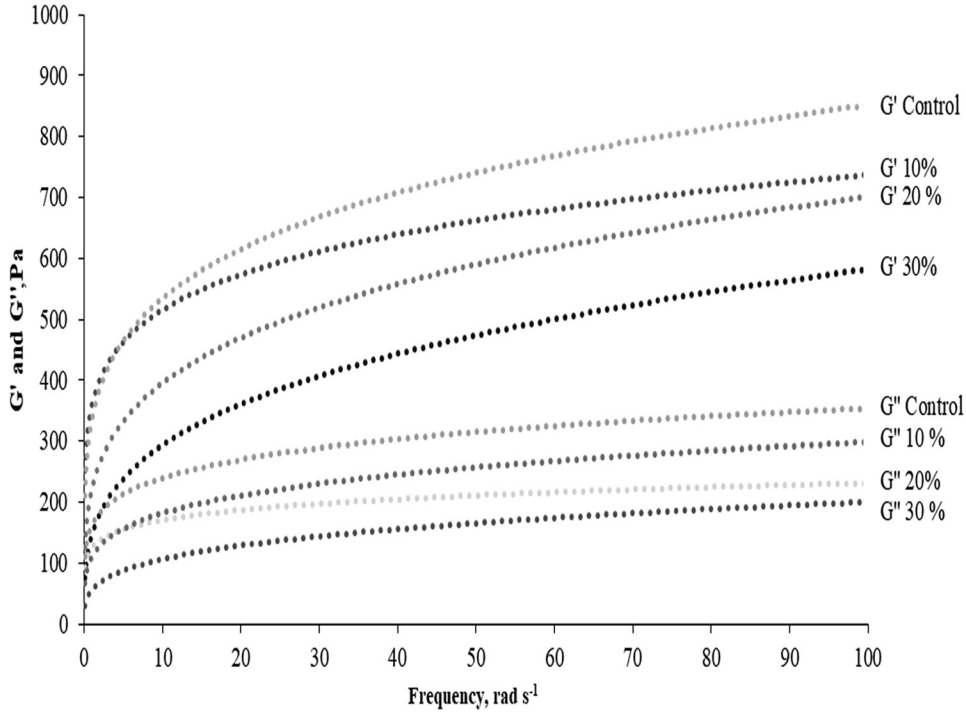

Fig. 5. Elastic (G′) and viscous (G″) moduli of liquid margarine

Table 3. Stability, textural properties, PVs, and TBA values of liquid margarines (25 ± 0.5 °C)

Samples	Firmness (g) 7th day	Spreadability (g. sec) 7th day	PV (meq O_2/kg) 7th day	TBA (mg MDA/kg) 7th day	Stability (28th day)
Control	33.76 ± 0.25[d]	24.73 ± 0.25[d]	0.49 ± 0.03[d]	0.42 ± 0.01[d]	+
10%	29.74 ± 0.35[c]	20.07 ± 0.39[c]	0.19 ± 0.03[c]	0.30 ± 0.02[c]	+
20%	25.38 ± 0.30[b]	16.47 ± 0.34[b]	0.15 ± 0.01[b]	0.25 ± 0.01[b]	+
30%	19.97 ± 0.15[a]	10.23 ± 0.15[a]	0.09 ± 0.01[a]	0.19 ± 0.02[a]	+

[a] Samples indicated with different superscripts in the same column are statistically different ($P < 0.05$).
PV: peroxide value; MDA: malondialdehyde.

incorporation, elevating oleic acid content while concurrently reducing palmitic acid content. Similar findings have been reported in previous studies (Fallahasgari et al., 2023).

Lipid oxidation is a significant concern due to its potential to produce undesirable aromas and flavours, which can adversely affect the nutritional quality of fats. PV and TBA value are widely acknowledged as critical parameters in assessing lipid oxidation (Frankel, 1991). Hence, the determination of these values in lipid-containing products is imperative.

Table 3 shows the PVs (meq O_2/kg) and TBA values (mg MDA/kg) for the investigated liquid margarines. Despite the decrease in palmitic acid content and increase in oleic acid content resulting from the substitution of the oil mixture with oleosomes, the PV and TBA values of the liquid margarines exhibited a reduction. This observation aligns with previous research findings (Nikiforidis and Kiosseoglou 2009; Gray et al., 2010), suggesting that oleosome-based emulsions may exhibit remarkable oxidative stability.

4. CONCLUSIONS

The findings of this study demonstrate that oleosomes extracted from hazelnuts using water exhibit high physical stability at a neutral pH value, suggesting their successful application in products with neutral pH. Moreover, the results suggest that hazelnut oleosome can effectively contribute to the chemical and physical stability of double emulsions. This presents promising prospects for their utilisation in diverse food applications, especially those necessitating stable emulsions and oxidative resilience, thereby serving as a valuable lipid source for the food industry. It is noteworthy that further investigations, particularly sensory evaluations, are imperative to corroborate the outcomes of these analyses.

Conflict of interest: The authors declare no competing interests.

Authors contributions: H.E. and F.B.T. conducted the study experiments. H.E. was responsible for the execution of the work. All authors were involved in writing the manuscript, and all authors read and approved the final manuscript.

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