



Original research

Structural and textural properties of apple cheese fortified with pomegranate seed oil emulsion stabilized by chitosan-zein particles

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A B S T R A C T

Apple production generates considerable waste, which can be reduced by processing it into apple cheese. However, nutritional losses during processing necessitate the addition of functional ingredients. Pomegranate seed oil, rich in unsaturated fatty acids but prone to oxidation, can be encapsulated to enhance oxidative stability and improve the nutritional value of the product. Chitosan-zein particles were prepared at a 1:10 ratio and used to stabilize oil-in-water emulsions containing 20% and 30% pomegranate seed oil. The 20% oil emulsion showed greater stability and was selected for functional apple cheese production. Three treatments were formulated: control (T1), chitosan-zein emulsion containing 0.2% oil (T2) and chitosan-zein emulsion containing 0.5% oil (T3). Textural properties, including color, hardness, brittleness, adhesiveness, cohesiveness and gumminess, were then evaluated. The Pickering emulsion droplets had an approximate size of 1 μm and exhibited Newtonian flow behavior. SEM images showed that chitosan-zein particles formed compact and cohesive structures during drying. The 20% Pickering emulsion stabilized with chitosan-zein particles demonstrated the lowest creaming after 14 days of storage. Textural analysis (hardness, firmness, adhesiveness, cohesiveness and gumminess) of apple cheese samples containing the pomegranate seed oil emulsion showed no significant difference in hardness between the control and the 0.2% emulsion sample ($P > 0.05$). The results showed that pomegranate seed oil emulsified with chitosan-zein particles can be used as a stable ingredient in functional apple cheese. Incorporation of the oil in emulsion form at levels up to 0.2% was found to be appropriate for the product formulation.

Keywords: Structural properties; Textural properties; Pomegranate seed oil; Pickering emulsion; Apple cheese.

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1. Introduction

Today, consumers increasingly prefer food products that not only fulfill their basic nutritional requirements but also contribute to the prevention of various diseases. The consumption of omega-3-fortified foods has shown a steadily increasing trend worldwide due to increasing consumer awareness of their health benefits (Mirzaee Moghaddam, 2019). Lipids are a diverse group of organic compounds that are insoluble in water yet soluble in organic solvents. They perform a wide range of biological functions, including serving as fundamental structural components of cellular membranes and acting as major reservoirs of metabolic energy (Fahy et al., 2011). Lipids are composed of structural units known as fatty acids, which are broadly classified into saturated and unsaturated

fatty acids based on the presence or absence of double bonds in their hydrocarbon chains. Among the most important unsaturated fatty acids, omega-3 fatty acids have attracted considerable attention because of their well-documented health-promoting effects (Khoshnoudi-Nia et al., 2022). Among plant-derived sources of bioactive unsaturated fatty acids, pomegranate seed oil (PSO) has gained increasing interest due to its high content of punicic acid and its unique nutritional and functional properties (Miran & Kamandloo, 2025). Pomegranate (*Punica granatum*) is one of the oldest known edible fruits and is characterized by a high concentration of polyphenolic compounds. PSO is a yellow-colored oil with a mild odor (Paul & Radhakrishnan, 2020). PSO contains high levels of unsaturated fatty acids, particularly omega-3 and omega-6 fatty acids (Paul & Radhakrishnan, 2020; Amri et al.,

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2017). It is also rich in punicic acid, a bioactive conjugated linolenic acid that has been reported to play a significant role in the prevention of cardiovascular diseases (Rajaei, & Mirzaee Moghaddam, 2021). However, oils rich in omega-3 fatty acids are highly susceptible to oxidative degradation (Mahesar et al., 2019). However, the direct incorporation of PSO into food systems is limited because of its susceptibility to oxidation and poor physical stability, highlighting the need for suitable protection strategies (Comunian et al., 2020; Eratte et al., 2018).

Encapsulation refers to a process or set of techniques employed in food and pharmaceutical delivery systems. The primary objective of encapsulation is to enhance safety, preserve quality and prevent deterioration or contamination of products during transportation and storage (Eratte et al., 2018). Encapsulation refers to the process of coating core materials with wall materials to form particles at the micro or nanoscale. The selection of appropriate wall materials plays a critical role in determining the encapsulation efficiency and stability of the resulting particles (Comunian et al., 2020). Among different encapsulation approaches, Pickering emulsions have emerged as one of the most promising systems for improving the stability of lipophilic bioactive compounds (Miran & Kamandloo, 2025). Emulsions consist of two immiscible phases: a dispersed phase composed of spherical droplets and a continuous phase in which these droplets are distributed. Emulsions are widely applied in cosmetic, food and pharmaceutical formulations. The stability of an emulsion is a critical factor in maintaining its physicochemical properties over time (Song et al., 2015). To stabilize emulsions, surfactants are commonly used; however, they may cause issues such as air entrapment, foaming and irritability. As a suitable alternative, solid particles adsorbed at the oil-water interface can be employed. Emulsions stabilized in this manner are known as Pickering emulsions (Mirzaee Moghaddam, 2019). Pickering emulsions represent an innovative approach in the food industry, where solid particles act as emulsion stabilizers. Recent research has demonstrated that these emulsions can significantly enhance the stability and quality of food products (Nazari et al., 2025; Miran & Kamandloo, 2025). The advantages of Pickering emulsions include a reduced need for chemical surfactants, enhanced stability against temperature and time variations and improved sensory and nutritional properties of food products. Studies have shown that various particles, such as proteins, starches and lipids, can serve as effective stabilizers in this process. Furthermore, research indicates that Pickering emulsions can play an effective role in developing novel formulations, such as dairy products, sauces and beverages, contributing to greater diversity and appeal in food products. Overall, this approach has been recognized as a promising strategy for optimizing the quality and stability of food products (Nazari et al., 2025). The effectiveness of Pickering emulsions largely depends on the characteristics of the stabilizing particles employed (Chevalier & Bolzinger, 2013; Zhu, 2019; Sharkawy et al., 2020).

Chitosan is a linear polysaccharide derived from the deacetylation of chitin and is found in the exoskeletons of crustaceans, insect cuticles and the cell walls of certain fungi. It is recognized as a naturally occurring cationic biopolymer (Sharkawy et al., 2020). Chitosan is insoluble in water at neutral pH but becomes soluble under acidic conditions. Due to its positive charges, chitosan can interact with negatively charged compounds, such as amino acids and anionic proteins (Aider, 2010). Zein is the major storage protein of corn endosperm and is rich in hydrophobic amino acids such as proline, leucine and alanine. Owing to its hydrophobic nature, excellent film-forming ability, biodegradability and biocompatibility, zein has been widely used as a natural biomaterial

for the encapsulation of bioactive compounds and the stabilization of Pickering emulsions in food systems (Luo & Wang, 2014; Corradini et al., 2014; Anderson & Lamsal, 2011). Therefore, chitosan-zein composite particles are considered promising stabilizers for incorporating bioactive oils into fruit-based food products such as apple cheese (Zhu, 2019).

Apple is one of the major commercial crops in temperate and cold regions and serves as a rich source of vitamin C, dietary fiber and phenolic compounds such as chlorogenic acid, procyanidin B2 and quercetin (Suna, 2019). Processing apples into value-added products such as apple cheese can help reduce post-harvest losses. Apple cheese is a traditional fruit-based confection originating from Lithuania and Poland. It is produced by prolonged heating of apple puree with sugar until a dense gel-like structure is formed. Its gel-like structure and pectin-rich matrix make it a promising carrier for the incorporation of functional bioactive ingredients in the development of value-added fruit-based products (Arnold & Gramza-Michałowska, 2024). Apple cheese is one method of processing horticultural products, prepared from apple puree, with a final sugar content ranging from 75% to 85%. After cooling, the puree solidifies into a block approximately 10 mm thick, which can be cut into sticks or cubes (Orrego et al., 2014). The use of thermal processes in the production of fruit leather or fruit cheese may lead to a reduction in certain nutrients. Therefore, to fortify and enhance the nutritional value of these products, nutrient-rich compounds such as PSO can be incorporated during their production (Javadi Farsani et al., 2023). However, the high susceptibility of these oils to oxidation poses challenges for their incorporation into food products. One effective approach to protect these compounds is through encapsulation techniques (Mirzaee Moghaddam, 2019). Oil-in-water emulsions are widely used as a means of encapsulating lipophilic compounds, including functional lipids and other bioactive substances, in the fields of food, nutrition and pharmaceuticals (Mirzaee Moghaddam & Rajaei, 2021). To the best of our knowledge, based on a comprehensive review of the available literature, no published study has investigated the structural and textural properties of apple cheese fortified with PSO emulsion stabilized by chitosan-zein particles. Therefore, this study aimed to develop apple cheese fortified with a chitosan-zein particle-stabilized PSO Pickering emulsion and to evaluate its structural and textural properties. The findings may contribute to the development of novel functional fruit-based products enriched with bioactive lipids while maintaining desirable quality attributes. In addition, this study provides useful information for designing stable Pickering emulsion-based delivery systems for lipophilic bioactive compounds in fruit-derived foods.

2. Materials and Methods

2.1. Materials

The materials used in this study included chitosan with a degree of deacetylation of 75-85% (Sigma-Aldrich, USA), corn zein (Zarrin Corn Co., Iran), 96% ethanol (Zakaria Jahrom Co., Iran), acetic acid (Merck, Germany), pomegranate seed oil (Kamjad Shahroud Co., Iran) and Lebanese yellow apples of the Golden Delicious variety. The composition of the pomegranate seed oil used consisted of punicic acid (50.05%), linoleic acid (9.88%), oleic acid (9.57%), palmitic acid (4.09%) and stearic acid (3.02%).

2.2. Pre-emulsion tests

To comprehensively characterize the chitosan-zein particles prior to emulsion preparation, a series of pre-emulsion analyses were conducted. These tests aimed to evaluate the physicochemical properties, particle size distribution, zeta potential and morphological characteristics of the particles, which are critical parameters influencing their emulsifying performance and stability in Pickering emulsions.

2.2.1. Zein purification.

Crude corn zein was obtained from Zarrin Corn Co., Shahroud and was purified before use. For this purpose, 200 g of crude zein was dissolved in two 600 mL Erlenmeyer flasks containing 70% ethanol at a 1:6 ratio and stirred continuously at 70 °C for 2 hours. After this step, the clear supernatant was separated from the precipitate by centrifugation and the resulting solution was concentrated under vacuum at 40 °C using a rotary evaporator. The concentrated solution was then poured into large trays and dried in an oven at 70 °C for 24 hours. Finally, the dried material was ground into a fine powder using a mortar and pestle (Anderson & Lamsal, 2011).

2.2.2. Preparation of chitosan-zein particle solution

To prepare the chitosan-zein solution, separate 0.5% (w/v) chitosan and 0.5% (w/v) zein solutions were initially prepared at a 1:10 ratio. First, 0.5 g of chitosan was dissolved in 100 mL of 1% (v/v) acetic acid solution and sonicated in an ultrasonic bath for 20 minutes. The chitosan solution was then stirred on a magnetic stirrer at 500 rpm. Subsequently, zein was dissolved in 20 mL of 70% ethanol and mixed at 30 °C for 5 minutes. The zein solution was then added to the chitosan solution under probe ultrasonication. Next, the ethanol present in the solution was removed using a rotary evaporator under vacuum and the final solution was subjected to probe ultrasonication again to ensure complete dispersion of the chitosan-zein particles (Sharkawy et al., 2020).

2.2.3. Particle morphology and structure

The morphology and structure of the chitosan-zein particles were examined using a scanning electron microscope (SEM). To prepare the samples, a drop of freshly diluted suspension was placed on a glass slide. After complete drying, SEM images were captured to observe the particle morphology (Javadi Farsani et al., 2023).

2.2.4. Particle size and distribution

The particle size of the chitosan-zein suspension was measured using a dynamic light scattering (DLS) instrument (ZEN 3600, UK). For sample preparation, a defined amount of the suspension was diluted with distilled water and sonicated for 2 minutes in an ultrasonic bath (Pipetec Lab 750, France) to ensure complete dispersion of the particles.

The particle size distribution was evaluated using the Span index, which was calculated according to Eq. (1). Lower Span values indicate a narrower particle size distribution.

$$\text{Span} = \frac{D(90\%) - D(10\%)}{D(50\%)} \quad (1)$$

Where represent the particle diameters below which 10%, 50% and 90% of the total particle volume are contained, respectively (Guerra-Rosas, et al., 2016).

2.2.5. Preparation of Pickering emulsions

To prepare Pickering emulsions, PSO was added to the chitosan-zein particle dispersion at three different ratios (20%, 30% and 50%). The resulting mixture was homogenized using a high-speed homogenizer at 11,000 rpm for 5 minutes. To further reduce particle size, the primary emulsion was subjected to probe ultrasonication at 65% amplitude. The ultrasonication was performed intermittently (5 seconds on, 5 seconds off) for a total treatment time of 5 minutes (Nazari et al., 2025).

2.3. Post-emulsion tests

Following the preparation of Pickering emulsions, several post-emulsion analyses were performed to assess emulsion stability, droplet size distribution, rheological behavior, microstructure and the quality characteristics of the fortified apple cheese samples. These analyses provide insight into the effectiveness of chitosan-zein particles as stabilizers and their impact on the final product properties.

2.3.1. Determination of creaming index (CI)

In this study, the creaming index (CI) of emulsions containing 20%, 30%, and 50% PSO was evaluated according to the methods described by Cheong et al. (2016) and Ju et al. (2020). For this test, 10 mL of each emulsion sample was transferred into glass tubes, which were tightly sealed to prevent evaporation. The samples were then stored for 14 days, after which two distinct layers were observed: a creamy layer at the top and a serum layer at the bottom. To enhance phase distinction, the samples were exposed to fluorescent light and the physical characteristics of the emulsions were recorded (Cheong & Nyam, 2016; Ju et al., 2020).

The creaming index (CI), representing emulsion stability against creaming, was calculated as a percentage using Eq. (2).

$$\text{CI (\%)} = \frac{\text{HS}}{\text{HE}} \times 100 \quad (2)$$

Where CI is the creaming index (%), HE is the total height of the emulsion (mm) and HS is the height of the serum layer (mm). A higher CI value indicates faster creaming and larger emulsion droplet size. In this study, the sample exhibiting the optimal CI was selected for subsequent experiments.

2.3.2. Emulsion viscosity measurement

The viscosity of emulsions containing PSO was measured using a rheometer (Compact Modular, Model 302 Physica, Austria) equipped with a concentric double-cylinder geometry. In this test,

the viscosity behavior of the emulsions was evaluated under different shear rates to assess their flow properties (Javadi Farsani et al., 2023; Shah et al., 2016).

2.3.3. Emulsion droplet size imaging and measurement

To investigate the droplet size distribution of the emulsion, (DLS) was employed. Additionally, to analyze the droplet morphology, a light microscope equipped with a digital camera was used. In this method, a droplet of the emulsion was collected using a sampler and placed on a glass slide. Then, a drop of distilled water was added to dilute the emulsion and was evenly distributed. Immediately, before the sample dried, images were captured using the light microscope at a magnification of 40x and the droplet size was measured using the DLS technique (Mirzaee Moghaddam & Rajaei, 2021).

2.4. Production of apple cheese containing pomegranate seed oil emulsion

To produce the apple cheese, after washing and peeling the fruit, the pulp was placed in a water bath at 65°C for 3 hours. The pulp was then ground and passed through a sieve. In this study, three types of apple cheese samples were prepared: the control apple cheese (T1), apple cheese with 0.2% emulsion (T2) and apple cheese with 0.5% emulsion (T3). For the preparation of the samples, 120 grams of the fruit pulp was mixed with two concentrations of the emulsion and placed on aluminium trays with dimensions of 2×32×25 mm. The samples were then dried at 60°C for 5 hours and slices of 2×10×10 mm with a thickness of 10 mm were cut. The samples were stored in zip-lock plastic bags and kept in the refrigerator until further analysis (Fu et al., 2019; Orrego et al., 2014).

2.4.1. Textural properties of the produced apple cheese samples

In this study, to analyze the texture profile (TPA) and determine the texture characteristics of the functional apple strips, a material testing device (model STM-20, Sentam Co., Iran) was used. For this purpose, samples of each treatment were prepared in 10×10×10 mm dimensions. Each sample was compressed in two cycles using a cylindrical probe with a diameter of 60 mm and a movement speed of 60 mm/min, until 80% of the initial height was reached and then the pressure was released. Six repetitions were performed for each

treatment. Using the data obtained from the device's memory, force-time curves for each sample were plotted (Fig. 1) and based on these curves, the textural properties including hardness, cohesiveness, springiness, gumminess and chewiness were determined for each sample (Caine et al., 2003; Mirzaee Moghaddam & Rajaei, 2021).

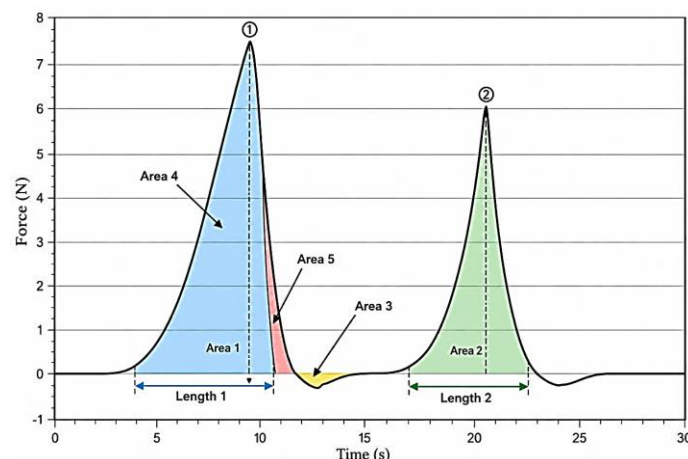


Fig. 1. Force-time plot to determine texture profile analysis parameters. Peak force is hardness; cohesiveness= Area2/Area1; springiness= Length2/Length1; gumminess= Hardness×cohesiveness; adhesiveness= Area 3.

2.5. Statistical analysis

In this study, the differences between the various treatments were analyzed using analysis of variance (ANOVA) at a 95% confidence level. Subsequently, the means of the data were compared using Duncan's test and the analysis was performed with SPSS software (version 22). The experimental results are presented in the corresponding figures, which were generated using Microsoft Excel (Version 2019).

3. Results and Discussion

3.1. Morphological analysis of the produced particles using SEM

The SEM micrographs of the chitosan-zein composite particles are presented in Fig. 2. SEM analysis provides valuable information

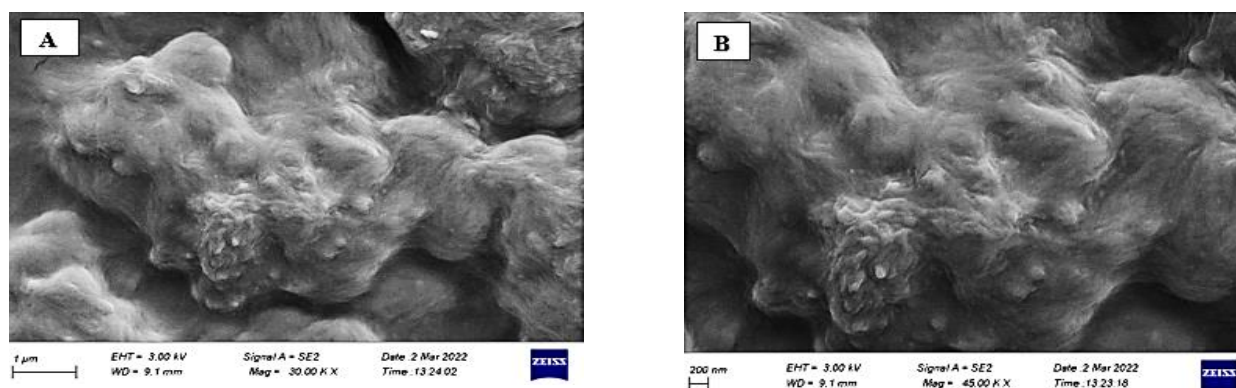


Fig. 2. Scanning electron microscope (SEM) images of chitosan-zein particles, showing particle morphology, surface texture and aggregation pattern after drying.

regarding particle morphology, surface characteristics, particle size and aggregation behavior. The obtained micrographs revealed that the particles possessed irregular shapes with relatively rough surfaces and formed compact aggregates after drying. The observed aggregation may be attributed to the intermolecular interactions between chitosan and zein as well as hydrogen bonding and hydrophobic interactions that became more pronounced during water removal.

SEM images provide valuable information about the morphology, surface characteristics and aggregation behavior of the produced particles. As shown in Fig. 2, the chitosan-zein composite particles exhibited irregular shapes and formed compact aggregated structures after the drying process. This aggregation can be attributed to the strong intermolecular interactions between chitosan and zein including hydrogen bonding, electrostatic attraction and hydrophobic interactions, which became more pronounced during water removal. Moreover, the presence of chitosan on the particle surface may have promoted particle-particle adhesion, resulting in the formation of dense structures. Similar irregular and quasi-spherical particle morphologies have also been reported for encapsulated bioactive compounds produced by spray drying, confirming the successful formation of stable particulate systems (Hasani et al., 2023).

The formation of compact and aggregated structures has also been reported for chitosan-protein composite particles, where dehydration promotes particle-particle interactions and enhances structural cohesion. Furthermore, the rough particle surface may improve the adsorption of particles at the oil-water interface, thereby increasing the stability of Pickering emulsions. Similar observations have been reported by Luo & Wang (2014), who demonstrated that zein-based composite particles exhibit irregular morphology and strong interparticle interactions after drying, contributing to improved emulsion stabilization.

Albadarin et al. (2017) demonstrated in their research that chitosan particles are irregular in size and exhibit adhesive properties. These particles, with an average size of approximately 30 micrometers, have a rough surface and pores, creating a clumping effect in the product (Albadarin et al., 2017). The enhanced stability of the Pickering emulsion can be attributed to the irreversible adsorption of chitosan-zein composite particles at the oil-water interface, where they form a dense and mechanically robust interfacial layer that effectively inhibits droplet coalescence and improves emulsion stability (Chevalier & Bolzinger, 2013; Zhu, 2019; Sharkawy et al., 2020).

3.2. Emulsion stability

In Fig. 3, a snapshot of the prepared Pickering emulsions is presented. Digital images taken on the first day, 2 hours after preparation, showed no visible differences in the appearance of the treatments and the emulsions appeared stable in terms of their visual characteristics. The prepared samples were then stored in the laboratory environment for 14 days without any movement. The results indicated that the 20% emulsion exhibited the best stability, while the 50% treatments showed phase separation or creaming more than the other treatments. The 20% and 30% treatments also displayed fewer visible changes.

The improved stability observed in the emulsions containing lower oil concentrations may be attributed to the availability of sufficient chitosan-zein particles to completely cover the oil droplets, resulting in the formation of a dense interfacial layer. In contrast, increasing the oil fraction without a proportional increase in

stabilizing particles may lead to incomplete surface coverage, thereby promoting droplet aggregation and creaming during storage (Chevalier & Bolzinger, 2013; Xiao et al., 2016).

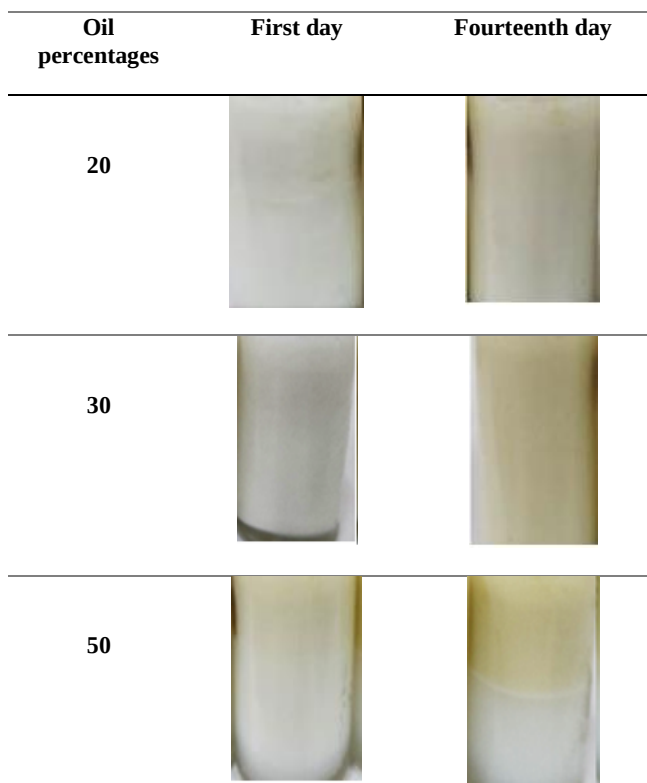


Fig. 3. Visual appearance of Pickering emulsions containing different concentrations of PSO (20, 30 and 50%) stabilized by chitosan-zein particles after 1 days and 14 days of storage at room temperature.

The creaming index results are presented in Fig. 4. Statistical analysis showed that the creaming index of the 20% oil emulsion was significantly lower than those of the 30% and 50% oil emulsions ($P < 0.05$ and $P < 0.01$), whereas the highest creaming index was observed for the 50% oil formulation. These findings indicate that increasing the oil concentration adversely affected the physical stability of the Pickering emulsions. This behavior can be explained by the increased frequency of droplet collisions at higher oil concentrations, which enhances droplet flocculation and accelerates gravitational separation during storage. Similar results were reported by Xiao et al. (2016), who demonstrated that increasing the oil concentration increased droplet size and reduced emulsion stability. Likewise, Shah et al. (2016) reported that increasing the oil fraction in chitosan-based Pickering emulsions enlarged droplet size because the available stabilizing particles became insufficient to completely cover the oil-water interface.

These findings are consistent with previous studies demonstrating that increasing the dispersed-phase volume fraction leads to larger droplet sizes and reduced emulsion stability, as the available chitosan-zein particles become insufficient to completely cover the oil-water interface (Chevalier & Bolzinger, 2013; Zhu, 2019; Sharkawy et al., 2020). Consequently, droplet flocculation and gravitational separation become more pronounced at higher oil concentrations.

Based on the visual stability observations and the significantly lower creaming index, the Pickering emulsion containing 20% pomegranate seed oil exhibited the highest physical stability among all treatments. Therefore, this formulation, stabilized by chitosan-zein composite particles, was selected for subsequent physicochemical characterization and incorporation into apple cheese. The high stability observed in the present study is consistent with previous findings indicating that Pickering emulsions stabilized by biopolymeric particles exhibit superior resistance to coalescence and creaming because of the formation of a rigid interfacial layer around oil droplets (Miran & Kamandloo, 2025).

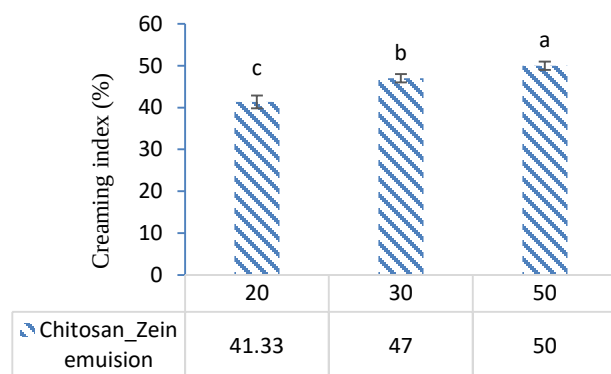


Fig. 4. Creaming index of different emulsions (T1, T2 and T3 corresponding to 0%, 0.2% and 0.5% PSO, respectively).

3.3. Emulsion droplet size

The droplet morphology and size of the prepared Pickering emulsion were evaluated using optical microscopy and (DLS). Optical microscopy was used to observe the morphology and distribution of emulsion droplets (Fig. 5), whereas DLS analysis provided a more accurate determination of droplet size distribution (Fig. 6).

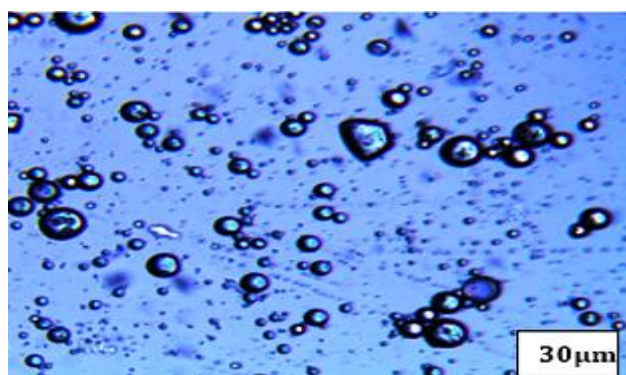


Fig. 5. Optical microscopy images of PSO Pickering emulsions stabilized with chitosan-zein composite particles.

In the present study, the average droplet size (D_{50}) of the emulsion was determined to be approximately 1 μm , which is considerably smaller than the typical droplet sizes observed in food industry emulsions. This particle size is comparable to that reported by Hasani et al. (2023), who obtained encapsulated particles with an

average diameter of approximately 1.04 μm . The obtained particle size distribution indicates the successful formation of the Pickering emulsion and suggests the development of a stable particulate system. Together with the SEM observations and the physical stability results, these findings support the effectiveness of the chitosan-zein composite particles as Pickering stabilizers (Zhu, 2019; Chevalier & Bolzinger, 2013). The relatively small droplet size observed in the present study can be attributed, at least in part, to the application of ultrasonic homogenization during emulsion preparation. Ultrasound waves, particularly at low frequencies, induce cavitation in the medium, generating intense shear forces that lead to the breakup and reduction of the dispersed phase droplets (oil droplets) (Albadarin et al., 2017).

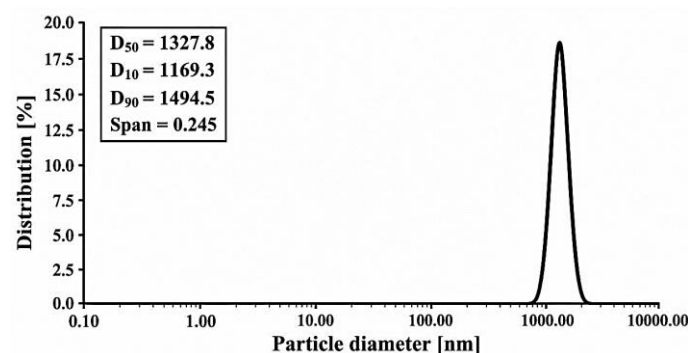


Fig. 6. Droplet size distribution of PSO Pickering emulsion stabilized with chitosan-zein composite particles determined by DLS.

The small droplet size obtained in the present study indicates the high emulsification efficiency of the chitosan-zein composite particles. Smaller droplets provide a larger interfacial surface area, allowing the stabilizing particles to form a compact protective layer around the oil droplets, which contributes to improved physical stability and reduced creaming. Moreover, the application of ultrasonic homogenization enhances cavitation-induced shear forces, leading to more efficient droplet disruption and a narrower droplet size distribution. Similar findings have been reported in previous studies, where ultrasound-assisted emulsification significantly reduced droplet size and improved the stability of Pickering emulsions (McClements, 2018; Zhu, 2019).

The obtained D_{50} value of approximately 1 μm confirms the effectiveness of the emulsification process and agrees with previous reports indicating that Pickering emulsions stabilized by protein-polysaccharide composite particles generally exhibit droplet sizes within the submicron to low-micron range. Such small droplets not only improve emulsion stability but also enhance the protection of encapsulated lipophilic bioactive compounds against coalescence and oxidative deterioration during storage.

3.4. Emulsion viscosity

The flow behavior of the Pickering emulsion containing 20% PSO stabilized by chitosan-zein composite particles is presented in Fig. 7. The apparent viscosity remained nearly constant over the investigated shear rate range, indicating that the emulsion exhibited Newtonian flow behavior. This behavior can be primarily attributed to the predominance of the aqueous continuous phase, which inherently possesses Newtonian characteristics. Furthermore, the

relatively low oil concentration (20%) and the relatively small droplet size limited droplet-droplet interactions, thereby preventing the formation of a three-dimensional particle network within the continuous phase (Nazari et al., 2025).

The observed Newtonian behavior suggests that the stabilizing particles effectively adsorbed at the oil-water interface without forming a highly interconnected structure capable of resisting shear deformation. At low dispersed phase concentrations, the viscosity of Pickering emulsions is mainly governed by the continuous aqueous phase, whereas increasing the oil volume fraction generally enhances particle interactions and results in non-Newtonian or shear-thinning behavior (McClements, 2015; Zhu, 2019).

These findings are consistent with previous studies reporting that Pickering emulsions containing relatively low oil fractions exhibit Newtonian flow because of limited droplet flocculation and weak interparticle interactions. In contrast, emulsions with higher dispersed phase concentrations usually develop stronger droplet networks, leading to increased viscosity and pseudoplastic behavior (Chevalier & Bolzinger, 2013; Zhu, 2019).

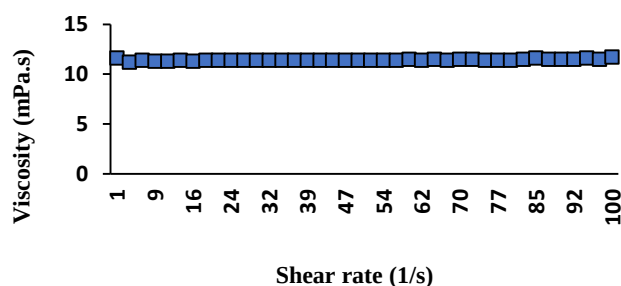


Fig. 7. Apparent viscosity as a function of shear rate for the Pickering emulsion containing 20% PSO stabilized by chitosan-zein composite particles.

3.5. Textural evaluation of the produced apple cheese samples

The results of the texture analysis indicated that the addition of PSO emulsion had a significant effect on the textural properties of the apple cheese (Fig. 8). Texture is one of the most important quality attributes affecting consumer acceptance of fruit-based confectionery products. The incorporation of oil emulsions stabilized by protein-polysaccharide particles may modify the gel network, thereby influencing hardness, cohesiveness, springiness, and gumminess. Therefore, evaluating these parameters provides valuable information regarding the structural integrity and overall quality of the developed apple cheese (Ma et al., 2023; McClements, 2018).

Incorporation of the PSO emulsion significantly increased the hardness of the apple cheese samples. As the emulsion concentration increased, hardness gradually increased with treatment T3 (0.5% oil) exhibiting the highest hardness value. This increase may be attributed to the formation of a more compact and interconnected gel network promoted by the chitosan-zein stabilized emulsion, which reinforced the structural integrity of the apple cheese matrix. Similar improvements in gel firmness following the incorporation of protein-polysaccharide stabilized emulsions have been reported in previous studies (Ma et al., 2023; Pavlátková et al., 2023).

These differences were significant compared to the control treatment (T1) at both $P < 0.01$ and $P < 0.05$ levels. Furthermore, the addition of the emulsion led to an increase in the springiness of the

samples, which was also statistically significant compared to the control. Treatment T2 exhibited the highest cohesiveness, followed by T3, whereas the control treatment (T1) showed the lowest cohesiveness. These results indicate that the incorporation of the chitosan-zein stabilized emulsion improved the internal structural integrity of the apple cheese matrix, although the highest cohesiveness was observed in T2 rather than T3.

An increase in springiness suggests that the emulsion droplets were effectively integrated into the gel matrix, enabling the structure to recover more efficiently after deformation. This behavior is commonly associated with enhanced elasticity and greater structural resilience in emulsion-filled food gels, where uniformly distributed oil droplets reinforce the three-dimensional gel network (Dickinson, 2017; McClements, 2018; Miran & Kamandloo, 2025).

The cohesiveness results indicated that the emulsion containing treatments exhibited higher cohesiveness than the control, with treatment T2 showing the highest value. This improvement may be attributed to the adsorption of chitosan-zein particles at the oil-water interface, where they formed a stable interfacial layer that strengthened the interactions between the dispersed oil droplets and the surrounding pectin rich fruit gel matrix. Consequently, the resistance of the gel network to structural breakdown during compression increased, resulting in higher cohesiveness. At the higher emulsion concentration (T3), the larger number of dispersed droplets may have partially disrupted the continuity of the gel network, leading to slightly lower cohesiveness than that observed for T2. Similar observations were reported by Pavlátková et al. (2023).

Treatment T3 showed a significant difference in this index compared to the control at both $P < 0.01$ and $P < 0.05$ levels. Finally, the evaluation of gumminess, which is calculated as the product of hardness, cohesiveness and springiness, revealed significant changes in the chewing energy required for the different treatments, particularly T3. In a study, Mirzaee Moghaddam (2019) reported that the addition of fish oil (encapsulated in chitosan-stearic acid nanogels) to a gummy formulation resulted in a decrease in the product's hardness, cohesiveness and gumminess, which contrasts with the findings of this study. These differences are likely due to the specific type of oil (PSO) and the matrix (apple puree) used, as well as the presence of the chitosan-zein complex as the emulsion stabilizer.

Since gumminess is influenced by hardness and cohesiveness, the observed changes indicate that the incorporation of the stabilized Pickering emulsion modified the mechanical properties of the apple cheese matrix. Such modifications may improve the sensory perception and chewing characteristics of the final product. These findings suggest that chitosan-zein stabilized Pickering emulsions can serve as effective delivery systems for bioactive oils while simultaneously improving the textural properties of fruit-based products (Zhu, 2019; Comunian et al., 2020; Miran & Kamandloo, 2025).

Among the fortified formulations, T2 provided the most balanced textural profile, exhibiting the highest cohesiveness while maintaining hardness and springiness close to those of the control. Although T3 contained a higher level of PSO, the greater increase in hardness and the slight reduction in cohesiveness indicated that T2 offered a more desirable balance between nutritional fortification and textural quality.

Overall, the results demonstrate that fortification of apple cheese with PSO emulsion stabilized by chitosan-zein particles improved several textural attributes without compromising the structural integrity of the product. These findings highlight the potential of

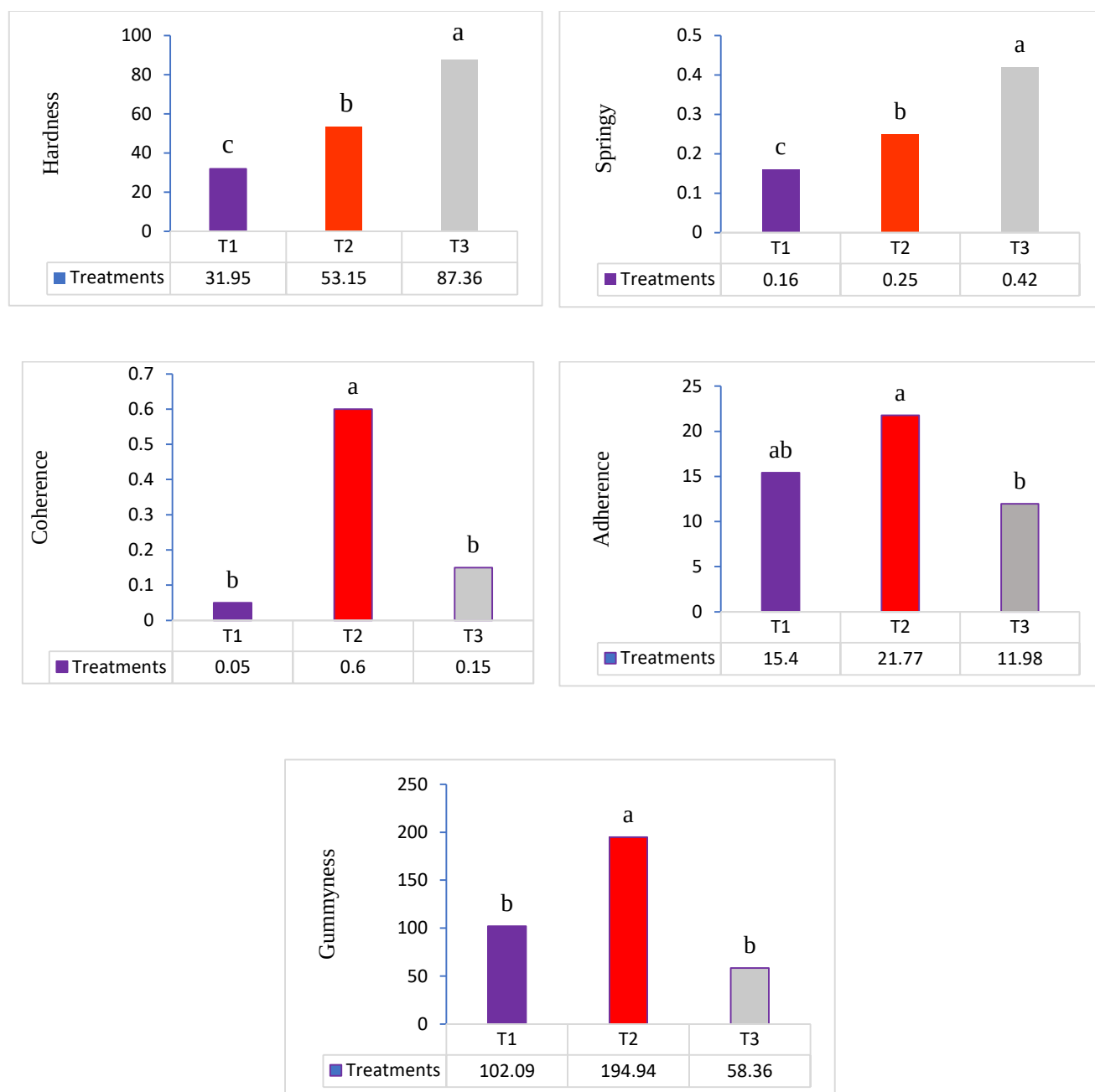


Fig. 8. Effect of PSO Pickering emulsion stabilized by chitosan-zein composite particles on the textural properties (hardness, springiness, cohesiveness and gumminess) of apple cheese. Different lowercase letters above the bars indicate significant differences among treatments according to Duncan's multiple range test ($P < 0.05$). Treatments included control (T1), chitosan-zein emulsion containing 0.2% PSO (T2) and chitosan-zein emulsion containing 0.5% PSO (T3).

chitosan-zein stabilized Pickering emulsions as efficient delivery systems for incorporating bioactive oils into functional fruit-based foods, consistent with the recent review by [Miran and Kamandloo \(2025\)](#).

4. Conclusion

This study demonstrated that chitosan-zein composite particles were effective stabilizers for the preparation of Pickering emulsions

containing PSO. Among the investigated formulations, the emulsion containing 20% oil exhibited the highest physical stability, as evidenced by its lower creaming index and desirable droplet characteristics. Incorporation of the stabilized emulsion into apple cheese significantly influenced its textural properties. Increasing the emulsion concentration enhanced hardness, springiness, and cohesiveness, with treatment T3 exhibiting the highest hardness (87.36 N), while treatment T2 showed the highest cohesiveness, indicating improved structural integrity of the product. Considering the overall balance among the evaluated textural parameters,

treatment T2 was identified as the most suitable formulation. The findings of this study demonstrate the potential of chitosan-zein stabilized Pickering emulsions as effective delivery systems for incorporating PSO into fruit-based functional foods while maintaining desirable structural and textural characteristics. Future studies are recommended to investigate the oxidative stability, sensory properties, and storage behavior of the developed product under different storage conditions.

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Conflict of interest

The authors declare that there is no conflict of interest.

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