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Functional flavored milk fortified with hexosomally encapsulated shrimp-derived astaxanthin: Physicochemical, color, and sensory stability

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ABSTRACT

This study investigated the production and characterization of a functional flavored milk fortified with shrimp-derived astaxanthin (ASX)-rich oil in free and nanoencapsulated (hexosomal) forms. ASX-rich oil was extracted from green tiger shrimp (Penaeus semisulcatus) processing by-products using supercritical CO_2 extraction and subsequently incorporated into food-grade hexosomes. The resulting ASX-loaded hexosomes exhibited a mean particle size of 164.3 nm, a polydispersity index of 0.29, and a ζ-potential of -36.5 mV. Encapsulation efficiency reached 85.1 ± 3.3%, demonstrating the effective incorporation of ASX-rich oil within the nanostructured lipid carriers. Fortified flavored milk samples were prepared using free and hexosomal ASX-rich oil and evaluated during 12 days of refrigerated storage (4 °C) in terms of physicochemical, rheological, color, and sensory properties. The hexosomal ASX-fortified samples showed pH and titratable acidity profiles comparable to the control, while samples enriched with free ASX exhibited a significantly greater pH decline and higher acidity. Apparent viscosity increased in fortified samples, particularly in those containing hexosomal ASX, reflecting enhanced structural interactions and dispersed phase contributions. Color analysis revealed that nanoencapsulation mitigated excessive increases in redness and yellowness, while maintaining higher lightness compared to free ASX-fortified samples. Sensory evaluation demonstrated that hexosomal encapsulation effectively masked undesirable fishy odor and preserved overall acceptability, whereas free ASX-fortified milk received significantly lower sensory scores. Overall, the results demonstrate that hexosomal nanoencapsulation is an effective strategy to improve the physicochemical stability, color quality, and sensory acceptance of flavored milk fortified with shrimp-derived ASX, highlighting its potential for developing value-added functional dairy products.

1. Introduction

The growing consumer demand for healthier and value-added foods has markedly accelerated the development of functional food products enriched with bioactive compounds. Functional foods are formulated to provide health benefits beyond basic nutrition and may contribute to the prevention or management of chronic diseases (Gies et al., 2019; Bagheri et al., 2026). Among various functional food categories, dairy products represent one of the most widely used and commercially successful matrices for bioactive fortification due to their high consumption rate, favorable sensory attributes, and excellent nutritional profile (Bagheri et al., 2026). It has been estimated that dairy-based products fortified with bioactives such as carotenoids, omega-3 polyunsaturated fatty acids (ω-3 PUFAs), probiotics, and plant-derived phytochemicals account for a major share of the global functional food market (Bagheri et al., 2026; Shahidi & Ambigaipalan, 2016).

Milk and milk-based beverages, including flavored milk, are particularly suitable carriers for lipophilic bioactives owing to their natural fat content, buffering capacity, and ability to protect sensitive compounds during gastrointestinal digestion, thereby enhancing bioaccessibility and absorption (Dima et al., 2020). In addition to nutritional advantages, flavored milk products exhibit higher consumer acceptance, especially among children and adolescents, due to their improved sensory appeal and attractive color and flavor profiles (Arab et al., 2019; De Pelsmaeker et al., 2013). Moreover, several studies have reported that consumption of flavored milk may contribute to higher intakes of calcium, vitamin A, phosphorus, magnesium, and potassium compared with plain milk, supporting its potential as a vehicle for functional fortification (De Pelsmaeker et al., 2013; Nagarajappa & Battula, 2017).

Astaxanthin ($C_{40}H_{52}O_4$, ASX) is a high-value xanthophyll carotenoid naturally synthesized by microalgae and subsequently accumulated in marine organisms such as crustaceans through the food chain. Structurally, ASX contains a long-conjugated polyene chain linking two β-ionone rings bearing hydroxyl and keto groups, a configuration that contributes to its strong antioxidant activity while also making the molecule highly lipophilic and sensitive to oxidation (Šimat et al.,

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2022). Shrimp processing by-products constitute an important and underutilized source of ASX-rich oil, characterized by a distinctive reddish-orange color and high biological activity (Šimat et al., 2022). ASX has been extensively recognized as one of the most potent natural antioxidants, exhibiting antioxidant activity several-fold higher than other carotenoids (e.g., lutein, zeaxanthin, canthaxanthin) and up to 100-fold greater than α-tocopherol (Ambati et al., 2014). In addition to its antioxidant capacity, ASX has been associated with anti-inflammatory, cardioprotective, neuroprotective, and metabolic health benefits, supporting its growing application in functional foods and nutraceuticals (Šimat et al., 2022; Ahmadkelayeh & Hawboldt, 2020).

Despite these promising bioactivities, the practical application of ASX in food systems remains challenging due to its extremely low water solubility, high susceptibility to degradation by heat, light, and oxygen, and limited bioavailability (Dima et al., 2020; Zhao et al., 2019). Furthermore, incorporation of marine-derived oils rich in PUFAs may lead to the development of undesirable off-flavors and fishy odors as a result of lipid oxidation and hydrolysis, thereby negatively affecting consumer acceptance of fortified dairy products (Gulzar et al., 2020; Robertson et al, 2016). To overcome these technological and sensory limitations, nanoencapsulation and lipid-based delivery systems have emerged as highly effective strategies to improve the stability, dispersibility, sensory masking, and bioaccessibility of lipophilic bioactives in food matrices (Yaghmur, 2019; Hosseini et al., 2022). Among advanced nanostructured systems, non-lamellar lyotropic liquid crystalline (LLC) nanoparticles, particularly cubosomes and hexosomes, have attracted increasing attention for food applications due to their high internal surface area, excellent loading capacity for hydrophobic compounds, and potential to enhance oxidative stability and controlled release (Yaghmur, 2019; Ramezanzade et al., 2024). Hexosomes are derived from inverse hexagonal liquid crystalline phases, in which amphiphilic lipids self-assemble into cylindrical structures arranged in a hexagonal lattice, forming extended hydrophobic lipid domains that can effectively accommodate lipophilic bioactive compounds such as ASX-rich oil (Mirzaee Kalaei et al., 2025). In a previous study, we successfully developed structurally tunable hexosomes using a food-grade binary lipid system composed of Dimodan U and citrem, and demonstrated their effectiveness for encapsulating shrimp-derived ASX-rich oil with improved physicochemical stability (Mirzaee Kalaei et al., 2025). However, to date, limited information is available regarding the application of hexosomal nanodispersions as both functional colorants and nutritional ingredients in dairy-based beverages, particularly flavored milk products. Most previous studies have focused on microencapsulated carotenoids (e.g., canthaxanthin), nanoliposomal shrimp oil, or algal lipid extracts in milk and yogurt systems (Arab et al., 2019; Robertson et al., 2016; Gulzar & Benjakul, 2020b), while the use of hexosomal carriers remains largely unexplored. Therefore, the objective of the present study was to develop flavored milk beverages fortified with hexosome-encapsulated shrimp-derived ASX-rich oil and to evaluate their physicochemical, rheological, color, and sensory properties during refrigerated storage. The performance of hexosomal ASX-rich oil was compared with that of free ASX-rich oil to elucidate the role of nanoencapsulation in improving product stability, quality, and consumer acceptability. This work aims to provide new insights into the application of advanced lipid-based nanocarriers for the development of value-added functional dairy beverages.

2. Materials and methods

2.1. Materials

Green tiger shrimp (*Penaeus semisulcatus*) processing by-products were obtained from a local shrimp processing plant in Bushehr Province, Iran, and transported to the laboratory under iced conditions. Upon arrival, the biomass was stored at -20 °C in the dark until further processing. Sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), potassium phosphate (KH_2PO_4), potassium chloride (KCl), phenolphthalein, and chloroform were purchased from Sigma-Aldrich (St. Louis, MO, USA). Grinsted® citrem LR10 and Dimodan® U were kindly provided by Danisco A/S (Copenhagen, Denmark).

2.2. Preparation of shrimp byproducts

Shrimp wastes, including cephalothorax, shells, and tails, were freeze-dried using a freeze-dryer (OPR-FDU-7012, Operon, Korea) for 72 h at -65 °C under vacuum conditions. The dried materials were subsequently ground using a laboratory mixer (Depose, Moulinex, Italy) and sieved through a stainless-steel sieve with a mesh size of 450 μm to obtain a uniform powder.

2.3. Supercritical fluid extraction (SFE) of ASX-rich oil

Supercritical fluid extraction was carried out using a bench-scale SEPAREX-SFE-500 system (CHIMIE FINE, Champigneulles, France). Approximately 35 g of freeze-dried shrimp by-product powder was loaded into a 100 mL extraction vessel. Liquid CO_2 was delivered to the extraction chamber using a high-pressure pump at a constant flow rate of 27 g/min. Based on preliminary optimization experiments, extraction conditions were set at a static equilibration time of 20 min, an extraction pressure of 300 bar, an extraction temperature of 55 °C, and ethanol as a co-solvent at 5% (w/w). After extraction, the recovered ASX-rich oil was subjected to solvent removal by gently purging with a nitrogen stream at ambient temperature to promote the evaporation of residual ethanol. The nitrogen flushing was continued until no visible solvent remained. The resulting ASX-rich oil was then stored at −20 °C in the dark until further use (Roy et al., 2020).

2.4. Preparation of ASX-rich oil-loaded hexosomes

ASX-rich oil-loaded hexosomes were prepared at a total lipid concentration of 5.0 wt% using a binary lipid mixture of Dimodan U and citrem at a 1:1 (w/w) ratio. A post-loading (post-application) method was employed, whereby ASX-rich oil was incorporated into pre-formed hexosomal nanodispersions at an oil concentration of 1.0 wt%. This oil concentration was selected based on the results of our previous optimization study (Mirzaee Kalaei et al., 2025). The resulting nanodispersions were subjected to probe ultrasonication for 15 min (8 s on, 2 s off pulse mode) until homogeneous milky-white dispersions were obtained (Prajapati et al., 2018). During hydration and ultrasonication, the amphiphilic lipids self-assemble into inverse hexagonal nanostructures, leading to the formation of hexosomal particles.

2.5. Particle size, size distribution, and ζ-potential characterizations

The mean particle size, polydispersity index (PDI), and ζ-potential of the ASX-rich oil-loaded hexosomes were determined using a Zetasizer (Malvern Instruments, UK) at 25 °C and a scattering angle of 173°, with a laser wavelength of 633 nm. Prior to analysis, the nanodispersions were diluted ten-fold with phosphate-buffered saline (PBS, pH 7.4) to minimize multiple scattering effects. The viscosity value used in the instrument settings corresponded to that of the dispersant (PBS) at 25 °C.

2.6. Encapsulation efficiency (EE)

Encapsulation efficiency (EE) of ASX-rich oil in hexosomal nanodispersions was determined according to the method described by Gulzar and Benjakul (2020a), with minor modifications. Briefly, unencapsulated (free) oil was separated from the nanodispersion by centrifugation at 9600 × g for 10 min. The concentration of free ASX-rich oil in the supernatant was quantified by measuring absorbance at 468 nm using a UV-Vis spectrophotometer (WPA Biowave II, Biochrom, Cambridge, UK). Encapsulation efficiency was calculated using Eq. (1):

$$\%EE = \frac{OD_{468}\ total\ ASX-rich\ oil\ - OD_{468}\ unencapsulated\ ASX-rich\ oil}{OD_{468}\ total\ ASX-rich\ oil} \times 100 \quad (1)$$

Where OD_{468} represents the optical density at 468 nm.

2.7. Preparation of fortified flavored milk samples

Freshly extracted ASX-rich oil was incorporated into pasteurized flavored milk (Alis Dairy Company, Mashhad, Iran) in two forms: free oil and hexosome-encapsulated oil, at a concentration of 2.5% (w/v). Samples were homogenized using an Ultra-Turrax homogenizer (3000 rpm, 60 s), cooled to 5 °C, packaged, and stored under refrigeration at 4 °C for 12 days. Analyses of pH, acidity, rheological properties, color, and sensory attributes were conducted on storage days 1, 4, 8, and 12 (Gulzar & Benjakul, 2020b). In the present study, fortification was performed after pasteurization in order to specifically evaluate the influence of free and hexosome-encapsulated ASX-rich oil on the physicochemical and sensory stability of flavored milk during refrigerated storage.

2.8. Physicochemical parameters of flavored milk during storage

2.8.1. pH and titratable acidity

The pH of the flavored milk samples was evaluated using a pH meter (GENWAY, Model 3510, England). Titrable acidity (TA) was analyzed according to AOAC method 947.05 (AOAC,1990). For the analysis, 20 mL of the sample were diluted with hot distilled water and titrating with 0.1 N NaOH using 1% phenolphthalein indicator. TA was expressed as % of lactic acid. These values were measured on days 1, 4, 8, and 12 (Arab et al., 2019).

2.8.2. Rheological measurements

Apparent viscosity of flavored milk samples was measured using a rheometer (MCR502, Anton Paar GmbH, Graz, Austria) at 25 °C. Flow curves were obtained over a shear rate range of 0.01-100 s^{-1}. Rheological behavior was evaluated on days 1, 4, 8, and 12 and compared with that of the control (unfortified) milk sample (Arab et al., 2019).

2.9. Color measurements

Color parameters of control and fortified flavored milk samples were measured using a reflectance spectrophotometer (X-Rite SP-64, USA). The CIELAB color coordinates L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) were recorded. Measurements were performed on days 1, 4, 8, and 12. Total color difference (ΔE*) relative to day 1 was calculated using Eq. (2): (Arab et al., 2019):

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (2)$$

2.10. Sensory evaluation

Sensory evaluation was conducted using 20 untrained panelists aged between 25 and 35 years. A five-point hedonic scale (1 = dislike extremely; 5 = like extremely) was used to evaluate appearance, odor, color, texture, and overall acceptability. Water was provided for palate cleansing between samples (Ghorbanzade et al., 2017).

2.11. Statistical analysis

Data were analyzed using analysis of variance

(ANOVA), followed by Duncan's multiple range test to determine significant differences among treatments. In addition, paired samples t-tests were used to evaluate changes within each treatment during storage by comparing values obtained at different storage times. Statistical significance was considered at the 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1. Characterization of ASX-rich oil-loaded hexosomes

3.1.1. Size and ζ-potential measurements

Dynamic light scattering was used to determine the mean particle size, size distribution, and ζ-potential of ASX-rich oil-loaded hexosomes. Particle size is a critical parameter governing the physical stability of colloidal nanodispersions and strongly influencing cellular uptake, interfacial behavior, and potential biodistribution of encapsulated bioactives (Yaghmur, 2019; McClements, 2018). Therefore, characterization of particle size is essential for evaluating the suitability of the prepared hexosomal system for food and nutraceutical applications. As shown in Fig. 1A, the ASX-rich oil-loaded hexosomes exhibited a mean hydrodynamic diameter of 164.3 nm with a PDI of 0.29, indicating the formation of relatively homogeneous and narrowly distributed nanoparticles (Hasanzadeh Baboli et al., 2023). These results are consistent with previously reported hexosomal systems intended for delivery of lipophilic compounds. For example, Eskandari et al. (2025) reported a mean diameter of approximately 137 nm for ω-3 PUFA-loaded chitosan-coated hexosomes, confirming that the particle size obtained in the present study falls within the typical range for food-grade hexosomal nanocarriers.

The ζ-potential is another key indicator of colloidal stability, as higher absolute ζ-potential values generate sufficient electrostatic repulsion to reduce particle aggregation and enhance dispersion stability (Hosseini et al., 2022; McClements, 2026). The measured ζ-potential of -36.5 mV (Fig. 1B) suggests good electrostatic stabilization of the hexosomal nanodispersion and is comparable to values reported for similar citrem-based systems (Ramezanzade et al., 2024; Prajapati et al., 2018). The negative surface charge can be primarily attributed to the presence of citrem, a negatively charged food-grade emulsifier, which contributes to surface charge modulation and improved colloidal stability of Dimodan U/citrem nanoparticles (Ramezanzade, 2024). Such highly negative ζ-potential values are generally associated with enhanced resistance to aggregation and improved physical stability during storage.

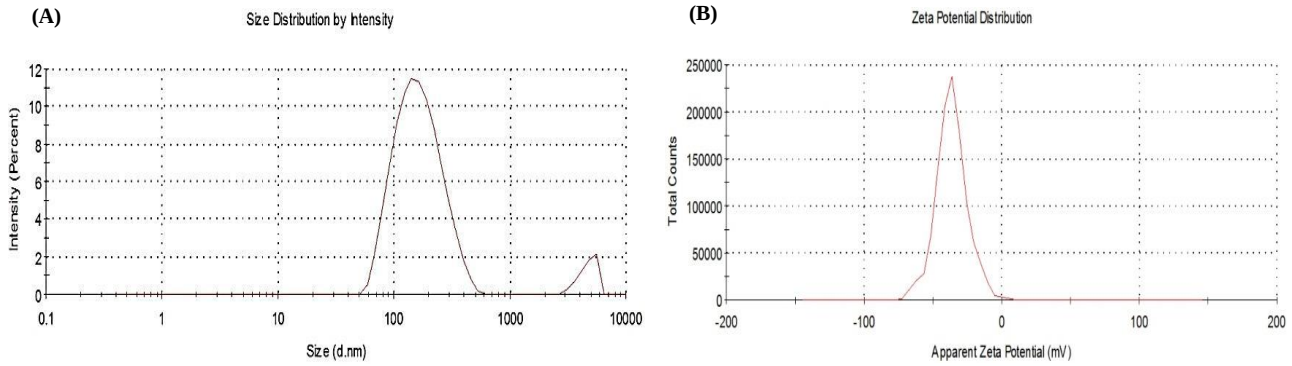

Fig. 1. (A) Size distribution and (B) ζ-potential of ASX-rich oil-loaded hexosomes prepared at ASX-rich oil concentration of 1wt%.

3.1.2. Encapsulation efficiency (EE)

Encapsulation efficiency (EE) is a critical parameter for assessing the loading capacity and practical applicability of delivery systems for lipophilic bioactives (Ramezanzade, 2024; McClements, 2018). In the present study, the EE of ASX-rich oil in hexosomal nanodispersions was 85.1 ± 3.3%, indicating highly efficient incorporation of shrimp-derived ASX-rich oil into the hexosomal matrix. This value is in good agreement with previously reported encapsulation efficiencies for shrimp oil-loaded nanoliposomes, which ranged from 75.2% to 93.6% depending on the preparation method (ultrasonication *vs.* microfluidization) Gulzar & Benjakul, 2020a). The high EE observed in this study can be attributed to several factors, including the small particle size and high internal surface area of hexosomes, which facilitate strong hydrophobic interactions between the lipophilic components of the ASX-rich oil, including ASX, and the internal lipid domains of the Dimodan U/citrem hexosomal matrix (Eskandari et al., 2025). Moreover, surface charge and electrostatic interactions are known to influence encapsulation efficiency and retention of hydrophobic compounds within nanocarriers (McClements, 2018; Miladi et al., 2015). The relatively high absolute ζ-potential observed in this study likely promoted strong interfacial interactions and reduced leakage of ASX-rich oil from the hexosomal structure, thereby contributing to the high EE. Similar relationships between surface charge, carrier structure, and improved encapsulation performance have been reported for lipid-based and polymeric nanocarriers used for food and pharmaceutical applications (Miladi et al., 2015; Jafari et al., 2008).

3.2. Physico-chemical parameters of flavored milk during storage

3.2.1. pH

Fig. 2. shows the changes in pH of control and fortified flavored milk samples during 12 days of refrigerated storage at 4 °C. The initial pH values were 6.53, 6.52, and 6.36 for the control, hexosomal ASX-fortified, and free ASX-fortified samples, respectively. These values are consistent with those reported for skim and flavored milk products stored under refrigerated conditions (Nagarajappa & Battula, 2017; Gulzar & Benjakul, 2020b). A gradual decline in pH was observed in all samples throughout storage. pH of control and fortified samples had no significant change within the first 4 days of storage ($P > 0.05$). Afterwards, pH of all samples decreased continuously up to the end of storage ($P < 0.05$). This decrease is commonly attributed to the metabolic activity of residual lactic acid bacteria or psychrotrophic microorganisms, which convert lactose into lactic acid during refrigerated storage, even in pasteurized milk products (Fox et al., 2015). Similar trends have been reported in fortified and non-fortified milk systems during cold storage (Nagarajappa & Battula, 2017; Gulzar et al., 2020).

Notably, while the control and hexosomal ASX-fortified samples exhibited comparable pH values at the end of storage, the free ASX-fortified samples showed a significantly greater pH reduction ($P < 0.05$), reaching a value of 5.22 (Figure 2). This pronounced decrease in pH can be attributed to the presence of unencapsulated shrimp oil, which is more susceptible to lipid oxidation and hydrolytic reactions. Oxidation of PUFAs leads to the formation of free fatty acids (FFAs) and secondary oxidation products, which can contribute to acidification of the milk matrix (Gulzar et al., 2020; Robertson, 2016). In contrast, nanoencapsulation of ASX-rich oil within hexosomes likely limited direct exposure of lipids to oxygen and pro-oxidative components in milk, thereby reducing oxidative degradation and mitigating excessive acid formation. Similar protective effects of encapsulation on pH stability have been reported for milk systems fortified with nanoliposomal marine oils (Gulzar & Benjakul, 2020b; Raju et al., 2022).

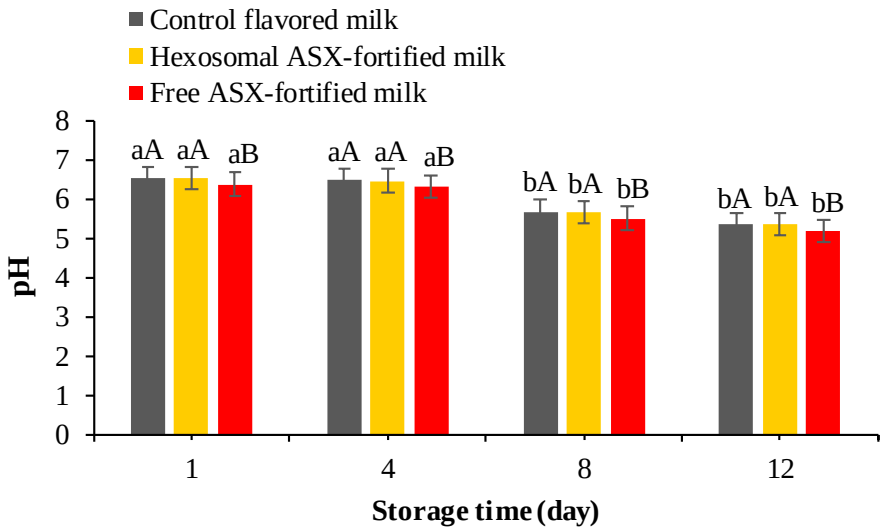

Fig. 2. Changes in pH values of different flavored milk samples during the storage period (4 °C). Different lowercase letters in the same columns denote significant differences for the same treatments within different days of storage ($p < 0.05$). Different uppercase letters in the same denote significant differences between treatments at the same storage day ($p < 0.05$). Values are given as mean ± standard deviation.

3.2.2. Acidity

Figure 3 illustrates changes in titratable acidity (TA) of control and fortified flavored milk samples during refrigerated storage. In all treatments, a slight increase in TA was observed over time, which is consistent with the observed decrease in pH and reflects gradual lactic acid formation during storage. The control and hexosomal ASX-fortified samples exhibited very similar acidity values throughout the storage period ($P > 0.05$). This similarity can be attributed to the protective effect of nanoencapsulation, which limits lipid oxidation and reduces the release of FFAs and acidic degradation products into the milk serum phase (Gulzar & Benjakul, 2020b; Raju et al., 2022). Encapsulation therefore helps maintain acidity levels comparable to those of the control milk, preserving physicochemical stability.

In contrast, the free ASX-fortified samples consistently exhibited significantly higher acidity values than both the control and hexosomal ASX-fortified samples ($P < 0.05$). This behavior is closely related to the enhanced susceptibility of free shrimp oil to oxidative and hydrolytic degradation, which generates FFAs and acidic secondary oxidation products, thereby contributing to higher TA and lower pH (Gulzar et al., 2020; Robertson et al., 2016). The parallel trends observed for pH and acidity confirm that free oil incorporation accelerates chemical and microbiological changes in the milk matrix, whereas hexosomal encapsulation effectively moderates these effects.

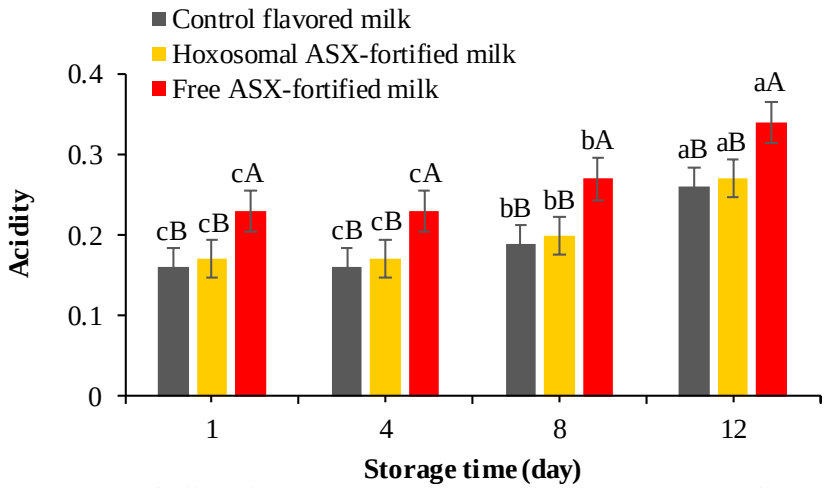

Fig. 3. Changes in titratable acidity values of different flavored milk samples during the storage period (4 °C). Different lowercase letters in the same columns denote significant differences for the same treatments within different days of storage ($p < 0.05$). Different uppercase letters in the same denote significant differences between treatments at the same storage day ($p < 0.05$). Values are given as mean ± standard deviation.

3.2.3. Apparent viscosity

The flow behavior of control and fortified flavored milk samples at different storage times is shown in Figure 4A-D. All samples exhibited shear-thinning (pseudoplastic) behavior, as evidenced by a decrease in apparent viscosity with increasing shear rate. This behavior is characteristic of milk-based colloidal systems and has been widely reported for skim and flavored milk products (Arab et al., 2019; Bienvenue et al., 2003). The apparent viscosity of the control sample was consistently lower than that of the fortified samples, while both hexosomal- and free ASX-fortified samples exhibited significantly higher apparent viscosity than the control sample, particularly at the end of the storage period. The increase in viscosity following addition of ASX-rich oil can be attributed to several mechanisms. First, incorporation of lipid-based nanodispersions increases the dispersed phase volume fraction, leading to enhanced particle-particle interactions and increased internal friction within the milk matrix (Gulzar et al., 2020; McClements, 2018). Second, the presence of nano- or micro-sized oil droplets can promote weak flocculation or droplet-protein interactions, which further contribute to viscosity enhancement. In addition, fortification increases the total solids content of the milk, which is known to increase viscosity through formation of transient junction zones and enhanced protein-fat interactions (Vasiljevic et al., 2007). Similar increases in viscosity have been reported in dairy products fortified with carotenoids, marine oils, and lipid-based delivery systems (Gulzar et al., 2020; Robertson, 2016; Soliman & Nasser, 2022). Collectively, these results indicate that both free and encapsulated ASX-rich oil modify the microstructure of flavored milk, leading to increased apparent viscosity and altered rheological behavior.

3.3. Color measurements

Table 1 presents the color parameters (L*, a*, b*, and ΔE*) of flavored milk samples during 12 days of storage at 4 °C. The highest initial L* (lightness) value was observed for the control sample (78.14) on day 1 ($P < 0.05$), which is consistent with typical values reported for skim and flavored milk systems (Arab et al., 2019; Soliman & Nasser, 2022). There were significant differences between the control and fortified samples at all time points ($P < 0.05$), in agreement with previous reports on dairy products fortified with lipid extracts and carotenoids (Robertson et al., 2016; Arab et al., 2019). A continuous increase in L* (whiteness) and a concomitant decrease in a* (redness) and b* (yellowness) values were observed for both the control and hexosomal ASX-fortified samples during storage ($P < 0.05$). This behavior can be attributed to several factors, including (i) structure of milk proteins and fat globules, (ii) dilution and redistribution of native pigments, and (iii) limited oxidation and degradation of residual-colored compounds during refrigerated storage (Bienvenue, 2003; Soliman & Nasser, 2022). In the hexosomal ASX-fortified samples, the augmented whiteness (higher L* values) is plausibly associated with enhanced light scattering caused by nanoscale hexosomal structures, which increase optical turbidity and mask intrinsic pigment color, thereby increasing perceived whiteness (Raju et al., 2022; Zhong et al., 2018).

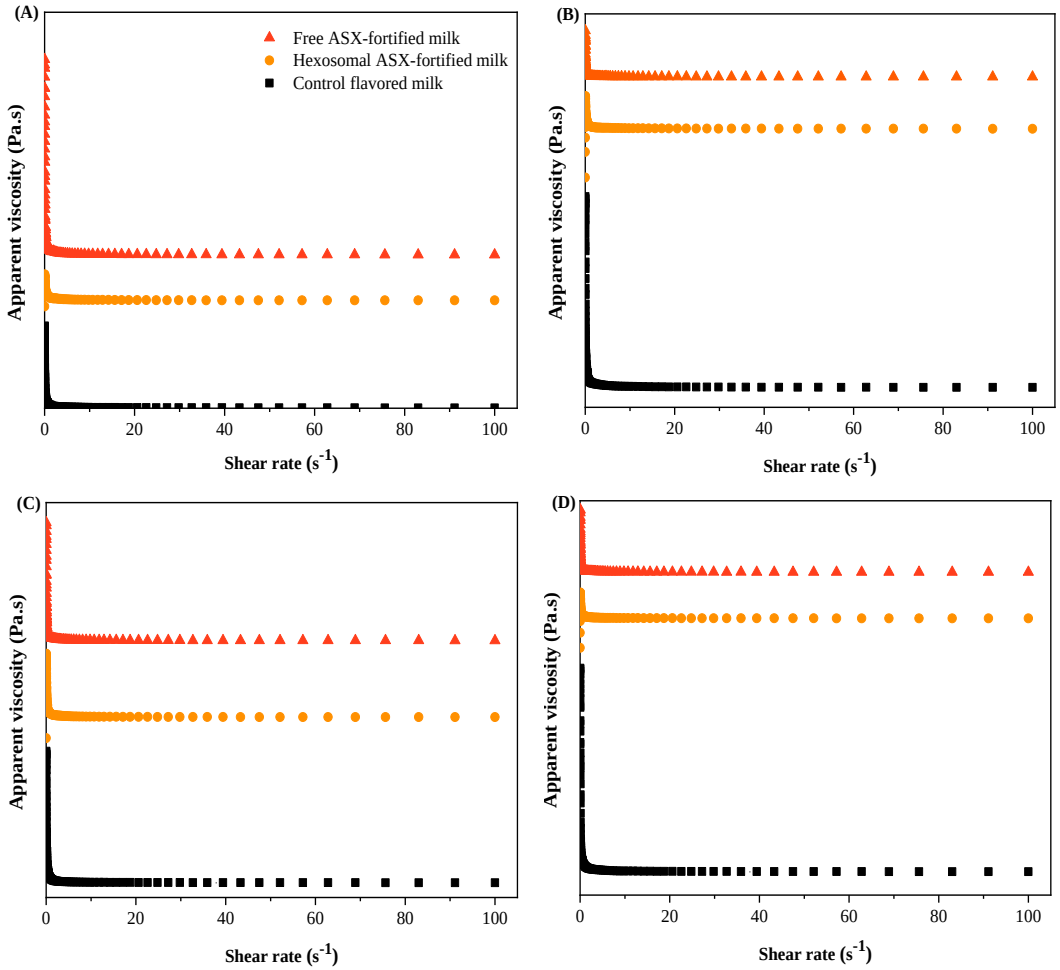

Fig. 4. Apparent viscosity of different flavored milk samples at day 1 (A), 4 (B), 8 (C), and 12 (D) during the storage period (4 °C).

Table 1. Color parameters of different flavored milk samples during the storage period (4 °C).

Parameters	Day	Treatments		
		Control flavored milk	Hexosomal ASX-fortified milk	Free ASX-fortified milk
L*	1	78.14 ± 0.02^{bA}	78.09 ± 0.05^{bA}	68.94 ± 0.02^{aB}
	4	78.40 ± 0.01^{bA}	77.86 ± 0.04^{bA}	67.89 ± 0.03^{aB}
	8	79.71 ± 0.07^{abA}	80.39 ± 0.09^{aA}	68.36 ± 0.05^{aB}
	12	80.53 ± 0.03^{aA}	80.60 ± 1.10^{aA}	67.81 ± 0.08^{aB}
a*	1	-1.42 ± 0.10^{aB}	-0.94 ± 0.02^{aC}	9.94 ± 0.06^{bA}
	4	-1.36 ± 0.08^{aB}	-0.83 ± 0.12^{aC}	13.10 ± 0.09^{aA}
	8	-1.32 ± 0.02^{aB}	-0.71 ± 0.07^{bC}	13.75 ± 0.11^{aA}
	12	-1.20 ± 0.09^{bB}	-0.60 ± 0.01^{bC}	14.31 ± 0.14^{aA}
b*	1	5.96 ± 0.03^{aB}	6.19 ± 0.10^{aB}	17.4 ± 0.09^{dA}
	4	5.88 ± 0.11^{bB}	6.02 ± 0.08^{aB}	22.07 ± 0.15^{cA}
	8	5.85 ± 0.07^{bB}	5.11 ± 0.02^{bC}	24.05 ± 0.11^{bA}
	12	4.76 ± 0.02^{cB}	4.58 ± 0.05^{bB}	25.58 ± 0.07^{aA}
ΔE*	1	00.0^{d}	00.0^{d}	00.0^{d}
	4	0.28 ± 0.01^{cB}	0.31 ± 0.02^{cB}	5.73 ± 0.05^{cA}
	8	1.57 ± 0.08^{bC}	2.55 ± 0.07^{bB}	7.68 ± 0.11^{bA}
	12	2.68 ± 0.04^{cB}	3.00 ± 0.11^{aB}	9.34 ± 0.14^{aA}

Different lowercase letters in the same columns denote significant differences for the same treatments within different days of storage ($p < 0.05$). Different uppercase letters in the same denote significant differences between treatments at the same storage day ($p < 0.05$). Values are given as mean ± standard deviation.

In contrast, the free ASX-fortified samples exhibited a significant decrease in L* and an increase in both a* and b* values with increasing storage time ($P < 0.05$). This trend is mainly attributed to the direct dispersion of non-

encapsulated ASX within the milk matrix. ASX is a highly colored red-orange carotenoid with strong light absorption in the visible region (λmax ≈ 470-480 nm), and its unencapsulated form can more effectively impart redness and yellowness to the dairy system (Soliman & Nasser, 2022; Takeungwongtrakul & Benjakul, 2017). Moreover, free ASX is more susceptible to aggregation and oxidative degradation, which may intensify color development and lead to progressive darkening and increased chroma during storage (Soliman & Nasser, 2022; Zhong et al., 2018). Accordingly, the reduced lightness and increased redness and yellowness in free ASX-fortified samples should be specifically attributed to ASX itself, rather than to β-carotene or ω-3 PUFAs. ASX is the dominant chromophore responsible for these color changes due to its extended conjugated double-bond system, which confers a strong red-orange hue (Takeungwongtrakul & Benjakul, 2017). Similar increases in a* and b* values following enrichment of milk with shrimp-derived lipid extracts have been reported previously [18, 30]. Consequently, the total color difference (ΔE*) increased progressively with storage time for all fortified samples, indicating perceptible color changes over time. These findings are consistent with previous studies on dairy systems fortified with marine oils and carotenoids, where increasing ΔE* values were associated with pigment redistribution and oxidative reactions during storage (Gulzar & Benjakul, 2020b; Soliman & Nasser, 2022).

3.4. Sensory attributes

The sensory profiles of control flavored milk, hexosomal ASX-fortified, and free ASX-fortified samples are presented in the spider-web diagram (Fig. 5.). Sensory analysis revealed no significant differences between the control and hexosomal ASX-fortified samples across all evaluated attributes throughout storage ($P > 0.05$), indicating that nanoencapsulation effectively masked the sensory impact of shrimp-derived oil. This observation is consistent with previous reports showing that nanoencapsulation of marine oils and carotenoids in dairy matrices significantly reduces fishy odor and improves overall acceptability (Gulzar & Benjakul, 2020b; Soliman & Nasser, 2022); Zhong et al., 2018).

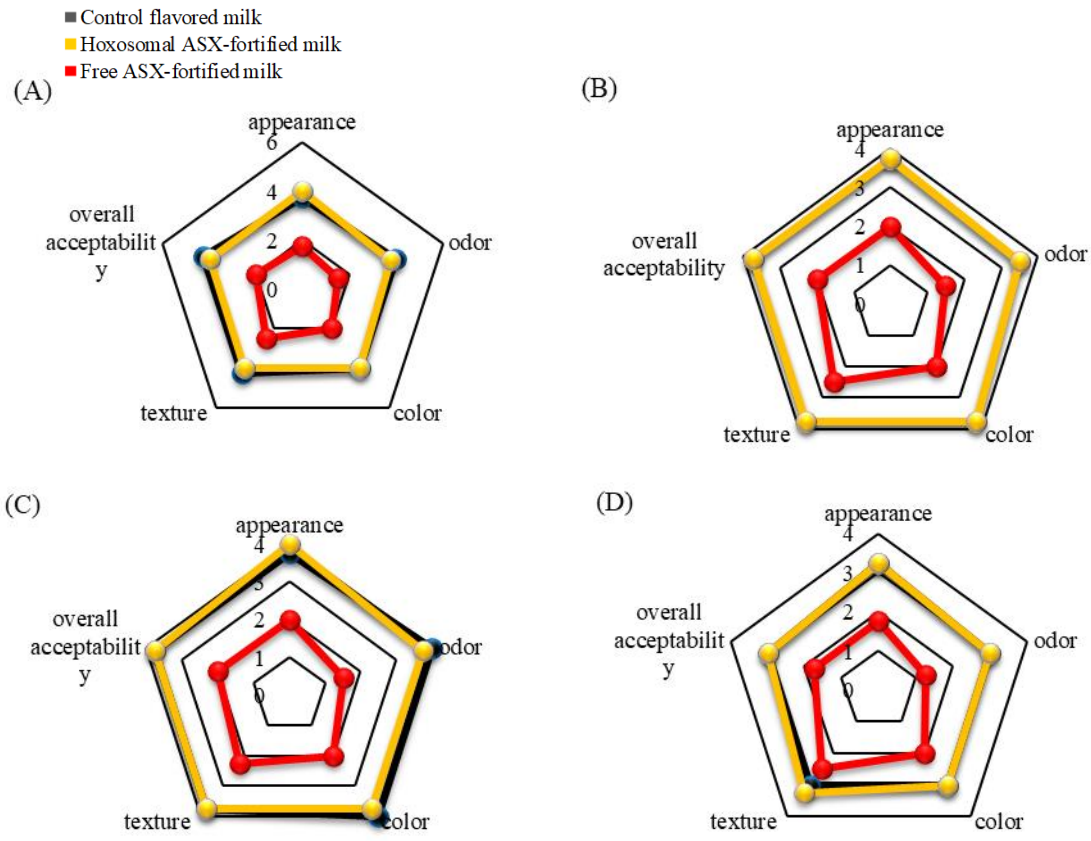

Fig. 5. Sensory analysis of different flavored milk samples at day 1 (A), 4 (B), 8 (C), and 12 (D) during the storage period (4 °C).

In contrast, free ASX-fortified samples scored significantly lower for most sensory attributes than both the control and hexosomal ASX-fortified samples ($P < 0.05$). The lower sensory scores can be attributed to the direct exposure of ASX-rich oil to the milk matrix, which facilitates the release of volatile oxidation products (e.g., aldehydes and ketones) associated with marine oils, thereby generating fishy and rancid off-flavors (Shahidi & Ambigaipalan, 2016; Zhong et al., 2018). Moreover, the absence of a protective encapsulation barrier increases

lipid oxidation rates, leading to the formation of secondary oxidation products that negatively impact aroma and taste (Robertson et al., 2016; Soliman & Nasser, 2022). Comparable findings have been reported for dairy systems fortified with non-encapsulated fish oil or carotenoid-rich lipid extracts, where significant deterioration in odor and flavor was observed relative to nanoencapsulated counterparts (Soliman & Nasser, 2022; Zhong et al., 2018). These results further confirm that nanoencapsulation plays a critical role in improving the sensory quality and consumer acceptability of marine oil-fortified dairy products.

At the end of the storage period, all samples exhibited a decrease in sensory scores, and this decline was statistically significant ($P < 0.05$), which is likely associated with general quality deterioration during refrigerated storage, including acidification, oxidative reactions, and changes in texture and flavor. Overall, the results demonstrate that nanoencapsulation of ASX-rich oil is an effective strategy to mitigate undesirable sensory attributes and enhance the feasibility of incorporating shrimp-derived ASX into flavored milk formulations.

4. Conclusion

This study demonstrated that hexosomal encapsulation is an effective strategy for the incorporation of ASX into flavored milk, significantly improving color stability and sensory acceptability compared with free ASX fortification. During refrigerated storage, hexosomal ASX-fortified samples exhibited higher L* values and more stable a* and b* parameters, indicating improved color uniformity and reduced pigment-related discoloration. In contrast, free ASX supplementation resulted in progressive decreases in lightness and increases in redness and yellowness, reflecting direct pigment dispersion and oxidative degradation effects. Sensory evaluation further confirmed the advantages of hexosomal delivery, as hexosomal ASX-fortified samples maintained sensory characteristics comparable to the control throughout storage, while free ASX-fortified samples showed significantly lower scores, particularly for odor and overall acceptability. These findings highlight the ability of nanoencapsulation to mask undesirable marine-derived sensory notes and to protect sensitive carotenoids against oxidative and environmental degradation. Overall, the results indicate that hexosomal delivery systems offer a promising technological approach for the development of stable, sensory-acceptable, and functionally enhanced dairy beverages fortified with ASX. The integration of nanoencapsulation with dairy matrices may facilitate the industrial production of value-added functional dairy products with improved consumer acceptance and extended shelf-life.

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References

Ambati, R. R., Phang, S. M., Ravi, S., & Aswathanarayana, R. G. (2014). Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications-A review. *Mar. Drugs.*, *12*(1), 128-152. https://doi.org/10.3390/md12010128

Ahmadkelayeh, S., & Hawboldt, K. (2020). Extraction of lipids and astaxanthin from crustacean by-products: A review on supercritical CO_2 extraction. *Trends Food Sci. Technol.*, *103*, 94-108. https://doi.org/10.1016/j.tifs.2020.07.016

AOAC. (1990). Official methods of analysis (17th ed., pp. 805–806). Washington, DC: Association of Official Agricultural Chemists.

Arab, M., Razavi, S. H., Hosseini, S. M., Nayebzadeh, K., Meybodi, N. M., Khanniri, E., Mardi, P., & Mortazavian, A. M. (2019). Production and characterization of functional flavored milk and flavored fermented milk using microencapsulated canthaxanthin. *LWT*, *114*, 108373. https://doi.org/10.1016/j.lwt.2019.108373

Bagheri, H., Akhavan-Mahdavi, S., Sarabi-Aghdam, V., Mirarab Razi, S., Singh Beniwal, A., & Rashidinejad, A. (2026). Targeted dairy fortification: leveraging bioactive compounds to enhance nutritional value. *Crit. Rev. Food Sci.*, *66*(2), 295-319. https://doi.org/10.1080/10408398.2025.2522991

Bienvenue, A., Jiménez-Flores, R., & Singh, H. (2003). Rheological properties of concentrated skim milk: importance of soluble minerals in the changes in viscosity during storage. *J. Dairy Sci.*, *86*(12), 3813-3821. https://doi.org/10.3168/JDS.S0022-0302(03)73988-5

De Pelsmaeker, S., Schouteten, J., & Gellynck, X. (2013). The consumption of flavored milk among a children population. The influence of beliefs and the association of brands with emotions. *Appetite*, *71*, 279-286. https://doi.org/10.1016/j.appet.2013.07.016

Dima, C., Assadpour, E., Dima, S., & Jafari, S. M. (2020). Bioavailability of nutraceuticals: Role of the food matrix, processing conditions, the gastrointestinal tract, and nanodelivery systems. *Crit. Rev. Food Sci.*, *19*(3), 954-994. https://doi.org/10.1111/1541-4337.12547

Eskandari, Z., Hosseini, S. F., Kalaycioglu, G. D., & Yaghmur, A. (2025). Layer-by-layer hybrid hexosomes with enhanced oxidative stability and bioaccessibility of omega-3 polyunsaturated fatty acids. *Food Chem.*, 145050. https://doi.org/10.1016/j.foodchem.2025.145050

Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., & O'mahony, J. A. (2015). Heat-induced changes in milk. In P. F. Fox, T. Uniacke-Lowe, P. L. H. McSweeney, J. A. O'Mahony (Eds.), *Dairy chemistry and biochemistry* (2nd ed., pp. 345-375). Springer Cham.

Ghorbanzade, T., Jafari, S. M., Akhavan, S., & Hadavi, R. (2017). Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. *Food Chem.*, *216*, 146-152. https://doi.org/10.1016/j.foodchem.2016.08.022

Gies, M., Descalzo, A. M., Servent, A., & Dhuique-Mayer, C. (2019). Incorporation and stability of carotenoids in a functional fermented maize yogurt-like product containing phytosterols. *LWT*, *111*, 105-110. https://doi.org/10.1016/j.lwt.2019.04.103

Gulzar, S., Benjakul, S., & Hozzein, W. N. (2020). Impact of β-glucan on debittering, bioaccessibility and storage stability of skim milk fortified with shrimp oil nanoliposomes. *Int. J. Food Sci. Technol.*, *55*(5), 2092-2103. https://doi.org/10.1111/ijfs.14452

Gulzar, S., & Benjakul, S. (2020a). Characteristics and storage stability of nanoliposomes loaded with shrimp oil as affected by ultrasonication and microfluidization. *Food Chem.*, *310*, 125916. https://doi.org/10.1016/j.foodchem.2019.125916

Gulzar, S., & Benjakul, S. (2020b). Fortification of skim milk with nanoliposomes loaded with shrimp oil: properties and storage stability. *J. Am. Oil Chem. Soc.*, *97*(8), 929-940. https://doi.org/10.1002/aocs.12370

Hasanzadeh Baboli, N., Hosseini, S. F., & Gharsallaoui, A. (2023). Antibacterial and anti-biofilm properties of cinnamaldehyde-loaded nanoliposomes against Listeria monocytogenes and Salmonella enteritidis adhered to stainless steel. *Int. J. Food Sci. Technol.*, *58*(10), 5275-5282. https://doi.org/10.1111/ijfs.16634

Hosseini, S. F., Rezaei, M., & McClements, D. J. (2022). Bioactive functional ingredients from aquatic origin: A review of recent progress in marine-derived nutraceuticals. *Crit. Rev. Food Sci.*, *62*(5), 1242-1269. https://doi.org/10.1080/10408398.2020.1829863

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Dry. Technol.*, *26*(7), 816-835. https://doi.org/10.1080/07373930802135972

McClements, D. J. (2018). Enhanced delivery of lipophilic bioactives using emulsions: a review of major factors affecting vitamin, nutraceutical, and lipid bioaccessibility. *Food Funct.*, *9*(1), 22-41. https://doi.org/10.1039/C7FO01593B

Miladi, K., Sfar, S., Fessi, H., & Elaissari, A. (2015). Enhancement of alendronate encapsulation in chitosan nanoparticles. *J. Drug Deliv. Sci.*, *30*, 391-396. https://doi.org/10.1016/j.jddst.2015.07.018

Mirzaee Kalaei, F., Hosseini, S. F., Rezaei, M., & Yamini, Y. (2025). Preparation and characterization of lyotropic liquid crystalline nanoparticles loaded with astaxanthin-rich oil from shrimp waste. *Food Bioprocess Technol.*, *18*(3), 2436-2446. https://doi.org/10.1007/s11947-024-03600-z

Nagarajappa, V., & Battula, S. N. (2017). Effect of fortification of milk with omega-3 fatty acids, phytosterols and soluble fibre on the sensory, physicochemical and microbiological properties of milk. *J. Sci. Food Agric.*, *97*(12), 4160-4168. https://doi.org/10.1002/jsfa.8260

Prajapati, R., Salentinig, S., & Yaghmur, A. (2018). Temperature triggering of kinetically trapped self-assemblies in citrem-phospholipid nanoparticles. *Chem. Phys. Lipids.*, *216*, 30-38. https://doi.org/10.1016/j.chemphyslip.2018.08.002

Raju, N., Gulzar, S., & Benjakul, S. (2022). Cholesterol-lowered shrimp lipid-loaded liposome stabilized by pectin/glycerol and its fortification in peach tea drink. *Int. J. Food Sci. Technol.*, *57*(3), 1563-1572. https://doi.org/10.1111/ijfs.15546

Ramezanzade, L., Hosseini, S. F., Sajedi, R. H., Nielsen, A. M., & Yaghmur, A. (2024). Food-grade hexosomes as efficient vehicles for delivery of fish-purified antioxidant peptide. *Food Chem.*, *434*, 137446. https://doi.org/10.1016/j.foodchem.2023.137446

Robertson, R. C., Mateo, M. R. G., O'Grady, M. N., Guihéneuf, F., Stengel, D. B., Ross, R. P., Fitzgerald, G. F., Kerry, J. P., & Stanton, C. (2016). An assessment of the techno-functional and sensory properties of yoghurt fortified with a lipid extract from the microalga *Pavlova lutheri*. *Innov. Food Sci. Emerg. Technol.*, *37*, 237–246. https://doi.org/10.1016/j.ifset.2016.09.018

Roy, V. C., Getachew, A. T., Cho, Y.-J., Park, J.-S., & Chun, B.-S. (2020). Recovery and bio-potentialities of astaxanthin-rich oil from shrimp (Penaeus monodon) wASXe and mackerel (*Scomberomous niphonius*) skin using concurrent supercritical CO_2 extraction. *J. Supercrit. Fluids.*, *159*, 104773. https://doi.org/10.1016/j.supflu.2020.104773

Shahidi, F., & Ambigaipalan, P. (2016). Beverages fortified with omega-3 fatty acids, dietary fiber, minerals, and vitamins. In F. Shahidi & C. Alasalvar (Eds.), *Handbook of functional beverages and human health* (1st ed., pp. 801-813). CRC Press.

Šimat, V., Rathod, N. B., Čagalj, M., Hamed, I., & Generalić Mekinić, I. (2022). Astaxanthin from crustaceans and their byproducts: a bioactive metabolite candidate for therapeutic application. *Mar. Drugs.*, *20*(3), 206. https://doi.org/10.3390/md20030206

Soliman, T. N., & Nasser, S. A. (2022). Characterization of carotenoids double-encapsulated and incorporate in functional stirred yogurt. *Front. Sustain. Food Syst.*, *6*, 979252. https://doi.org/10.3389/fsufs.2022.979252

Takeungwongtrakul, S., & Benjakul, S. (2017). Biscuits fortified with micro-encapsulated shrimp oil: characteristics and storage stability. *J. Food Sci. Technol.*, *54*, 1126-1136. https://doi.org/10.1007/s13197-017-2535-6

Vasiljevic, T., Kealy, T., & Mishra, V. K. (2007). Effects of β-glucan addition to a probiotic containing yogurt. *J. Food Sci.*, *72*(7), C405-C411. https://doi.org/10.1111/j.1750-3841.2007.00454.x

Yaghmur, A. (2019). Nanoencapsulation of food ingredients by cubosomes and hexosomes. In S.M. Jafari (Eds.), *Lipid-based nanostructures for food encapsulation purposes* (1st ed., pp. 483-522). Elsevier. https://doi.org/10.1016/B978-0-12-815673-0.00013-9

Zhao, T., Yan, X., Sun, L., Yang, T., Hu, X., He, Z., Liu, F, & Liu, X. (2019). Research progress on extraction, biological activities and delivery systems of natural astaxanthin. *Trends Food Sci. Technol.*, *91*, 354-361. https://doi.org/10.1016/j.tifs.2019.07.046

Zhong, J., Yang, R., Cao, X., Liu, X., & Qin, X. (2018). Improved physicochemical properties of yogurt fortified with fish oil/γ-oryzanol by nanoemulsion technology. *Molecules*, *23*(1), 56. https://doi.org/10.3390/molecules23010056