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Research Article

Effect of Pickering Emulsion Stabilized by Soy Protein Nanoparticles on Physical and Rheological Properties of Gluten-Free Cake Batter

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In this research, Pickering emulsion (PE) stabilized by soy protein nanoparticles (SPNs) and xanthan gum, was added to gluten-free cake batter based on rice flour. The physical and rheological properties of the PE-containing batter were quantified, compared with the control sample. The results revealed that the addition of the PE to the batter decreased its specific gravity, which in turn increased the volume and reduced the hardness of the cake. The power-law model with a high determination coefficient (R^2) could best describe the flow behavior of the batter. Measurement of apparent viscosity, consistency coefficient, and flow behavior index indicated that the gluten-free cake batter had shear-thinning behavior. Moreover, the oscillatory test results showed that the PE-containing batter had more liquid-like behavior than the PE-free sample.

1. Introduction

Cereal-based products have drawn enormous attention from ancient times and are considered a group of the most consumed food products in the household basket. Nowadays, with the development of technology, following an appropriate diet is of great importance, and different people go on various diets. Moreover, some food intolerance causes people to change their diets inevitably, with celiac disease being one of them. In celiac, which is an autoimmune and hereditary disease, the mucosal membrane of the patient's small intestine is damaged and inflamed after the consumption of gluten-containing cereal-based products. With respect to this fact that the gluten-free diet is the only way of curing the celiac, the development of gluten-free foods, particularly the bakery products has attracted a lot of attention [1, 2]. Cake is a wheat flour-based bakery product appealing to differently aged people, owing to its low production cost, easy preparation, long shelf-life, desirability, and integrated gluten network structure [3, 4].

Rice flour is typically used in the production of gluten-free cake, and given the qualitative defects of gluten-free products, gluten substitutes should be employed. Application of Pickering emulsion (PE) is a way of enhancing the properties of cake batter. This type of emulsion is stabilized by amphiphilic nanoparticles such as proteins and polysaccharides which physically prevent the fusion of the emulsion phases [5]. The Pickering oil-in-water emulsion stabilized by modified Longan shell cellulose nanofibers (LSCNF) was used in the batter and increased its viscosity and also improved the surface attributes and gelation properties of the batter. In addition to the improve of the dough texture, using this system as a fat replacer can prevent the release of free fatty acids during the digestion [6]. By being irreversibly adsorbed onto the oil-water interface, such particles properly stabilize surfactant-free emulsions. In addition, application of the two major fractions of soy protein (glycinin and β-conglycinin) in the preparation of PE, without surface modification, improves its emulsifying and gelling properties [7, 8]. This was investigated by some studies that the quality attributes of the bakery products can be improved by incorporating of PE. The cellulose-based PE was employed successfully to produce reduced-fat biscuits. Moreover, the dietary fibers of this PE also increased the dough viscoelasticity by changing the gluten network resulting in a disintegrated and heterogeneous matrice [9]. Furthermore, the effect of PE, stabilized by zein nanoparticles and enriched with cinnamon oil, as a fat replacer, improved the cake viscoelastic properties by interacting with the zein nanoparticles and hence forming a gel-like network [10].

Xanthan gum is applied as polysaccharide-based amphiphilic particles in emulsion preparation. Due to its high apparent viscosity and shear-thickening behavior, it is usually utilized as a thickening agent in PEs to inhibit droplet aggregation and develop desirable textural properties [11]. In protein-stabilized emulsions, the emulsion stability depends on the interaction between xanthan and the protein, and as the protein charge converts from positive to negative with a rise in pH, this interaction is highly dependent on pH [12].

Considering the studies conducted so far, PE based on plant proteins like soy protein and microbial polysaccharides like xanthan, has not ever been reported in the preparation of gluten-free cake. Therefore, the purpose of this research is to stabilize PE by soy protein nanoparticles (SPNs), as well as investigating its effects on gluten-free rice flour-based cake batter.

2. Materials and Methods

2.1. Materials. Soy protein isolate (SPI) (90.032% protein) was supplied from wonderful Shandong LTD., China. Sunflower oil, for emulsion preparation, and the cake ingredients (icing sugar, vegetable oil, egg, water, vanilla, and baking powder) were purchased from local market (Gorgan, Iran). Half-grain rice (Hashemi cultivar) was bought from local market (Ghaemshahr, Iran). In order to prepare the rice flour, it was soaked in water, dried, and finally grounded. All the chemicals utilized in this study were supplied from Merck Co., Germany.

2.2. Preparation of SPNs. SPNs were produced according to the method previously described by Zhang et al. [13] with slight modifications. The SPI powder was suspended in distilled water at 10 mg/ml and stirred at room temperature for 30 min. The suspension pH was increased up to 12 using NaOH 0.1 N and kept constant for 30 min. To denature the SPI, the suspension was heated at 85°C for 30 min and cooled down thereafter. Then, its pH was reduced to 8 using HCL 0.1 N. The suspension was diluted (distilled water) until the protein concentration reached 6 mg/ml. After that, $CaCl_2$ 2.5 mM was added to the suspension to induce particles formation and modify the suspension ionic strength. The final suspensions exhibited pH values in the range of 6.8–8.8 as the addition of $CaCl_2$ decreased the pH. Therefore, the NaOH 0.1 N was used to adjust the pH, and the protein concentration was further decreased to 5 mg/ml. The resulting solution was kept at 4°C overnight, and natamycin was added at 20 ppm to restrain microbial growth. Eventually, the solution was sonicated (UP200H, Dr. Hilelscher GmbH, Germany) at a power of 80 W for 2 min (pulsation time: 0.5 s ON and 0.5 s OFF).

2.3. PE Preparation. The PE was prepared based on the method presented by Liu & Tang [14] with some modifications. The SPNs and xanthan solution 0.1% (w/v) were utilized to prepare the PE. For this purpose, sunflower oil (20%) was gently added to the mixture of xanthan solution (20%) and SPNs (60%), which was subsequently agitated using a homogenizer (Ultra Turrax, Miccra D-9, Germany) at 11000 rpm. Afterwards, homogenization continued at 16000 rpm for 2.5 min. The resulting emulsion (20 ml) was sonicated at 80 W for 2 min (pulsation time: 0.5 s ON and 0.5 s OFF) in a glass beaker covered by ice to prevent the temperature rise above 50°C during the ultrasonication process for better homogenization.

2.4. Particle Size Distribution. The hydrodynamic diameter and polydispersity index (PDI) of the SPNs and the PE were measured through dynamic light scattering (DLS) (Malvern Instruments, the U.K.) at 25°C [15]. In brief, samples were diluted by 100-folds using distilled water with a same pH as initial continuous phase and then measured by DLS instrument.

2.5. Determination of Zeta Potential. The zeta potential of the SPNs and the PE was quantified using a zeta sizer (Zeta Compac, CAD, France) [15]. Briefly, small amounts of samples were diluted in distilled water at a ratio of 1 : 100 until it was transparent and then measured by Zeta Compac instrument.

2.6. Measurement of Emulsifying Activity (EA). The EA test was performed according to the method presented by Dalev & Simeonova [16] with slight changes. The emulsion was centrifuged (EBA200, Hettich, Germany) at 2600 g for 5 min. Next, the system total volume (w/v) and the emulsion phase volume (EPV) were measured. EA was calculated using

$$\mathrm{EA}\ (\%) = \frac{\mathrm{EPV}}{\mathrm{WV}} \times 100. \tag{1}$$

2.7. Emulsion Stability Index (ESI). This index was determined through turbidity measurement [17]. The emulsion was diluted with sodium dodecyl sulfate (SDS), and its absorbance value was measured at 500 nm using a spectrophotometer (Jenway 6300, England). After that, the emulsion was kept at 25°C for 10 min, and its absorbance value was quantified again. ESI was computed according to

$$\mathrm{ESI}\ (\mathrm{min}) = \frac{A_0}{(A_0 - A_{10})} \times 10, \tag{2}$$

Where A_0 denotes the absorbance value immediately after emulsion preparation, and A_{10} stands for the absorbance value after 10 min of storage.

2.8. Creaming Index (CI). After emulsion preparation, 10 ml of the emulsion was poured into a 15 ml falcon tube, and after sealing the tube, it was stored at a refrigerator which its temperature was set on 4°C for 21 days. The emulsion creaming was examined after 7, 14, and 21 days. The total height of the emulsion (HE) and the height of the serum (HS) were measured. The CI percentage was obtained using [17]:

$$\text{CI}\,(\%) = \left(\frac{\text{HS}}{\text{HE}}\right) \times 100. \tag{3}$$

2.9. Cake Batter Preparation. The sugar-batter method Bennion & Bamford [18] with some changes in ingredients, was applied to cake preparation. The cake formula was comprised of 100 g rice flour, 72 g icing sugar, 57 g oil, 0.5 g vanilla, 72 g egg, 2 g baking powder, 30 g water, and 20 ml of the PE. The percentage of PE added based on each 100 grams of flour is 20% by volume. The total weight of the cake batter was 358.3 g. In order to prepare the batter, the oil and icing sugar were firstly mixed and stirred until changing into a cream-colored liquid. Then, the previously whipped egg was added, and the mixture was vigorously agitated for 2 min. Next, half of the water was added while stirring. After that, the previously blended powdered ingredients were incorporated into the mixture while being gently stirred. Afterwards, the PE was added while slowly stirring the mixture for 1 min. Finally, the remaining half of the water was added, and the mixture was agitated at a medium rate for 30 min. The control (PE-free) sample was also prepared as mentioned above.

2.10. Batter Specific Gravity. This batter specific gravity was determined based on [19]. A specific volume of the batter was weighed in a container and divided by the weight of the same volume of water having the same temperature.

2.11. Batter Consistency. In order to quantify the batter consistency, it was poured into Perspex cylindrical container with a depth of 50 mm, height of 69 mm, inner diameter of 50 mm, and outer diameter of 60 mm. The batter consistency was measured through back extrusion using TA.XT plus texture analyzer (Stable Micro Systems, the U.K). A/B-d35 (35 mm in diameter) was employed for this test. The probe speed before, during, and after the test was set at 1 mm/s, 1 mm/s, and 10 mm/s, respectively [20].

2.12. Batter Rheological Properties. The rheological tests were conducted using MCR301 parallel-plate rheometer (Anton Paar, Austria) at 20°C. The tests included steady shear rheological properties (flow behavior) and oscillatory tests (strain sweep and frequency sweep).

2.12.1. Determination of Batter Flow Behavior. This test was performed to draw the flow curves and determine the changes in the batter apparent viscosity. In this test, the gap between the plates was considered to be 1 mm, and the shear rate varied between 0.05-250 s^{-1}. In order to determine the time-dependent behavior, the shear rate was elevated up to 250 s^{-1}, held constant for 20 s, and reduced to its initial value. The power-law model was fitted to the obtained data, and the model parameters were determined. Equation (4) denotes this model:

$$\tau = k(\gamma)^n, \tag{4}$$

Where τ, γ, k, and n, respectively, stand for shear stress (Pa), shear rate (s^{-1}), consistency coefficient ($Pa.s^n$), and flow behavior index (dimensionless).

2.12.2. Oscillatory Tests. Strain sweep test was carried out in the strain range of 0.1-1000 (%) at a constant frequency of 1 Hz to determine the linear viscoelastic region (LVR). Frequency sweep test was conducted in the frequency range of 0-100 Hz at a constant strain of 0.1 within LVR [21]. The dynamic rheological properties of the samples were examined using the storage modulus (G') and loss modulus (G'') which, respectively, describe the elastic and viscous behavior of a sample. In this test, if $G' > G''$, the sample shows the solid-like behavior, and if $G'' > G'$, it shows the liquid-like behavior. The frequency dependency of G' (Equation (5)) and G'' (Equation (6)) can be expressed using the power-law model:

$$G' = k'.\omega^b \tag{5}$$

$$G'' = k''.\omega^d \tag{6}$$

where ω denotes the oscillatory frequency (Hz), k' and k'' are intercepts ($Pa.s^n$), and b and d denote the slopes of the curves of G' and G''.

2.13. Statistical Analyses. In this research, all the experiments of the data were accomplished in triplicate to investigate the sample to compare with control. In order to analyze the obtained data, one-way analysis of variance (ANOVA) was performed by SAS version 9.0 at $P < 0.05$. Mean comparison was conducted through the least significant difference (LSD) test at 95% confidence level. The rheological data were plotted by Rheoplus version 3.40 (Anton Paar, Germany).

3. Results and Discussion

3.1. Particle Size and Zeta Potential. The sizes and PDI of the SPNs and PE, which indicate the homogeneity and appropriate size of the nanoparticles, are summarized in Table 1. Many qualitative properties of an emulsion depend on its particle size distribution. Therefore, knowledge about the particle sizes of emulsions is of utmost importance. Zhang et al. [13] reported the sizes and PDI of SPI nanoparticles in the ranges of 28-179 nm and 0.23-0.29, respectively. The results of the present study revealed that the prepared PE had an acceptable dispersity, because the lower the PDI is, the more suitable the dispersity is, and the more homogenous the particles are.

Table 1: The particle size distribution of SPNs and PE.

Samples	Z-average (d.nm)	PDI	Zeta potential (mV)
SPNs	61.213 ± 4.65	0.397 ± 0.003	-13.166 ± 1.560
PE	7752 ± 0.65	0.504 ± 0.024	-30.696 ± 1.671

Zeta potential is the best index to determine the electrical charge of the particle surfaces in a colloidal system, as it indicates the degree of particle aggregation in the stationary layer and the extent of the adsorption of opposite ions onto the particle surfaces [15].

The zeta potentials of the SPNs and the PE were equal to -13.166 and -30.696 mV, respectively. The high zeta potential of colloidal particles causes an increase in electrostatic repulsive forces between them, thus raising the system physical stability at pH values above its isoelectric point. On the other hand, the emulsion prepared using the SPNs and the xanthan solution showed a higher zeta potential. Generally, xanthan solution is negatively charged at neutral pH, due to the occurrence of acidic groups in the structure of this gum. The presence of negative charge in both the SPNs and the gum elevated the zeta potential of the emulsion. As a result, they repelled each other because of their same electrical charge, thus restraining instability mechanisms such as coalescence in the emulsion. Naji-Tabasi et al. [22] and Zhang et al. [13] reported the zeta potential of SPI nanoparticles to be -10.32 and -15.8 mV, respectively, which conform to the findings of the present study.

3.2. Emulsion Stability. Centrifugation can also accelerate droplet coalescence rate in an emulsion, as it makes the droplets approach one another [23]. The PE stability results are summarized in Table 2.

The presence of protein nanoparticles improves the emulsifying activity in addition to stabilizing the oil droplets and preventing their coalescence. The size of the nanoparticles produces a profound effect on PE stability. Since the SPNs were smaller than the emulsion dispersed phase, they adsorbed onto the oil droplet surfaces, thus inhibiting their aggregation and stabilizing the dispersed phase.

Emulsion stability demonstrates its capability of resisting to changes in its properties during storage. The changes include coagulation, creaming, and flocculation [24]. Investigation of the PE stability during the 21 days of storage indicated that no phase separation occurred in the first two weeks (Figure 1), because xanthan prevented the oil droplets from aggregating, owing to its large molecular size, which led to consistency development and network formation in the emulsion (Table 2). However, at the end of the storage period, an insignificant phase separation (about 3%) was observed.

Zeta potential is another factor affecting emulsion stability. The farther from zero (positive or negative) this parameter is, the more stable the emulsion is, because of the electrostatic repulsion between the particles. In the present research, the fresh PE zeta potential was -30.696. In general, a zeta potential of lower than -30 mV or higher than +30 mV represents appropriate conditions for inhibiting the occurrence of instability mechanisms such as coalescence and flocculation. Under such conditions, the oil droplets do not tend to aggregate with each other, due to the development of repulsive forces. This contributes to the emulsion stability [25].

3.3. Batter Specific Gravity and Consistency. Batter specific gravity is an indicator of its air holding capacity; however, it does not denote the size and homogeneity of the air bubbles [26]. The results demonstrated that the batter specific gravity lowered, compared with the control sample ($P < 0.05$), after the addition of the PE (Figure 1), because the PE raised the gas holding ability of the batter, and consequently, the batter was lightened and its specific gravity decreased. This result is in agreement with those previously presented by Sowmya et al. [19] who reported the inverse correlation between cake batter specific gravity and its ability to retain air bubbles.

Batter consistency is essential for the physical retention of air during mixing. Our findings showed that the incorporation of the PE into the batter significantly reduced its consistency, compared with the control sample (Figure 2). Furthermore, the direct correlation between the batter specific gravity and consistency implied that the presence of the PE in the batter formula made it more homogenous, smoother, softer, and lighter. The adsorption of the PE onto the air bubble walls brought about a rise in the extensibility of the bubbles, thus restraining their collapse. Low consistency leads to the expulsion of air bubbles from the batter surface, whereas high consistency results in the retention of the bubbles in the batter matrix [27]. Moreover, the higher the batter consistency, the smaller the amount of air introduced into it; hence, its specific gravity rises.

3.4. Batter Rheological Properties

3.4.1. Apparent Viscosity. Batter apparent viscosity is an influential factor in the cake final quality, especially in its volume. The results indicated that as shear rate increased, apparent viscosity decreased in both of the batter samples (Figure 3). This could be attributed to the probable breakdown of the batter structure such that the macromolecular chains in the batter would be coherent in the direction of the shear rate rather than being entangled. As a result, the batter shows non-Newtonian shear-thinning behavior [28].

Tsatsaragkou et al. [29] and Turabi et al. [30] accomplished similar results on gluten-free rice flour-based cake batter. The results show that the viscosity of a sample decreases with a rise in its water content. Addition of the emulsion to the batter caused it to become diluted, which could be the reason behind the viscosity reduction. In addition to apparent viscosity, the PE decreased the batter consistency. Accordingly, the low-viscosity batter could not retain the air bubbles during baking, which resulted in a decrease in the specific gravity and volume of the final product.

3.4.2. Flow Behavior. Flow behavior index shows how a food product behaves in stress and rate of shear conditions. The power-law model with a high R^2 was employed to determine the flow behavior of the batter samples. It was fitted to the

Table 2: Emulsion stability of PE stabilized with SPNs during 21 day at 4°C.

Samples	Creaming index (%)			Emulsion activity (%)	Emulsion stability index
	One weeks (%)	Two weeks (%)	Three week (%)		
PE	0	0	3.77 ± 0.516	78 ± 4.358	4877 ± 28.116

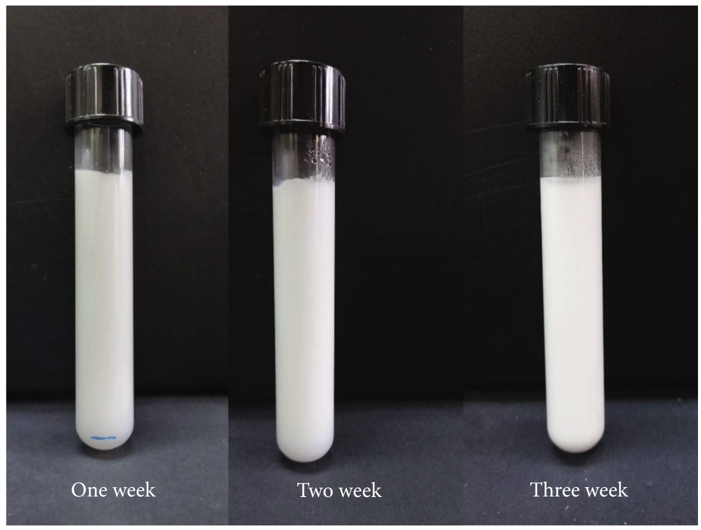

Figure 1: Visual images of the Pickering emulsions during the storage.

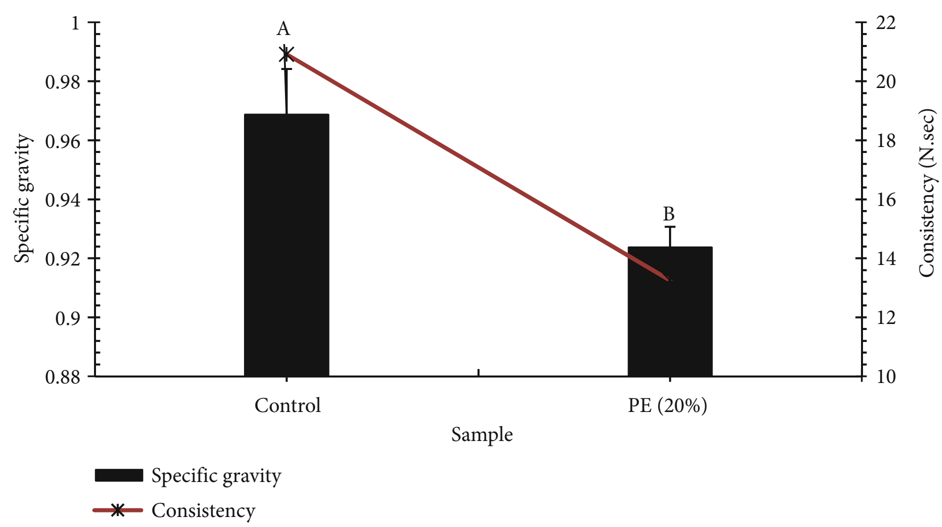

Figure 2: Evaluation of specific gravity and consistency of PE (20%) in comparison to the control sample.

shear rate-shear stress data (Table 3(a)). The n and k indices were, respectively, lower and higher for the PE-containing batter relative to the control sample. This demonstrated the shear-thinning behavior of the samples [31].

Baixauli et al. [32] applied the power-law model to investigate the effect of replacing wheat flour with resistant starch on muffin. Turabi et al. [30] used the same model to examine the effects of different gums and emulsifiers on rice flour-based cake, and Tsatsaragkou et al. [29] also employed this model to describe the flow behavior of batter in the production of gluten-free cake containing resistant starch. Murugkar et al. [33] used the Power law rheological model to study the effect of the incorporation of nanoencapsulated flaxseed oil on the flow behavior on the eggless cake batter and reported that the rheological properties of the batter were improved by adding nanoparticles. Yildiz et al. [34] also investigated the flow behavior properties of the soy protein-enriched cake batter and reported that the addition of soy proteins increased the consistency of the batter resulted from the formation of disulphide bonds.

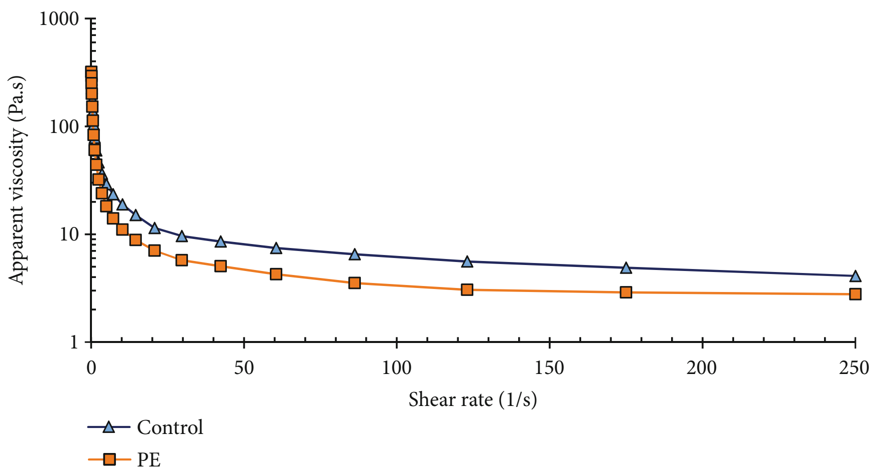

Figure 3: Apparent viscosity changes of cake batter samples by shear rate increasing.

Table 3: Power law model parameters for cake batter samples (a) and parameters of the power-law functions describing dependence of storage and loss moduli on angular frequency of cake batter samples (b).

(a)

Samples	R^2	n	K (pa.s^n)
Control	0.967	0.421	82.49
PE	0.932	0.346	64.908

(b)

Samples	R^2	$G' = k'.\omega^b$		R^2	$G'' = k''.\omega^d$	
		k'	b		k''	d
Control	0.77	522	0.152	0.79	251.6	0.243
PE	0.81	363.19	0.534	0.93	120.8	0.369

3.4.3. Oscillatory Test. Frequency sweep test was conducted within LVR (strain of 0.1%) in the frequency range of 0-100 Hz. The dependence of the batter dynamic rheological properties on frequency as a function of the PE effect is illustrated in Figure 4(a).

The results showed that G' was more than G'' in both the control and PE-containing samples. Hence, the solid-like behavior was dominant in the batter. The batter samples prepared using different flours differ in viscoelastic behavior. Depending on the flour type and frequency, the behavior type varies [35]. Renzetti et al. [36] declared that the batters prepared from wheat, rice, oat, and sorghum flours showed solid-like viscoelastic behavior. Our findings demonstrated that the G' of the PE-containing sample was lower than that of the control one, revealing the PE-containing sample showed more liquid-like behavior than the control. G' and G'' gradually increased as the frequency was elevated. In the control sample, both of the moduli were altered in the same direction with a rise in the frequency. Nevertheless, in the PE-containing sample, G' suddenly rose, while G'' decreased more than that of the control. Indeed, an interaction was developed in the batter structure, and a macromolecular network was created, which brought about an increase in the sample G'. Therefore, it can be suggested that the solid-like behavior of the control sample was associated with its consistency and apparent viscosity. Martínez-Cervera et al. [37] investigated the effect of replacing oil with the emulsion prepared using hydroxypropyl methylcellulose in muffin batter. The results of the oscillatory test denoted that the presence of the emulsion caused the batter G' to decrease. The reason behind the direct correlation between G'' and frequency can be interpreted as follows: when low frequencies are applied to a material, it has enough time to reconstruct the dissociated bonds in the frequency cycle. However, the material does not have sufficient time to do so at high frequencies. Consequently, G'' is raised as a result of the bonds dissociation, and the material shows the liquid-like behavior.

The dependence of G' and G'' on frequency can be explained by the power-law model (Table 3(b)). ω represents oscillatory frequency, and b and d stand for the power-law indices in G' and G'' [38]. Hence, G' and G'' do not vary with frequency at b and d values near zero, showing the high elasticity of the structure [39]. As summarized in Table 3(b), the control sample had lower b and d values than the PE-containing one, which indicated the higher elasticity of the control. Moreover, the higher k' and k'' values of the control sample confirmed this finding.

Complex viscosity denotes the resistance of the elastic or viscous flow to oscillatory movement. Complex viscosity denotes the elastic and viscous resistance of a structured material to flow once oscillatory motions are applied to it. If a structured material displays higher resistance to flow, its complex viscosity will be higher and vice versa [40]. Comparison between the complex viscosity values (Figure 4(b)) proved that as the frequency increased, the complex viscosity was reduced, representing the non-Newtonian behavior of the batter samples. The PE-containing sample had a lower complex viscosity than the

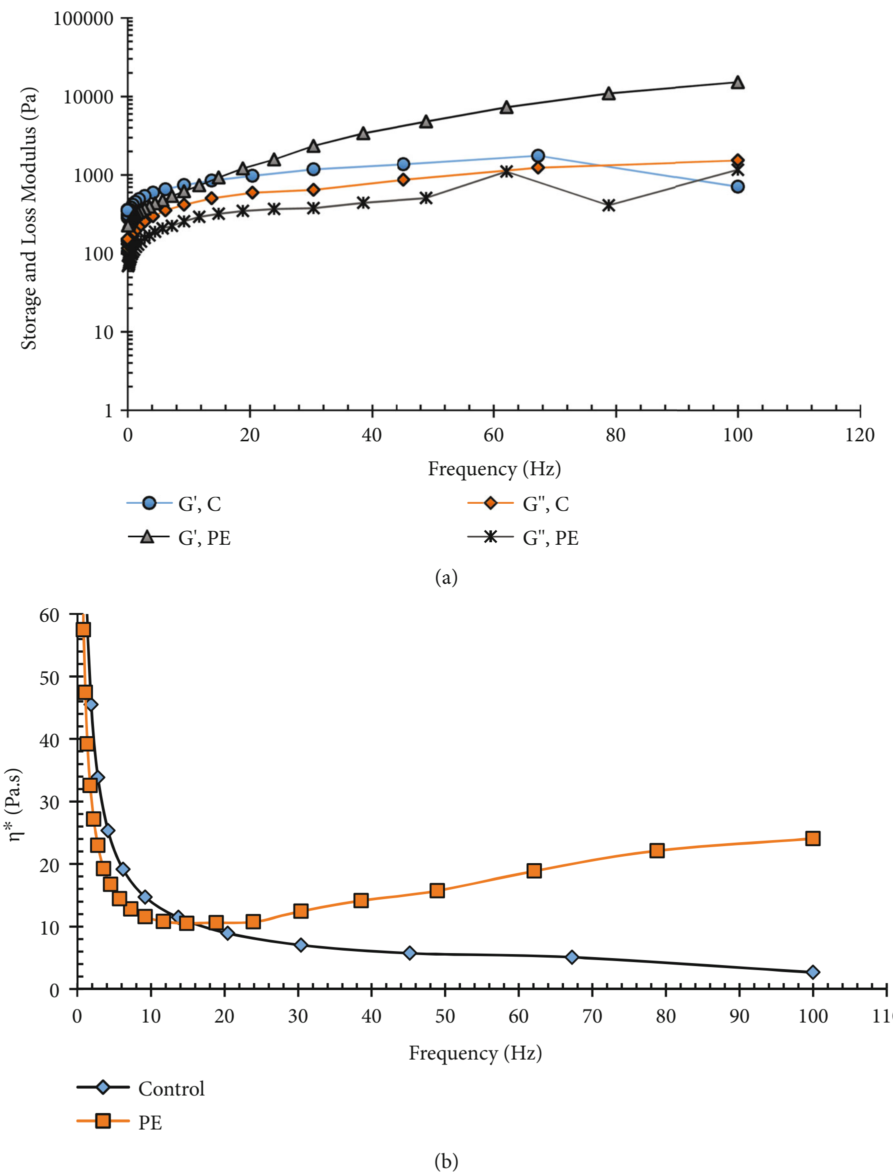

FIGURE 4: (a, b). Comparison of storage modulus and loss modulus of cake batter samples C (Control), PE (sample containing Pickering emulsion) (a) and comparison of complex viscosity of cake batter samples (b).

control one. Both G' and G'' are involved in the calculation of complex viscosity. Thus, it is obvious that the higher the values of G' and G'', the more the complex viscosity. The increase in the complex viscosity of the PE-containing sample at higher frequencies was due to the rise in its G'. Since the control sample had higher G' and G'' values than the PE-containing one, its complex viscosity was higher, too.

4. Conclusions

Based on the results of this research, SPNs improved the PE stability. Furthermore, xanthan gum inhibited the aggregation of the oil droplets by increasing the PE consistency and forming a network in the emulsion. The addition of the PE to the batter caused a reduction in its specific gravity, leading to volume increase and hardness decrease in the final product. The results of the rheological tests showed that the PE-containing sample was less viscous than the control and showed more liquid-like behavior based on the oscillatory tests. Eventually, although the batter viscosity was reduced after the addition of the PE, the presence of the emulsion could play a remarkable role in the distribution of air bubbles in the batter.

Data Availability

Our data is available on request through a data access committee, institutional review board, or the authors themselves.

Additional Points

Practical Applications. Gluten-free food products are important for individuals with celiac disease or gluten-related disorders since they need to follow a strict gluten-free diet. This study provides information on a Pickering emulsion stabilized by soy protein nanoparticles use to boost the physical and rheological properties of gluten-free cake batter. Results showed that use of these additives improved the techno-functional properties of the gluten-free cake batter. Therefore, the findings of this study can be useful for the production of functional free-gluten food products with health-promoting attributes.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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