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Ultrasonication-Enhanced High-Energy Emulsification for Improved Physicochemical Properties of Mangosteen Pericarp Nanoemulsions

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ABSTRACT

Formulating stable emulsions encapsulating hydrophobic plant bioactives remains a significant challenge due to poor solubility and chemical instability. This study compares two high-energy emulsification methods, high-speed homogenisation followed by microfluidisation (HSH-MF) and high-speed homogenisation followed by ultrasonication and microfluidisation (HSH-USP-MF) for preparing emulsions containing optimised dried mangosteen pericarp extract (ODMPE). Using identical formulations with food-grade biopolymers as emulsifiers, emulsions were characterised by droplet size, polydispersity index (PDI), zeta potential, antioxidant activity, and encapsulation efficiency (EE) after preparation and during storage. Results showed that both the HSH-USP-MF and HSH-MF methods produced emulsions C, C7 and D with similarly small droplet sizes in the nanometer range (296-336 nm), low polydispersity index (0.3-0.4), and acceptable zeta potentials (-36 to -40 mV), indicating effective droplet size reduction uniformity, and electrostatic stability. However, the HSH-USP-MF method combined with pea protein isolate (PPI) at pH 7 yielded emulsions with the highest antioxidant activity, reflecting superior electrostatic stability and bioactive protection compared to HSH-MF emulsions. Emulsion C7 (HSH-USP-MF at pH 7) exhibited the highest EE (87.6%) and superior antioxidant retention, maintaining over 80% EE after one month. In contrast, emulsions prepared by HSH-MF showed moderate encapsulation (54–59%) and slightly lower stability. The improved performance of HSH-USP-MF emulsions is attributed to ultrasonic cavitation, which enhances emulsifier adsorption and interfacial film strength, coupled with optimised protein charge at neutral pH that promotes electrostatic repulsion and droplet stabilisation. Overall, this study demonstrates that integrating ultrasonication within the emulsification sequence significantly improves the physicochemical properties and functional stability of ODMPE-loaded emulsions. These findings provide valuable insights for designing scalable, efficient delivery systems for hydrophobic bioactives in functional foods and nutraceuticals.

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1. Introduction

The development of bioactive-loaded emulsions is a growing area within process and chemical engineering, especially for applications in functional foods, pharmaceuticals, and nutraceuticals. However, incorporating hydrophobic compounds such as mangosteen (*Garcinia mangostana* L.) pericarp extract into aqueous systems poses formulation challenges due to their poor solubility and chemical instability. These limitations hinder the bioavailability and functional efficacy of such compounds in food, pharmaceutical, and nutraceutical applications. Emulsion-based delivery systems have therefore gained significant attention as versatile platforms for improving the dispersion, protection, stability, and controlled release of hydrophobic bioactives [1].

Oil-in-water (O/W) emulsions are particularly suitable for encapsulating hydrophobic substances, as they facilitate the incorporation of lipophilic compounds within the dispersed oil phase, thereby enhancing their solubility and stability in aqueous environments [2]. The physicochemical properties of emulsions, especially droplet size, size distribution uniformity, and interfacial charge, are critical determinants of their physical stability and functional performance. Smaller droplet sizes and narrow size distributions reduce gravitational separation and coalescence, while appropriate interfacial charge (zeta potential) imparts electrostatic repulsion that further stabilises the system [1]. High-energy emulsification techniques such as high-speed homogenisation (HSH), ultrasonication (USP), and microfluidisation (MF) are widely employed to produce nanoemulsions with improved stability and bioactive protection. These methods, particularly USP, disrupt oil droplets through intense shear forces and cavitation phenomena, enhancing emulsifier adsorption and interfacial film strength [3]. While MF employs high-pressure intensification to force fluids through a microstructured interaction chamber, where shear and impact forces generated by rapid stream collisions and wall interactions disrupt droplets, achieving submicron sizes and narrow size distributions. The sequence and combination of these techniques influence the extent of droplet size reduction and uniformity, with ultrasonication providing unique cavitation effects that can further improve emulsification efficiency and bioactive encapsulation [4].

Recent studies have demonstrated that the incorporation of ultrasonication between HSH and MF steps (HSH-USP-MF) can produce emulsions with superior physicochemical properties compared to dual-stage methods (HSH-MF) alone, including smaller droplet sizes, lower polydispersity index (PDI), and enhanced encapsulation efficiency [5]. However, direct comparisons of these methods using consistent formulations remain limited, particularly regarding their influence on short-term emulsion stability.

This study aims to fill this gap by comparing emulsions prepared by HSH-USP-MF and HSH-MF methods using ODMPE as the bioactive ingredient. Key parameters such as droplet size, PDI, zeta potential, antioxidant capacity, and encapsulation efficiency were evaluated immediately after preparation and during storage. The findings will provide insights into the influence of ultrasonic cavitation during emulsification on droplet stability, which is critical for guiding formulation strategies in functional food and nutraceutical applications.

2. Methodology

2.1 Preparation of Optimum Dried Mangosteen Pericarp Extract (ODMPE)

The optimum dried mangosteen pericarp extract (ODMPE) was prepared via microwave-assisted extraction under previously established conditions: 2.24 minutes irradiation time, a solvent-to-solid ratio of 25 mL/g, and 71% ethanol concentration [6].

2.2 Preparation of Pea Protein Isolate Solution as Aqueous Phase 1 (W_1)

PPI solutions (1% w/w) were prepared by dispersing pea protein isolate in deionised water, supplemented with 0.01% (w/w) sodium azide as an antimicrobial agent. The pH of the solutions was adjusted to 6.0 or 7.0 using 0.1 M HCl, followed by 24 h of stirring at 25°C to ensure complete hydration. The hydrated solutions were centrifuged (10,000 rpm, 25°C, 15 min), and the supernatant was collected and stored at 4°C until use. Based on prior solubility assessments [7], the PPI solution was assumed to exhibit 68% solubility. This solution served as the primary aqueous phase (W_1) in the oil-in-water ($O/W_1/W_2$) emulsion formulation.

2.3 Preparation of Soluble Soybean Polysaccharide Solution as Aqueous Phase 2 (W_2)

A 1% (w/w) SSPS solution was prepared by dissolving SSPS in deionised water at 70 °C, with 0.01% (w/w) sodium azide added as an antimicrobial agent. After complete dissolution, the pH was adjusted to 6.0 using 0.1 M NaOH. The solution was stirred continuously at 70 °C for 2 hours, then allowed to hydrate overnight at room temperature before storage at 4 °C. While previous studies reported that heating SSPS conjugated with soy protein isolate enhances emulsion stability via covalent bonding and facilitates controlled release of hydrophobic compounds Yang *et al.,* [8], this study employed the individually heated SSPS solution as the secondary aqueous phase (W_2) in the oil-in-water-in-water ($O/W_1/W_2$) emulsion, serving as the second emulsifier layer through non-covalent interactions.

2.4 Preparation of Oil-in-Water Emulsion ($O/W_1/W_2$)

Oil-in-water-in-water ($O/W_1/W_2$) emulsions were prepared following modified protocols based on Cheong *et al.,* [9] and Esfanjani *et al.,* [10]. Each emulsion was produced in 100 g batches in accordance with the procedure described by Mohammad *et al.,* [5]. The oil phase (O) was formed by mixing 40 mg of optimum dried mangosteen pericarp extract (ODMPE) with 1 g of soybean oil using a vortex mixer for 5 minutes. This oil phase was added dropwise into the primary aqueous phase (W_1, 49.48 g) containing pea protein isolate (PPI), and the mixture was stirred at 800 rpm for 5 minutes to form an O/W_1 mixed solution. Subsequently, the O/W_1 mixed solution was added dropwise into the secondary aqueous phase (W_2, 49.48 g) containing soluble soybean polysaccharide (SSPS) and stirred at 800 rpm for 5 minutes to form the final $O/W_1/W_2$ pre-emulsion. PPI solutions were prepared at pH 6.0 and 7.0 to investigate the effect of protein charge near and away from its isoelectric point. Two homogenisation protocols were applied to finalise the emulsions, adapted from Qamar, Bhandari, and Prakash [11]: 1. a combined high-speed homogeniser, ultrasonic processor, and microfluidiser (HSH-USP-MF) (emulsion C and C7); 2. a combined high-speed homogeniser and microfluidiser (HSH-MF) (emulsion D and D7). Figure 1 displays the conditions for each homogenisation tool. Control emulsions (C-Control, D-Control, C7-Control, D7-Control) were prepared identically but without ODMPE in the oil phase. All emulsions were stored at room temperature (25 °C) for characterisation.

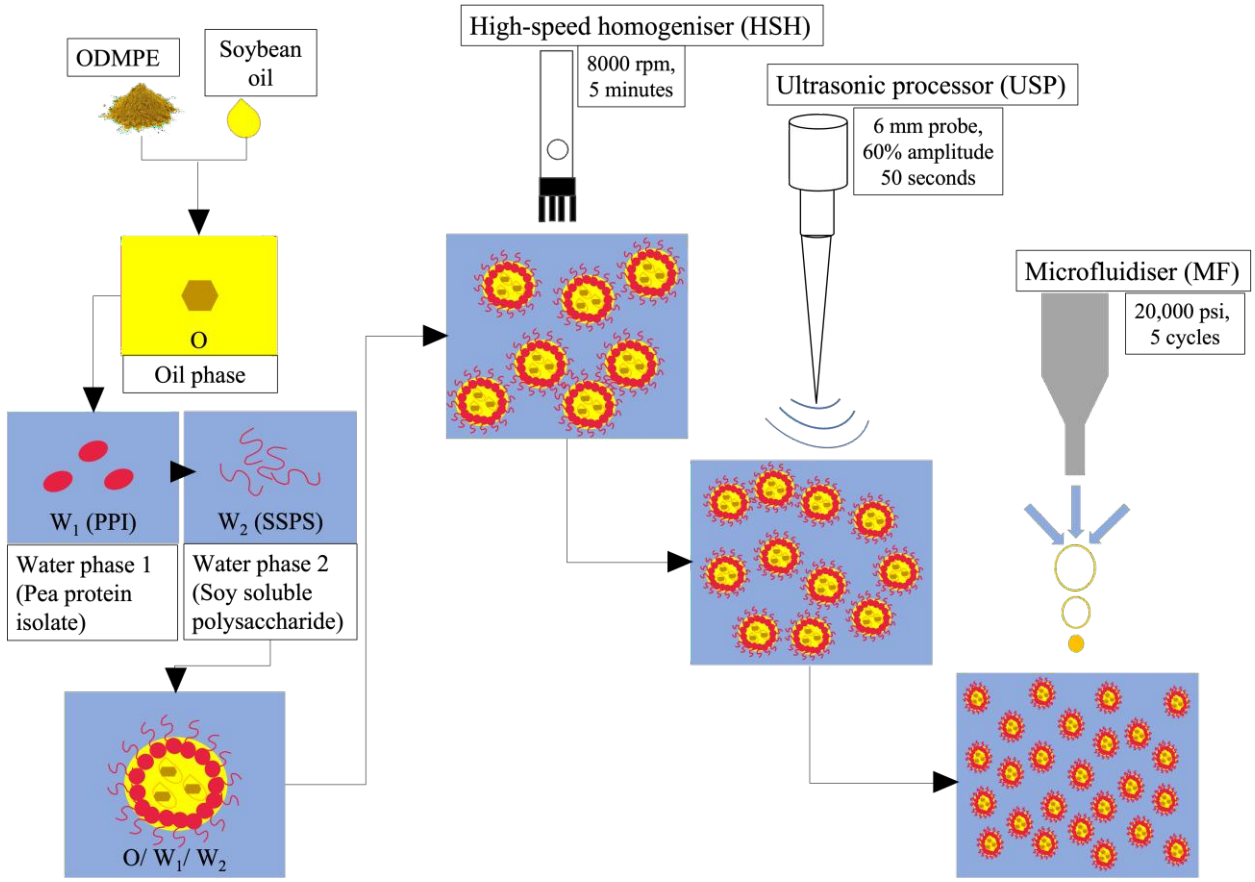

Fig. 1. Schematic diagram of the preparation of oil-in-water ($O/W_1/W_2$) of emulsion

2.5 Physical Properties of Emulsions

2.5.1 Mean droplet size and polydispersity index (PDI)

The droplet size and polydispersity index (PDI) of the emulsions were measured using dynamic light scattering with a Zetasizer Nano ZS (Malvern Panalytical, UK) at 25 °C [12]. Samples were diluted at a 1:9 ratio with deionised water prior to analysis. The average droplet size is reported as the Z-Average diameter (d.nm), while the PDI, indicating size distribution uniformity, ranges from 0 (monodisperse) to 1 (highly polydisperse).

2.5.2 Zeta potential

The zeta potential of emulsions was determined by laser Doppler micro-electrophoresis using a Malvern Zetasizer Nano ZS (Malvern Panalytical, UK) [12]. Samples were diluted at a 1:9 ratio with deionised water before measurement. An 800 µL aliquot was placed into a folded capillary zeta cell (DTS1070), and measurements were performed at 25 °C using the instrument's software. The zeta potential, expressed in millivolts (mV), reflects the surface charge and stability of the colloidal system.

2.6 Antioxidant Activity of Emulsions

The antioxidant activity of emulsions and controls was assessed using the ABTS radical scavenging assay following the method of Mohammad *et al.,* [6]. The $ABTS^{\bullet+}$ stock solution was prepared by mixing equal volumes of 7.0 mM ABTS and 2.45 mM potassium persulfate, then incubated in the dark at room temperature for 12 hours. The solution was diluted with 95% ethanol to achieve an

absorbance of 0.70 ± 0.05 at 734 nm. Sample dilutions (50–400 µg/mL) were prepared, and 50 µL of each was mixed with 2.5 mL of the ABTS reagent. Absorbance was measured at 734 nm using a microplate reader to determine radical scavenging activity. The ABTS radical scavenging activity (%) of emulsions and controls was first determined. The antioxidant capacity was then expressed as milligrams of α-mangostin equivalents (mg α-mangostin/g emulsion) and milligrams of Trolox equivalents (mg TE/g emulsion), calculated using a logarithmic regression equation for α-mangostin and a linear regression equation for Trolox, respectively.

2.6 Encapsulation Efficiency of Emulsions

Encapsulation efficiencies of the nanoemulsions ($O/W_1/W_2$) were measured following a modified method based on Guo *et al.,* [13]. Briefly, 1 mL of ODMPE or nanoemulsion was mixed with 3 mL of 95% ethanol and vortexed for 2 minutes. The mixture was centrifuged at 10,000 rpm and 25 °C for 15 minutes to remove large particles and free α-mangostin. The supernatant was filtered through a 0.45 µm PTFE syringe filter before HPLC analysis. Quantification of α-mangostin was performed using an Agilent 1260 Infinity HPLC system equipped with a diode array detector (DAD) and a C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase was delivered at 1.0 mL/min. α-Mangostin concentration was determined using a calibration curve generated from standard solutions by plotting peak area against concentration. Encapsulation efficiency (%) was calculated using the ratio of encapsulated α-mangostin in the nanoemulsion to the total α-mangostin content, as expressed in Eq. (1):

$$Encapsulation\ efficiency, EE\ (\%) = \frac{m_b}{m_a} \times 100 \quad (1)$$

Where m_b represents the amount of α-mangostin encapsulated within the nanoemulsion, and m_a denotes the initial α-mangostin content added (ODMPE). Encapsulation efficiency (EE, %) was evaluated at 0 and 1 month during storage at 4 °C.

3. Results

Following up on earlier work, which demonstrated that the synergistic interaction between ODMPE, pea protein isolate (PPI), and soluble soybean polysaccharide (SSPS) significantly enhances the physicochemical properties and stability of emulsions encapsulating optimum dried mangosteen pericarp extract (ODMPE), a hydrophobic bioactive compound rich in a-mangostin [5], this study narrows its focus to the emulsions prepared using the most effective homogenisation methods identified, high-speed homogenisation followed by microfluidisation (HSH-MF) and high-speed homogenisation followed by ultrasonication and microfluidisation (HSH-USP-MF). Specifically, emulsions C and C7 represent those produced by HSH-USP-MF, while D and D7 were formulated via HSH-MF, using identical formulation components and processing parameters. The inclusion of pH variation in the pea protein isolate (PPI) phase, represented by emulsions C and D at pH 6 and emulsions C7 and D7 at pH 7, allowed further assessment of protein-emulsifier interaction under two different process conditions. The HSH-USP-MF method, which integrates ultrasonication between high-speed homogenisation and microfluidisation, produced emulsions with notably smaller droplet sizes and enhanced interfacial stability compared to the simpler HSH-MF approach. The key variables examined were droplet size, polydispersity index (PDI), zeta potential, antioxidant properties, and encapsulation efficiency. This targeted comparison aims to elucidate the effect of sequential high-

energy processing steps, particularly the role of ultrasonication, on the physical characteristics and antioxidant retention of ODMPE-loaded emulsions.

3.1 Particle Size Measurement

In our previous work, we evaluated several homogenisation methods, including HSH (emulsions A/A7) and HSH coupled with ultrasonication (HSH-USP, B/B7) and found that emulsions produced by HSH-USP-MF (emulsions C/C7) and HSH-MF (emulsions D/D7) exhibited the lowest droplet sizes and PDI values [5]. Notably, all emulsions showed significantly smaller droplet sizes and narrower distributions compared to their respective controls. This article focuses on comparing these two methods (HSH-USP-MF and HSH-MF) due to their exceptional performance in achieving nanoscale emulsions with enhanced physical stability. Table 1 shows the mean droplet size, polydispersity index (PDI), and zeta potential of emulsions C and D and emulsions C7 and D7, along with their respective emulsion controls.

3.1.1 Mean droplet size

Both the HSH-USP-MF and HSH-MF techniques yield comparable results in generating nanoemulsions that encapsulate α-mangostin, with emulsions C, C7, and D7 exhibiting mean droplet sizes of 295.6 to 335.7 nm, along with similarly low polydispersity index (PDI). Remarkably, while both methods produce emulsions with droplet sizes in the nanometre range and low size dispersion, the HSH-USP-MF approach further amplifies pH-dependent benefits. Specifically, in the emulsion controls (formulations without ODMPE), the HSH-USP-MF method yielded smaller droplet sizes under both PPI pH conditions compared to the HSH-MF method. This suggests that the additional ultrasonication step in the combined process enhances the initial droplet breakup via acoustic cavitation, thereby improving overall interfacial stabilisation even in the absence of bioactives. Consequently, this approach not only ensures effective nanoemulsion formation but also potentially confers superior physical stability that may further benefit the encapsulation and protection of sensitive compounds. This size reduction can be attributed to the synergistic effect of ultrasonication and microfluidisation. These observations are in line with the findings of Wang *et al.,* [4], who reported that combining microfluidisation with ultrasonication when modifying citrus pectin resulted in a significant reduction of the hydrodynamic diameter from over 600 nm in untreated samples to approximately 418 nm in treated pectin. Although the systems differ (pectin solution versus oil-in-water emulsions), both studies highlight the effectiveness of combining these techniques to achieve nanoscale particle formation and enhance interfacial characteristics.

Table 1
Mean droplet sizes (nm), polydispersity index, and zeta potential (mV) of emulsions and their controls

Emulsions	Mean droplet size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
C	295.6 ± 7.5^{a}	0.363 ± 0.01^{a}	-39.57 ± 0.4^{a}
D	649.6 ± 16.6^{d}	0.649 ± 0.02^{c}	-35.67 ± 0.6^{cd}
C7	335.7 ± 19.5^{a}	0.409 ± 0.01^{a}	-37.79 ± 0.5^{ab}
D7	327.9 ± 17.6^{a}	0.364 ± 0.01^{a}	-36.07 ± 0.8^{bc}
C-Control	551.7 ± 18.8^{c}	0.648 ± 0.03^{c}	-33.43 ± 0.7^{e}
D-Control	1033.8 ± 38.4^{f}	1.00 ± 0.00^{e}	-34.63 ± 0.4^{cde}
C7- Control	463.1 ± 18.1^{b}	0.484 ± 0.02^{b}	-34.10 ± 0.9^{de}
D7- Control	815.5 ± 22.2^{e}	0.796 ± 0.03^{d}	-33.83 ± 0.7^{e}

Values represent mean ± standard deviation. For each analysis, different letters emulsions indicate significant differences ($p < 0.05$)

3.1.2 Polydispersity index

Alongside the reduction in mean droplet size, our emulsions processed via the combined method exhibited substantially lower polydispersity index compared to those prepared by less intensive methods. A narrow droplet size distribution is crucial for enhancing emulsion stability, as it promotes uniform interfacial coverage and minimises coalescence. Wang *et al.,* [4] similarly observed that after applying microfluidisation and ultrasonication, citrus pectin samples achieved a polydispersity as low as 0.12, indicating a homogeneous size distribution. This reduction in polydispersity can be linked to both the mechanical breakup of larger aggregates and the molecular-level modifications induced by ultrasound. In our formulation, using improvised conditions likely enhanced the efficiency of emulsifier adsorption at the oil–water interface, thereby contributing to uniform droplet stabilisation. Together, these findings validate the concept that a hybrid processing approach can not only minimise mean droplet size but also yield a more consistent droplet distribution, ultimately leading to emulsions with enhanced physical stability and better encapsulation performance.

The observed influence of PPI pH on emulsion characteristics in this study is strongly supported by the recent findings of Zhao *et al.,* [14], who systematically investigated the structural and functional modifications of pea protein isolate under various treatments, including pH shift and ultrasound. Zhao *et al.,* [14] reported that native PPI exhibits low solubility (approximately 10%), which limits its emulsifying performance in aqueous systems. However, both ultrasound and pH shift treatments, especially when combined, substantially increased PPI solubility, with ultrasound alone raising solubility to over 70% and the combined approach achieving more than 85%. Enhanced solubility was accompanied by a reduction in particle size and greater exposure of surface hydrophobic groups, both of which are critical for improving emulsifying activity and interfacial adsorption.

In the context of emulsion formation, Zhao *et al.,* [14] demonstrated that ultrasound and pH shift treatments not only reduced the particle size of PPI but also significantly improved its emulsifying activity index (EAI) and the uniformity of emulsion droplets. These structural changes facilitate better coverage of the oil-water interface, leading to smaller droplet sizes and lower polydispersity index, which are directly in line with our observation that PPI at pH 7 yields emulsions with superior droplet size and PDI values compared to pH 6, across both HSH-USP-MF and HSH-MF methods. Thus, Zhao *et al.,* [14] provide compelling mechanistic evidence that optimising PPI structure and charge state, through pH adjustment and/or ultrasound, directly translates to improved emulsification outcomes. In our experiments, this mechanistic synergy explains why emulsion controls at pH 7 consistently outperformed those at pH 6 and why the integration of ultrasound in the HSH-USP-MF method further amplified these benefits by promoting protein unfolding, solubility, and interfacial functionality.

3.1.2 Zeta potential

Our findings indicate that while emulsion controls prepared with PPI at pH 7 consistently exhibit smaller droplet sizes and lower PDI values compared to those at pH 6, the zeta potential measurements did not differ significantly between pH conditions or homogenisation methods, with the exception that emulsion C (HSH-USP-MF) demonstrated a distinctive zeta potential relative to emulsion D (HSH-MF).

Zhang *et al.,* [15] demonstrated that pH can significantly affect the zeta potential of emulsions stabilised by jackfruit seed protein, with variations in charge influencing stability. In our study, although the physical stabilisation reflected by droplet size and PDI supports the role of neutral pH

in enhancing the emulsifying properties of PPI, the similar zeta potential values across treatments suggest that net surface charge is not the major differentiating factor. Interestingly, while overall zeta potential values did not vary significantly between different pH conditions or homogenisation methods, emulsion C (produced using the HSH-USP-MF method) exhibited a higher zeta potential than emulsion D (produced by the HSH-MF method). This enhanced zeta potential in emulsion C may reflect subtle changes in the interfacial composition or protein configuration resulting from the additional ultrasonication step. The acoustic cavitation likely improves emulsifier adsorption and alignment at the oil–water interface, forming a more robust electrical double layer. Consequently, even though pH-dependent modifications did not lead to large differences in net surface charge, the higher zeta potential in emulsion C may enhance electrostatic repulsion between droplets, contributing to improved physical stability.

3.2 Antioxidant Properties

Following up on earlier work that the synergistic interaction between ODMPE, pea protein isolate (PPI), and soluble soybean polysaccharide (SSPS) significantly enhances antioxidant potential [5], the current study highlights the critical role of ultrasonic cavitation in further improving emulsion quality and bioactive retention. Table 2 summarises the ABTS radical scavenging activity of emulsions C and D, emulsions C7 and D7, and their respective emulsion controls (without ODMPE). The antioxidant activities were expressed as ABTS inhibition (%), a-mangostin equivalent (mg a-mangostin/g), and a-tocopherol equivalent (mg a-tocopherol/g).

The ABTS radical scavenging results indicate that the emulsification method and pH conditions significantly affect the antioxidant performance of ODMPE-loaded emulsions. Emulsion C7 demonstrated the highest ABTS inhibition, a-mangostin equivalent, and a-tocopherol equivalent (85.5%, 2534.7 mg a-mangostin/g, and 200.6 mg a-tocopherol/g, respectively). This outcome not only outperforms its pH 6 counterpart (emulsion C: 80.7%, 1772.9 mg a-mangostin/g, and 189.4 mg a-tocopherol/g, respectively) but also exceeds the performance of emulsions produced by the HSH-MF method (D/D7). Lee *et al.,* [16] showed that using ultrasonication for eight hours to extract crude polysaccharides from *Undaria pinnatifida sporophyll* with subsequent ethanol precipitation resulted in an extract (UPE_8) that not only had a higher yield and more polysaccharides but also markedly improved antioxidant activity in Vero cells. Moreover, the ethanol-precipitated fraction enriched in these polysaccharides (UPE_8P) exhibited better antioxidant performance than the supernatant. This approximately 5% increase in ABTS inhibition, along with higher levels of α-mangostin and α-tocopherol equivalents observed in our study, aligns with these findings and supports the conclusion that ultrasonication effectively preserves and enhances the antioxidant potential of bioactive compounds.

All emulsions formulated with ODMPE exhibited significantly higher antioxidant activities compared to their respective controls, corroborating our earlier findings [5]. In the absence of ODMPE, the controls formulated with the HSH-USP-MF method (C-Control and C7-Control) showed higher antioxidant activity than those with the HSH-MF method (D-Control and D7-Control), indicating that while the biopolymers in the continuous phase contribute to antioxidant activity, either by stabilising the emulsion or by direct radical scavenging, the incorporation of ultrasonication (i.e., the HSH-USP-MF method) markedly improves the overall performance.

Table 2
ABTS radical scavenging activity of emulsions and their controls

Emulsions	ABTS inhibition (%)	α-mangostin equivalent (mg α-mangostin /g)	α-tocopherol equivalent (mg α-tocopherol /g)
C	80.74 ± 0.37^{bc}	1772.88 ± 42.27^{b}	189.36 ± 0.75^{bc}
D	80.19 ± 0.28^{c}	1710.81 ± 35.25^{b}	188.25 ± 0.64^{c}
C7	85.50 ± 0.56^{a}	2534.74 ± 103.23^{a}	200.57 ± 1.29^{a}
D7	81.44 ± 0.62^{b}	1877.06 ± 86.58^{b}	191.14 ± 1.45^{b}
C-Control	78.83 ± 0.54^{d}	-	185.08 ± 1.26^{d}
D-Control	74.37 ± 0.39^{e}	-	174.73 ± 0.90^{e}
C7- Control	77.63 ± 0.24^{d}	-	182.30 ± 0.56^{d}
D7- Control	75.35 ± 0.26^{e}	-	177.00 ± 0.61^{e}
ODMPE	88.97 ± 1.09	3282.38 ± 261.69	394.66 ± 5.35

Values represent mean ± standard deviation. For each analysis, different letters between emulsions indicate significant differences ($p < 0.05$)

The superior performance of the HSH-USP-MF emulsions can be attributed to the effects of ultrasonic cavitation during processing. The intense shear forces and microturbulence generated by cavitation promote more efficient droplet breakup and a more uniform distribution of emulsifiers. This results in a homogeneous interfacial layer that effectively reduces the oxidative degradation of α-mangostin. Additionally, the pH 7 condition may enhance the emulsifying efficiency of PPI by aligning more closely with its optimal solubility range and promoting better interfacial adsorption.

These outcomes are consistent with studies that have reported enhanced bioactive retention in emulsions processed with ultrasonication. For instance, research on curcumin-loaded emulsions demonstrated that the combination of microfluidisation and ultrasonication led to reduced droplet sizes and improved oxidative stability [17]. Ultrasonic treatment facilitated the formation of nanoscale droplets with uniform distribution, enhancing encapsulation efficiency and minimising lipid oxidation. Notably, peroxide values were significantly lower in ultrasonicated emulsions, indicating improved protection against oxidative degradation. These findings mirror the improvements observed with α-mangostin, where ultrasonication similarly contributed to enhanced stability and bioactive retention.

Moreover, ultrasonication modifies the structure of protein emulsifiers such as PPI, increasing surface hydrophobicity and exposing antioxidant amino acid residues. For instance, the pH-dependent behaviour of plant proteins such as PPI aligns with previous findings in Khan *et al.,* [18], where neutral pH conditions of whey protein concentrate subjected to ultrasonic treatment exhibited an increase in ABTS inhibition from 47% to 68%, indicating improved antioxidant functionality. This mechanistic insight helps explain why emulsions prepared with PPI at neutral pH (pH 7) under ultrasonication showed superior antioxidant activity, as protein conformation at this pH optimises emulsifying and radical scavenging properties.

Importantly, the combined effect of ultrasonic cavitation and the biopolymer stabilisers (PPI and SSPS) creates a synergistic system where antioxidant activity is enhanced beyond the sum of individual components. Our previous work demonstrated that emulsions containing ODMPE had IC_{50} values up to 25.5% lower than controls without ODMPE, indicating stronger antioxidant potential [5]. Ultrasonication amplifies this effect by improving droplet size distribution and interfacial film robustness, thus protecting α-mangostin from oxidative degradation more effectively.

3.3 Encapsulation Efficiency

The encapsulation efficiency (EE) of emulsions C, D, C7, and D7 varied significantly, ranging from 51.6% to 87.6% (Figure 2). Emulsion C7 exhibited the highest EE at 87.6%, followed by D7 (59.1%), C

(57.9%), and D (53.5%). However, emulsion D7 had the same level of EE as emulsion C. This trend highlights the critical influence of both the homogenisation method and the pH of pea protein isolate (PPI) on the ability to retain hydrophobic bioactives such as α-mangostin within the emulsion droplets. The superior EE of emulsion C7, prepared using the HSH-USP-MF method at pH 7, can be attributed to the synergistic effects of ultrasonication and the optimised protein charge environment. Ultrasonication generates acoustic cavitation, producing intense localised shear forces that promote finer droplet formation and enhance emulsifier adsorption at the oil-water interface, resulting in a more cohesive and resilient interfacial film [19]. Simultaneously, the neutral pH condition increases the net negative charge on PPI molecules, strengthening electrostatic repulsion between droplets and between PPI and soluble soybean polysaccharide (SSPS), thereby reducing droplet aggregation and bioactive leakage [5].

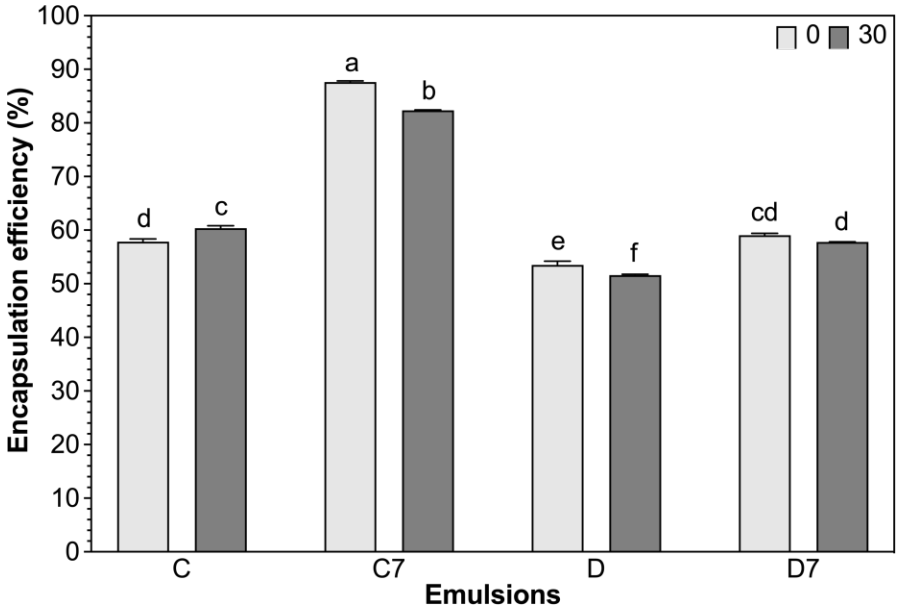

Fig. 2. Encapsulation efficiency (EE %) of emulsions C, D, C7, and D7 during 1 week (0) and 1 month (30) of storage. Values represent mean ± standard error. Different letters indicate significant differences ($p < 0.05$) according to Tukey's HSD test

After one month of storage, emulsion C7 retained 82.3% EE (6% decrease), while D7 maintained its initial EE. In contrast, emulsion D showed a minor EE decline (4%), and emulsion C exhibited a slight increase (5%) in EE, as previously reported in Mohammad *et al.,* [5]. The stability of D7 may arise from the synergistic effects of pH 7 and HSH-MF processing, which optimise protein-polysaccharide interactions and droplet uniformity, minimising coalescence and bioactive expulsion. The unexpected increase in EE for emulsion C could reflect structural rearrangements during storage, such as Ostwald ripening or interfacial film consolidation, which enhance retention of α-mangostin over time.

Overall, the data indicate that the combined HSH-USP-MF method, particularly as applied in generating emulsion C7 not only offers exceptional initial encapsulation but also confers enhanced stability, which is likely attributable to the favourable reductions in droplet size and PDI, as well as increased antioxidant capacity that collectively contribute to superior emulsion stability and bioactive protection. The HSH-USP-MF method combined with PPI at pH 7 offers a promising strategy

for producing emulsions with high encapsulation efficiency and excellent storage stability, making it suitable for functional delivery systems targeting hydrophobic phytochemicals.

In summary, these quantitative comparisons substantiate that ultrasonic cavitation is not merely a physical processing step but a vital intensification technique that enhances both the physicochemical and functional properties of emulsions. By reducing droplet size, improving interfacial stability, and boosting protein emulsifier antioxidant activity, ultrasonication ensures higher retention and efficacy of hydrophobic bioactives like α-mangostin, advancing the development of scalable, effective functional delivery systems.

4. Conclusions

This study demonstrates that the combined high-speed homogenisation, ultrasonication, and microfluidisation (HSH-USP-MF) method, particularly when applied with pea protein isolate (PPI) at pH 7, effectively produces nanoscale emulsions with superior physicochemical and functional properties. Emulsions prepared via HSH-USP-MF exhibited significantly smaller droplet sizes, lower polydispersity indices, and enhanced zeta potential compared to those produced by the simpler HSH-MF method, indicating improved droplet uniformity and electrostatic stability. These structural advantages resulted in higher encapsulation efficiencies and greater antioxidant retention, highlighting the method's ability to protect sensitive hydrophobic bioactives such as α-mangostin. Furthermore, emulsions maintained their stability and bioactive content over extended storage, highlighting the practical potential of this approach for functional food and nutraceutical applications. Overall, the integration of ultrasonication within the emulsification sequence, combined with optimal protein pH adjustment, offers a robust and scalable strategy for developing stable, bioactive-rich nanoemulsions.

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