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Research Article

Physical and Rheological Properties of Egg Albumin Foams Are Affected by Ionic Strength and Basil Seed Gum Supplementation

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In this study, the effect of ionic strength and basil seed gum (BSG) on the foaming properties of egg white albumin (EWA) was studied. The foam was prepared with 1% EWA (w/v) in the presence of different concentrations of sodium chloride (NaCl; 0, 0.5, and 1% w/v) and BSG (0, 0.1, and 0.3% w/v). The results showed that foam density and foam stability of EWA significantly (p < 0.05) increased with an increase in BSG concentration (from 0 to 0.3% w/v). On the contrary, an increase in NaCl concentration (from 0 to 1% w/v) increased foam overrun but decreased foam density. Amplitude rheological parameters indicated an improvement in foam structure with increasing NaCl concentration. In addition, an elastic structure was obtained in the case of the foams with the higher concentrations of NaCl. Elastic modulus (G') was higher than loss modulus (G'') in the frequency range, and there was low frequency dependency in all of the samples. In constant frequency of 1 Hz, tangent (δ) was the lowest in the sample containing 1% NaCl (w/v), but without BSG. There was a decrease in yield stress values with increasing BSG concentration; however, the increase in NaCl concentration led to an increase in yield stress. The highest yield stress (37 Pa) belonged to the sample containing 1% NaCl, but without BSG. Overall, it was found that both NaCl and BSG could substantially improve the rheological and foaming properties (in particular, foam stability) of egg white albumin.

1. Introduction

Foams are heterogenic systems, where gas is dispersed in a liquid phase [1]. The most common components for foam production are proteins, such as those present in egg. Foaming is considered as one of the most important properties of food proteins. Foaming properties of proteins depend on their intrinsic properties and extrinsic factors such as temperature, ionic strength, pH, and the interactions with food components [2]. Proteins and anionic polysaccharide mixtures have better surface-active properties compared to proteins alone, meaning that such mixtures can lead to formation of a more stable foam [2], mostly due to the increase in the viscosity of the corresponding solutions [2]. Many researchers have reported substantial improvement in

the foaming and the rheological properties of food proteins in the presence of some of polysaccharides [3–5]. One aspect of the effect of polysaccharides on foaming properties of proteins is by modification of gas bubble distribution in the liquid that changes gas volume fraction and foam density [1]. During the mixing of a polysaccharide with a protein, both polymers can interact with each other by means of hydrogen bonding and electrostatic and/or hydrophobic interactions [6]. Furthermore, the presence of polysaccharide can change the rheological properties of a solution because of its non-Newtonian rheological properties (e.g., viscoelasticity and thixotropy).

Egg white albumin (EWA) is the most important foam agent used in the food industry with a lot of recent research interests on its foamability in regard to the effect of different conditions and additives. Raikos et al. [7] studied the effect

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of sucrose and sodium chloride (NaCl) on foaming properties of egg white proteins at different ratios of sucrose to sodium chloride (12:0, 0:12, and 6:6 w/w). These researchers [7] found that the addition of sodium chloride to egg white protein solutions improved their foaming properties (e.g., foam overrun and foam stability), compared to the samples containing sucrose or the control sample (without sucrose or sodium chloride).

The foams made of EWA generally tend to destruct during the manufacture process and storage. Consequently, in order to improve the foam stability of EWA, different stabilizers such as polysaccharides have been used. For example, Miquelim et al. [6] studied the effect of guar, xanthan, and kappa-carrageenan on the foaming properties of EWA and reported that all of these polysaccharides improved foam stability of this protein. Hu et al [8]. reported that the addition of konjac glucomannan to EWA increased its foam stability.

Basil seed gum (BSG) is a novel polysaccharide that has recently been used in the food industry for its various functional roles such as surface activity, stabilizing, emulsifying, foaming, thickening, and gelling properties [9]. Rafe et al. [10] reported a fibril structure in BSG containing a globular structure same as that in scattered cotton. A shear thinning behavior, besides high consistency and yield stress, has also been reported for BSG [9, 11].

Although the properties of the foams made with proteins, with or without stabilizers, have already been extensively studied in the past, majority of these investigations have focused on the understanding of the mechanisms involved in the formation and stabilization of the foams so that the rheological properties of the foams have received less attention. One of the reasons for such a scarcity of the information around this field is the problematic process of using the conventional rheometers (e.g., cup and bob or parallel plate systems) for measuring the rheological properties, because of foam destruction and inevitable wall slip [12]. Barnes and Nguyen [13] proposed vane geometry as a solution to the wall-slip problem by placing the shear surface within the material. By using this technique, the yield surface of the material is well-defined during the measurement by the cylindrical surface carved out of the material by the vane located in the material itself, as opposed to lying along the surface of the bob. Additionally, the blades of the vane are quite thin and have very little cross-sectional area, so the vane can be lowered into the sample without much of the damage to the material (in this case, foam) structure [14]. Pernell et al. [14] used the vane geometry for determination of yield stress in the foams manufactured using white and whey protein isolate.

The effect of BSG on the physical properties (foam capacity and foam stability) of EWA has been previously studied [15], but there is no report on the use of BSG, as a novel, natural, and cost-effective stabilizer, for improvement of the rheological properties of EWA foams. In addition, as far as the authors are concerned, there is no study on the effect of ionic strength on the foamability and rheological properties of EWA, especially in the presence of another hydrocolloid (i.e., BSG). Hence, the aim of the present study was to investigate the effect of NaCl and the

novel polysaccharide BSG alone and/or in combination, on the physical (e.g., overrun, density, and stability) and rheological (e.g., amplitude sweep, frequency sweep, and yield stress) properties of EWA foams. Because in many of the food products, proteins and polysaccharides are present together, where ionic strength is one of the most important parameters that can influence the electrostatic interactions between proteins and polysaccharides. Correspondingly, such interactions can affect foamability, foam stability, and the rheological parameters of EWA. This is of a special interest for both researchers and food manufacturers, as the structure of the foam-based food products substantially depends on salts and stabilizers, as two of the most important parameters in such systems. It is hypothesized that the solubility, unfolding, viscosity, and aggregation of EWA, which have an effect on its foaming properties, can be altered by the addition of salt (NaCl) and/or polysaccharides such as BSG. Therefore, understanding the effect of both NaCl and BSG on different properties of EWA can guide food manufacturers to a better production of the foam-based food products with some desirable foaming properties.

2. Materials and Methods

2.1. Materials. Basil (Ocimum bacilicum L.) seeds were purchased from a local market in Neka, Iran, and the gum was extracted according to the method reported by Hosseini-Parvar et al. [11] at optimum conditions (i.e., 1 g of the seed in 65 mL water at pH = 8.0 and temperature of 68°C, stirring for 20 min at 300 rpm). Egg white albumin powder (analytical grade ovalbumin, >80% purity, CS: 35021190) was purchased from Applichem (Darmstadt, Germany). Sodium chloride was purchased from Pronalys Chemicals (Auckland, New Zealand).

2.2. Manufacture of Egg Albumin Solutions and the Corresponding Foams. A stock solution of albumin was prepared at a constant concentration of 2% (w/v) with stirring (300 rpm) the powder in distilled water for 2 h (25°C). A stock solution of BSG was also prepared at a concentration of 1% (w/v) by dispersing the gum in distilled water and stirring for 2h at room temperature and then stirring overnight at 4°C to complete the hydration. The stock solutions of EWA and BSG were then mixed to obtain a mixture solution with the protein content of 1% (w/v) and BSG concentration of 0.1 to 0.3% (w/v). To investigate the effect of ionic strength on the physical and rheological properties of foams, EWA-BSG mixture solutions were prepared in the presence of 0.5 (85.6 mM) and 1% NaCl (171 mM). To manufacture the fresh foams, different solutions were whipped in a glass container (diameter of 7 cm and height of 9 cm) with an electric kitchen mixer (Black & Decker, 250 W, maximum rpm) for 180 seconds.

2.3. Physical Properties of the Manufactured Foams

2.3.1. Foam Overrun. Foam overrun was determined according to the method from Hu et al. [8] with some

modification. 30 cc of each sample (solution) was used for the production of the foam, and the fresh foam was transferred to a graduated cylinder. The foam overrun was calculated using the following formula:

Foam Overrun =
$$\frac{V_{\rm F} - V_0}{V_0} \times 100, \tag{1}$$

where V_F is the foam volume reached at the end of the whipping process and V_0 is the initial volume of the sample.

2.3.2. Foam Density. Foam density was calculated according to a previous published method [16]. The fresh foam was transferred to a cylindrical container, and the foam density was calculated based on

Density
$$(g/cm^3) = \frac{m}{v} \times 100,$$
 (2)

where m and v are weight (g) and volume (cm³) of the foam, respectively.

- 2.3.3. Foam Drainage. The stability of the foams (drainage) was evaluated in terms of the volume fraction of the drained liquid after 30 min, according to the method from Kampf et al. [17] with some modification. The fresh foam was transferred into a gradual cylinder, and liquid drainage was recorded during 30 min of the storage.
- 2.4. Rheological Properties of the Manufactured Foams. The rheological properties of the foams were measured with an Anton Paar Physica rheometer (Physica, MCR 301, Anton Paar GmbH, Germany) equipped with a vane geometry (height of 3.8 cm and diameter of 1.9 cm), in four different parts as described in the following.
- 2.4.1. Amplitude Sweep Measurements. The strain sweep test was done in the stain range of 0.01–100% at 20°C and frequency of 1 Hz. The G' (storage modulus), G'' (loss modulus) plotted versus strain, $\tan{(\delta)}$ in linear viscoelastic region (LVE), and stress at the critical strain (τ_y) were determined correspondingly [18].
- 2.4.2. Frequency Sweep Measurements. The frequency sweep test was done in the frequency range of $0.1-100\,\mathrm{Hz}$, in the LVE range and at 20°C. G' and G'' were plotted versus frequency. The tan (δ) was determined at a constant frequency of 1 Hz, according to our previous method [19].
- 2.4.3. Yield Stress Measurements. The yield stress was determined using the steady shear test. The amount of stress rose up continuously from 0.1 to 100 Pa at 20°C. The amount of shear stress at the highest amount of viscosity, after which the viscosity decreased gradually, was considered as yield stress [20].
- 2.5. Data Analysis. The experiments were carried out in triplicates, and the statistical analyses were conducted using SPSS software (Version 16, IBM, Armonk, NY, USA).

Duncan test was performed to determine the significant difference between the samples at the 5% probability level (p < 0.05). The rheological data were analyzed using RheoPlus software (Version 3.4, Ostfildern, Germany), and graphs were plotted using Excel 2010.

3. Results and Discussion

3.1. Physical Properties of the Manufactured Foams. Foam capacity and foam stability are the most important features of the foams. Foam capacity can be determined by the rise in foam volume, while foam stability can be measured by the rate of liquid drainage from the manufactured foam [21]. The data obtained for the physical properties of the foams are presented and discussed as follows.

3.1.1. Foam Capacity (Overrun and Density). The effect of NaCl and BSG concentration on foam overrun and foam density is shown in Figures 1(a) and 1(b), respectively. According to Figure 1(a), foam overrun was significantly higher (p < 0.05) in the case of the foam supplemented with 1% NaCl and without BSG. For the sample supplemented with 0.3% BSG and without NaCl, foam overrun was 190%, which is significantly (p < 0.05) lower than that for the other samples (p < 0.05). Foam density demonstrated a reverse trend with overrun and it was significantly (p < 0.05) higher in the sample supplemented with 0.3% BSG and without NaCl (0.296 g/cm³). The lowest foam density belonged to the sample containing 1% NaCl and without the presence of BSG (0.193 g/cm³).

The results showed that while NaCl promoted foam overrun, BSG caused a decrease in this property. For example, in the case of the samples with constant amount of 1% NaCl and variable amounts of BSG (i.e., 0, 0.1, and 0.3%), foam overrun ranged from 370% (0% BSG) to 225% (0.3% BSG), whereas it was 271%, 295%, and 322% for the samples with constant amount of 0.1% BSG and containing 0%, 0.5%, and 1% NaCl, respectively (Figure 1(a)).

Figure 1(b) shows that foam density of the samples with 1% NaCl and containing 0%, 0.1%, and 0.3% BSG was 0.193, 0.208, and 0.272 g/cm³, respectively, while in the case of the samples with constant amount of BSG (0.1%) and containing 0, 0.5, and 1% NaCl, it was 0.242, 0.226, and 0.208 g/cm³, respectively. The increase in foam density with increase in BSG concentration is due to the water-bonding and thickening properties of BSG, which makes the foam heavier. Water binding can result in technological quality of the fresh foam, e.g., ease of manufacture and pumping, as well as the resistance to destruction [22].

In order to form a foam, proteins need to be water-soluble and have adsorption ability at the air-water interface [5, 21]. In this regard, the presence of NaCl can influence upon the protein solubility and adsorption at the air-water interface [5]. Since foamability is linked with protein solubility, in the case of the current experiment, overrun of the albumin foams increased with increasing the concentration of NaCl. NaCl can have an effect on the charged protein molecules as it improves the adsorption of the protein at the air-water interface with reduction of the electrostatic repulsion between

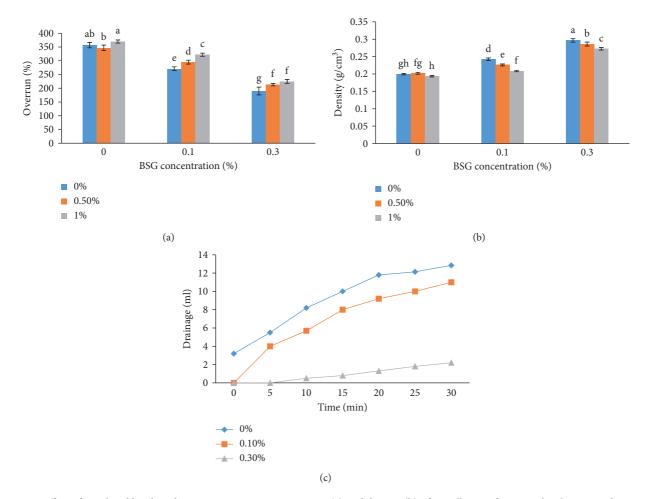


FIGURE 1: Effect of NaCl and basil seed gum concentration on overrun (a) and density (b) of egg albumin foams in the absence and presence of basil seed gum, as well as the effect of basil seed gum on foam stability in the absence of NaCl (c). Columns with different letters are significantly (p < 0.05) different.

nonadsorbed and adsorbed protein molecules [5]. Davis et al. [12] and Ercelebi and Ibanoğlu [5] similarly reported that the addition of NaCl significantly improved the adsorption of whey protein isolate at the air-water interface. Raikos et al. [7] also stated that the addition of NaCl increased foam overrun of EWA, due to facilitation of the protein adsorption at airwater interfaces. Davis et al. [12] reported similar results with respect to the effect of NaCl on whey protein isolate adsorption at the air-water interface. Interestingly, in the case of the current study and in the presence of BSG, the addition of NaCl represented the same effect on foam overrun of EWA as in the absence of BSG (Figure 1(a)). Increase in NaCl concentration increased foam overrun and decreased foam density. On the contrary, the addition of BSG and increasing its concentration significantly (p < 0.05) decreased foam overrun. Decrease in foam overrun with increase in BSG concentration can be attributed to the increase in the viscosity of the solution that prevents air incorporation. A similar behavior has been reported for whey protein isolate and in the presence of pectin/guar gum [5]. Protein-polysaccharide interactions can improve the stabilization of the interfacial layer, which can result in a more stable foam against bubble coalescence [23].

3.1.2. Foam Stability. It was found that while an increase in BSG concentration improved foam stability, NaCl had no significant effect on this parameter. Figure 1(c) shows the effect of BSG concentration on foam stability in the absence of NaCl. Increase in BSG concentration increased aqueous phase viscosity, which may have influenced the thickness and viscoelastic behavior of the adsorbed macromolecular layer that led to a decrease in the thinning rate of lamella and improvement in foam stability [8]. In the case of higher concentrations of BSG, an insoluble complex may form, which in turns can facilitate the development of a viscoelastic interfacial network at the air-water interface [16].

3.2. Amplitude Sweep Measurements. Strain sweep measurements were made over a strain range of 0.01-100% at 20°C and a constant frequency of 1 Hz to determine the linear viscoelastic region (LVE). In order to better understand the dynamic rheological properties, loss tangent in LVE region (tan $(\delta)_{\text{LVE}}$) and shear stress at the end of LVE region (τ_y) were determined. According to these results, two regions were obtained with increasing the strain: LVE region and nonlinear region. In the LVE region, G' and G'' were

constant, while in the nonlinear region both G' and G''decreased with increasing strain (data not shown). Tan (δ) and τ_{ν} were extracted from amplitude sweep data. Tan $(\delta)_{\text{LVE}}$ is G' to G'' ratio in the LVE region, and this parameter can be an indicator of the physical properties of the manufactured foams [16]. The effect of BSG and NaCl concentration on tan $(\delta)_{LVE}$ is shown in Figure 2(a). Accordingly, the sample without BSG and NaCl (control foam) showed the highest $\tan (\delta)_{LVE}$ (0.241). Increase in NaCl and BSG concentration caused a decrease in tan $(\delta)_{LVE}$ with NaCl showing a greater effect. The structure of the samples with lower amount of tan $(\delta)_{\text{LVE}}$ was stronger compared to the other samples (Figure 2(a)). The higher storage modulus can be due to forming a network across the lamella that causes high moduli [24]. τ_{ν} is stress in the end of LVE region that the nonlinear region starts after this stress [25]. On the contrary, the weakening structure of the samples will start when stress value is more than τ_y . τ_y value was significantly lower (p < 0.05) in the samples with both BSG and NaCl (0.281 Pa), compared to the control, and it increased with increasing NaCl concentration. The highest amount of τ_{ν} belonged to the samples containing 0.3% BSG (Figure 2(b)). τ_{ν} value increased with increasing BSG concentration.

According to the rheological parameters obtained in the amplitude sweep test, an increase in the concentration of either NaCl or BSG improved the structure of the manufactured foams. The function of salt in this case is that it can bind to the charged groups of proteins, causing a change in electrostatic interactions between biopolymers [5]. Decrease in the electrostatic repulsion can improve the foam structure. BSG can possibly form some flexible film around the foam bubbles and improve the foam structure [2]. BSG has surfaceactive properties that reduce surface tension and form a film at the surface of the gas bubbles [26]. This polysaccharide can substantially stimulate the viscoelastic properties and thickness of the adsorbed macromolecular layer (lower tan δ), which can result in decreasing the thinning rate of lamella and improving the foam stability (higher τ_{ν}) [8]. In the presence of sodium chloride, especially in the higher concentrations, most of the water molecules can bind to salt ions while reorganization of water molecules can occur around the protein (i.e., EWA). This might result in stronger surface hydrophobic protein-protein interactions compared to the protein-water interactions. The ions in salt can decrease the electrostatic interactions between the protein (EWA) and the polysaccharide (BSG), because they can interact with oppositely charged groups on the EWA to form a double layer that in turn leads to an increase in protein solubility with salting in [27]. This behavior can improve the rheological properties of the foams due to formation of the foams with solid behavior (lower tan δ) and strong structure (higher τ_{ν}).

3.3. Frequency Sweep Measurements. In the present study, small-amplitude oscillatory techniques were used in the case of the foams containing different amounts of BSG and NaCl in the LVE region (obtained from amplitude sweep test). To evaluate the effect of NaCl and BSG concentration on the rheological properties of EWA foams, dynamic measurements

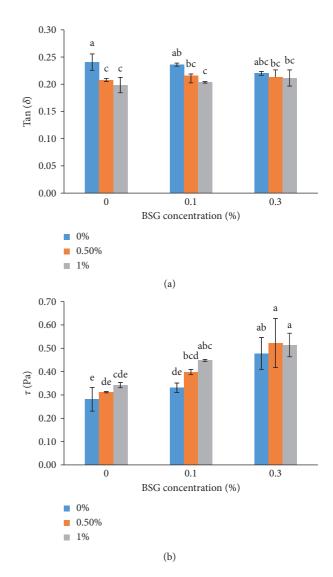


FIGURE 2: Effect of NaCl and basil seed gum on $\tan{(\delta)_{LVE}}$ (a) and τ_y (b) of the foams made of egg albumin. Columns with different letters are significantly (p < 0.05) different.

were performed over a frequency range of $0.1-100\,\mathrm{Hz}$; the mechanical spectra of samples are shown in Figures 3(a)-3(c). These findings demonstrated that G' dominated over the G'' in all of the frequency sweep range, representing a solid-like behavior in all of the samples [28]. As can be seen in Figure 3, in every case, G' and G'' values increased with a low slope as frequency increased, indicating a low frequency dependency in all of the foam samples. A low frequency dependency is characteristic of elastic materials [18]. These results indicate that frequency does not have a significance difference on the structure of the foams. There was no crossover point at the frequency sweep test, which indicates the solid-like behavior of the manufactured foams [29].

The effect of NaCl and BSG concentration of $\tan (\delta)$ in constant frequency of 1 Hz is shown in Figure 4. Tan (δ) is an important parameter that can describe the structure of the samples. When $\tan (\delta)$ is more than 1, the structure is viscous and G'' value is greater than G' [16]. The lowest amount of \tan

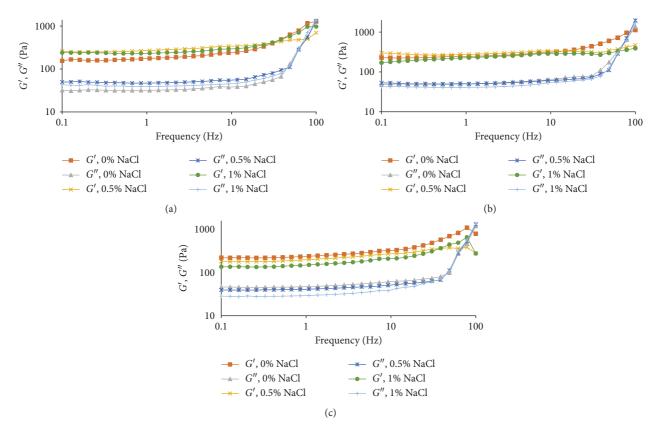


FIGURE 3: Effect of NaCl and basil seed gum concentration on storage and loss moduli (in frequency sweep test) of egg albumin foams supplemented with 0 (a), 0.1 (b), and 0.3% (c) basil seed gum.

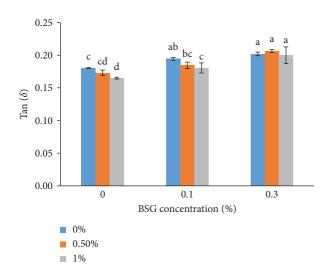


FIGURE 4: Effect of NaCl and basil seed gum concentration on tan (δ) in constant frequency of 1 Hz in the foams made of egg albumin. Columns with different letters are significantly (p < 0.05) different.

 (δ) (i.e., 0.165) belonged to the sample containing %1 NaCl, with no BSG, while the samples with 0.3% BSG showed the highest tan (δ) . According to the results presented in Figure 4, an increase in NaCl concentration decreased tan (δ) , while increasing BSG concentration increased tan (δ) . This means that the foam samples containing higher value of NaCl presented a more elastic characteristic versus the samples

containing higher value of BSG with a viscose behavior. Such behaviors can be explained with foam overrun; i.e., the lower overrun is a consequence of the reduced bubble formation, which has an effect on the rheological properties of the manufactured foams. The lower air bubble formation resulted in a more viscose behavior, higher tan (δ) , and lower solid properties [16]. Generally, protein-protein interactions and protein coagulation can be improved by the addition of salt, and thus, it leads to the interactions of the adsorbed protein at interfaces, and consequently, the increase in foamability [30]. Increase in air formation in the presence of NaCl may result in a lower tan (δ) , higher elastic behavior, and higher solid properties [16]. Increase in BSG concentration, on the contrary, can cause the decrease in foam formation and air fraction, which may lead to the viscose behavior of the manufactured foams.

3.4. Yield Stress Measurements. It was important to study the yield stress of the foams acquired from EWA containing different concentration of BSG and NaCl, because yield stress is one of the most important rheological properties of protein foams. Foams can endure small value of stress, but at the higher amount of stress, they will become flow-like fluids [31]. The structure of a foam, in general, has an important effect on the transition of solid-like behavior to fluid-like behavior. Yield stress can be described as a stress below flow observation [31].

The amount of yield stress in the foams manufactured in the presence of different concentrations of BSG and NaCl is

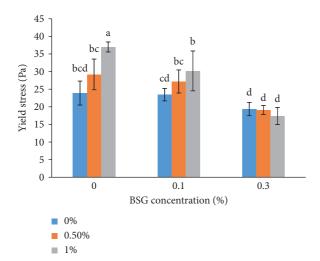


FIGURE 5: Effect of NaCl and basil seed gum concentration on yield stress in the foams made of egg albumin in the absence and presence of basil seed gum. Columns with different letters are significantly (p < 0.05) different.

shown in Figure 5. The foams supplemented with 1% NaCl, without BSG, showed the highest yield stress (37 Pa), while the samples containing 0.3% BSG showed the lowest amount of yield stress. Increase in NaCl concentration increased yield stress, while increasing BSG concentration decreased the yield stress. Davis et al. [12] reported that yield stress of the foams made with whey protein isolate significantly increased with the addition of NaCl (pH 7.0). Luck et al. [32] too reported that the addition of NaCl and CaCl₂ increased yield stress in the whey protein isolate foams. These researchers reported that when ionic strength was more than 100 mM, the diffuse double layer of ions surrounding colloids was so small as to not provide any stabilization against aggregation. This can decrease the electrostatic barrier of protein adsorption at the interface.

4. Conclusion

In the current study, the effect of ionic strength and BSG concentration on the physical and the rheological properties of EWA foams was investigated. The results showed that increasing ionic strength (by addition of sodium chloride) significantly (p < 0.05) improved foaming capacity of EWA. With an increase in the concentration of NaCl, foam overrun increased while foam density decreased. The increase in BSG concentration increased both foam density and foam stability, whereas it decreased foam overrun. Amplitude test results showed that G' was dominated over G'' in all of the strain ranges. Stress at the end of the LVE region (τ_v) increased with increasing concentration of both NaCl and BSG. The results of frequency sweep confirmed the findings from amplitude sweep, and thus, an elastic structure for all of the samples is confirmed. Yield stress increased with increasing ionic strength while an increase in BSG concentration decreased this parameter. Therefore, according to the results of this study, the addition of both NaCl and BSG is suggested for the improvement of the foaming properties

(especially, foam stability) and the rheological properties of EWA. However, in regard to the effect of BSG, understanding the optimum concentration is crucial, as at the high concentrations tested in the current experiment, this hydrocolloid increases the viscosity, which can negatively affect some parameters of the manufactured foams (e.g., overrun). The findings of this study also confirm that the supplementation of a EWA solution with sodium chloride alone is a simple and cost-effective way for improving the foaming properties of such a solution, due to the effect of salt on the physical and rheological properties of the manufactured foam.

Globular proteins such as egg white albumin are the main component for foam production. Foaming properties of EWA are dependent on solubility, unfolding, viscosity, and aggregation of proteins, and all of these can be altered by the addition of different concentrations of salt and polysaccharides such as BSG. The foam-based food products such as nougat, meringues, bravadoes, whipped cream, and chocolate mousses are popular nourishments, especially in Western countries. Therefore, understanding the properties of the foams made of egg albumin is an interesting topic for both researchers and food manufacturers.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. Increase in NaCl concentration increased foam overrun and decreased foam density. BSG increased foam stability and foam density while it decreased foam overrun. Amplitude sweep test indicated that sodium chloride improved foam structure. Storage and loss moduli increased with a low slope with increasing frequency. Yield stress value increased due to NaCl addition, but decreased with BSG supplementation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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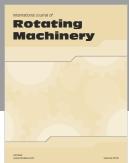
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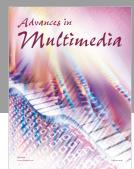




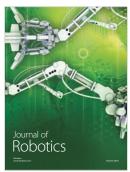














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