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On the physicochemical properties and foaming characteristics of proteins in cement environment

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ABSTRACT

This paper investigates the physicochemical properties of proteins and their foaming characteristics in a synthetic pore solution (SPS) of cementitious materials to aid in understanding their air-entraining performance. A total of 13 proteins with different molecular structures and physicochemical properties were studied. Hydrodynamic size, charge, viscosity, surface tension, and foam capacity and stability of the proteins at different concentrations were characterized. Optical microscopy was employed to study the foam bubble size and structure. It was shown that proteins gained an increased negative charge and their hydrodynamic size generally decreased at high pH relevant to cementitious materials. The effect of SPS on surface tension of the proteins did not show a specific trend. The proteins demonstrated improved foaming in SPS compared to in deionized water (DI) due to the change in their physicochemical properties. The bubble size distributions of the foams were analyzed. Optical microscopy showed increased roughness and thickness of the bubble film in the foam in SPS, indicating enhanced stability compared to the foam in DI. The effect of surface tension of the proteins on their foaming behavior was shown to be less in SPS than in DI.

1. Introduction

The use of air-entraining admixtures has made a valuable improvement in the freeze–thaw resistance and durability of concrete structures [1,2]. The capillary pores can be filled with water and freeze during the freeze–thaw cycles in harsh climates. During the freezing phase, the water in the capillary pores expands by approximately 9 % in volume and this can result in cracking in the microstructure and compromising the material integrity of concrete [1,3]. If there are macro voids nearby, the excess water is pushed out by the ice crystals in the capillary pores into the macro voids, and as a result, the damage to the microstructure will be minimal [4]. Otherwise, the hydraulic pressure in the water inside the capillary pores increases as a result of ice formation and can severely crush the microstructure of the concrete [1]. Parameters such as porosity, void size and their distribution in concrete are influential in the freeze–thaw performance [4] and there is a momentum towards controlling these properties in the concrete components through admixtures. In addition to the improved freeze–thaw performance, air entrainment has many other benefits to the concrete structures. These include improved workability and lower bleeding, leading to less heterogeneity in the material [2,5,6]. Since air entrainment can potentially increase the workability of concrete, the water/cement ratio (W/C) can be slightly lowered to restore the strength loss due to the air voids [4]. However, some studies have shown the opposite effect, where a reduction in flowability occurs in higher air content due to particle bridging of ionic bubbles [7].

Surfactants (surface-active agents) are chemical compounds that reduce surface tension at air-liquid interfaces and improve the conditions for bubble formation and stability, and as a result, are used as air-entraining admixtures in concrete. The molecular structure of surfactants consists of a nonpolar hydrophobic tail and a polar hydrophilic head, which helps them adhere to different chemical particles [8,9]. Surfactants adsorb onto the bubble film – air-liquid interface – in the solution, position themselves in such a way that the hydrophobic tails are away from water pointing into the air inside the bubble and the hydrophilic heads are attached to the interface. This reduces the surface tension of the interface, allowing bubbles to form, or existing bubbles to become more stable [8,10]. Most conventional surfactants are classified based on the composition of their hydrophilic and hydrophobic sections, called the head and the tail, respectively. Surfactants can be anionic, cationic, nonionic or zwitterionic based on the charge of their hydrophilic heads and this is influential on the stability of foams [8]. Anionic

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surfactants usually provide the highest degree of stability for the bubbles in cementitious materials [11]. Therefore, surfactant properties like surface tension and charge are attributed to the physiochemical process of bubble formation [1,8].

Biomolecules in the form of animal and plant products have been used for centuries in mortars [12]. Egyptians and Greeks utilized natural animal and plant products in different mortars of clay, lime and primitive cement to improve their performance [12]. In recent decades, the effect of protein-based agents in cementitious materials has been investigated [13–20]. The molecular structure of proteins consists of a large number of amino acids with varied functional groups including charged, hydrophobic, and hydrogen-bond forming groups [20]. The presence of hydrophobic functional groups in the molecular structure of proteins allows them to act as surfactants [21].

Parameters that influence protein behavior in a liquid solution include temperature, ionic strength, and pH [22]. pH specifically has a significant impact on proteins compared to synthetic surfactants, as it can cause chain breakage in large proteins in the high alkaline environment, leaving smaller hydrophobic chains segments that increase air content of the solution through lowering surface tension and establishing more hydrogen bonds [14]. Globular proteins can unfold, exposing their hydrophobic and hydrophilic residues that are hidden in the interior of the proteins; as a result, certain proteins will have an increased surface hydrophobicity and potentially increased tendency to adsorb onto the air-liquid interfaces [23].

There have been a few prior studies related to the use of proteins as foaming agents in cementitious materials; however, in most of these cases, insufficient attention was directed at the characteristics of the proteins and their molecular structures [15–18]. Panesar [14] used a protein-based foaming agent, labelled CF 200 alongside two other synthetic foaming agents, to manufacture cellular concrete specimens. The three foaming agents were shown to result in similar properties in hardened concrete, but the protein-based agent created smaller isolated air voids in the microstructure compared to the other agents studied in that paper. Chandra and Aavik [19] were one of the first researchers who directly studied the influence of three protein admixtures, namely gluten from flour, milk casein and an oil-based Purina 500 E, on the properties of cement mortar. Water to cement ratio (W/C) was shown to influence the effectiveness of the proteins in air entraining, where a minimum amount was observed at W/C = 0.45 for almost all proteins. The authors mentioned three main changes in protein molecular structures when it is exposed to cement paste [19,24]; these are the denaturation and unfolding of the secondary structure, hydrolysis of the protein, and the formation of salts with metal ions. Chandni and Anand [25] incorporated an unidentified vegetable oil protein as a foaming agent in concrete containing recycled waste and studied how different amounts of this foaming agent affected void area and void distribution. Studies on blood-based proteins including hemoglobin [26–32], milk proteins including whey and casein [24,33–37] and proteins extracted from plants and oils [38–40] can be found in the literature.

However, a systematic study focused on characterizing the physicochemical properties of proteins with different molecular structures and investigating their foaming behavior in a cementitious environment is currently not available in the literature. To address this knowledge gap, in this study, the physiochemical properties of 13 proteins with different molecular structures in deionized water (DI) and in a synthetic pore solution (SPS) simulating the chemistry of cementitious environment were evaluated. The physiochemical characteristics including surface tension, charge, hydrodynamic size, and viscosity, as well as their foaming characteristics were systematically studied. The aim is to decouple and understand the complex interactions that govern the air-entraining properties of proteins in solutions similar to that of the cement pore solution.

2. Experiments

2.1. Materials

2.1.1. Proteins

The proteins studied in this investigation were obtained from various commercial vendors and used as received. These proteins are listed in Table 1. Lysozyme and ovalbumin are globular proteins obtained from egg white [41,42]. NFMP is prepared by extracting moisture from non-fat milk [43] and is primarily composed of two main protein families of whey and casein [44]. Collagen peptide is one of the most notable proteins found in mammals, constituting around 25–30 % of the entire protein composition of the body [45]. Hemoglobin and immunoglobulins, two of the primary proteins found in the body, possess distinct surface activity and as such have been of interest to researchers [46,47]. Since a large amount of these proteins were needed in the experiments and in practical use with cementitious materials, obtaining very pure proteins at the scale needed was cost prohibitive and the proteins were obtained in bulk with expected impurities.

2.1.2. Synthetic pore solution

The proteins were tested in two solutions including deionized water (DI) and synthetic pore solution (SPS). SPS was prepared with 0.1062 M KOH, 0.0489 M Na_2SO_4, 0.037 M K_2SO_4 and 0.0212 M $Ca(OH)_2$, following the composition provided in [48]. SPS mixture was agitated and then centrifuged for 5 min at 4400 rpm to separate the undissolved particles from the mixture, leaving only dissolved ions in the solution. The final pH of the solution was measured to be about 13.6. The protein powders were weighed and dissolved in DI and SPS at room temperature before being used in the physiochemical tests. All physiochemical and foam experiments were performed in SPS, except for zeta potential and hydrodynamic size tests, which were performed in a high pH solution prepared by dissolving NaOH in DI until a pH of about 13 was achieved. The reason for this was to investigate the effect of high pH on zeta potential and hydrodynamic size of the proteins without interference from ions.

2.2. Methods

2.2.1. Fourier transform infrared spectroscopy (FTIR) of proteins

FTIR was performed using a PerkinElmer Frontier spectrometer with a Universal Attenuated Total Reflectance (UATR) accessory, to determine the structure and protein bond configuration in DI and SPS. For the FTIR analysis, 12 % concentration of protein by mass of DI and SPS was prepared. The protein solutions were mixed using a stirring rod for 30 min to ensure total dissolution of protein particles prior to testing. After thorough mixing, the sample was loaded into the FTIR device and scanned 3 to 5 times, based on the amount of variation observed. The data collection was done with a resolution of 4 cm^{-1} in the mid-range of 4000–600 cm^{-1}, since such a resolution achieves satisfactory signal to noise ratio for a liquid solution. The background noise was also collected and removed from the protein signal. The subtraction factor was adjusted to minimize similar peaks in both the sample and reference

Table 1
List of proteins and their abbreviations used in the study.

Name	Abbreviation	Name	Abbreviation
Sodium Caseinate	SC	Non-Fat Milk Powder	NFMP
Collagen Peptide	CP	Soy Protein Isolate	SPI
Whey Protein	WP	Mild Silver Protein	MSP
Sodium Immunoglobulin	Ig	Trypsin	Try
Lysozyme	Lys	Pepsin	Pep
Hemoglobin	Hem	Bovine Gelatin	BG
Egg White Ovalbumin	Alb		

spectra, using the peak heights in the 3500–3000 cm^{-1} region. Next, baseline correction was applied on the spectra to fix both the sloping shape of the spectra and the offset in absorbance.

2.2.2. Zeta potential and hydrodynamic size

Malvern Zetasizer Nano ZS (Malvern instruments Ltd., Malvern, U.K) was used to measure the net surface charge and hydrodynamic size of proteins in DI and in alkaline solution with a pH of 13 using Dynamic Light Scattering. SPS was not used as the mixing medium to prevent the effect of ions on protein surface charge and hydrodynamic size. The instrument had a size measurement range of 0.3 nm to 10 μm. Protein solutions with a concentration of 0.15 g/100 mL were prepared and equilibrated for 10 min prior to testing. The equilibrated sample was filled in a disposable folded capillary cell (DTS1070) and loaded into the instrument. Each sample was tested three times and in between each test, data was collected after 100 to 110 runs at a temperature of 22 °C.

2.2.3. Protein molecular structure characterization by polyacrylamide gel electrophoresis using sodium dodecyl sulfate (SDS-PAGE)

SDS-PAGE is a method of separating protein chains from each other based on their molecular weight between two opposite electrical poles. In this method, SDS is used to denature the proteins and break down the secondary and tertiary structures of the proteins and give them a negative charge [49]. The denatured protein solution is then added to the polyacrylamide gel and the separation of the protein constituents occurs as a result of their size-mediated movement subjected to an electrical potential difference. Since the gel has a meshed network, larger chains have a slower rate of movement than the smaller ones [49]. Different solvents were tried to achieve the best solubility for all proteins (some of which were hard to dissolve). The final chemical solvents include: β-mercaptoethanol for SC, 5 % ethanol for WP and NFMP, 1X phosphate buffered saline for Ig and Hem, 50 % glycerol for Alb and Try, dimethyl sulfoxide for Lys and 0.1 M acetic acid for Pep. The protein solutions were measured through Bradford assay which is a dye-based method to measure protein content [50] to ensure sufficient concentrations for the SDS-PAGE. For SDS-PAGE characterization, 5 μL of the protein solution was added to the same volume of a 10X tris/glycine/SDS buffer and loaded into a gradient 4–15 % precast polyacrylamide gel from Bio-Rad, capable of separating weights from 5 to 200 kDa. The test was performed at 22 °C at a voltage of 169 V, and the weight was determined using a reference protein marker. The gels were then stained for an hour using a Coomassie Brilliant Blue G-250 staining agent and were washed and destained repeatedly with a fixing solution of 30 % ethanol and 10 % acetone for a few days, until they became clear enough for observation. Since some proteins were not suitable for SDS-PAGE, only 9 proteins were used in this test.

2.2.4. Surface tension

The surface tension measurement was performed using a BZY 201 instrument with the platinum plate method. The equilibrium surface tension was recorded for both protein solutions in DI and in SPS, with 3–5 measurements to alleviate the unstable nature of some proteins in DI. Protein solutions at different concentrations of 0.625 %, 1.25 % and 2.5 % were tested.

2.2.5. Viscosity

The kinematic viscosity measurement of proteins at different concentrations of 0.625 %, 1.25 % and 2.5 % was carried out using the Cannon UBC-0B Ubbelohde glass viscometer. The viscous solution was allowed to freely flow down through a very thin tube in a controlled temperature under gravity, and the time it took for a certain amount of solution to flow was recorded in seconds. This time is then multiplied by a calibrated constant of the Ubbelohde glass viscometer to find the kinematic viscosity.

2.2.6. Foam properties

The ability of proteins to create foams and maintain them over time was evaluated through the foamability measurements. The foam capacity (or foamability) was evaluated by preparing 10 mL of DI or SPS with different protein concentrations of 0.625 %, 1.25 % and 2.5 % by mass of the respective solutions in a 50 mL graduated tube and then, shaken vigorously for 15 s. After that, the volume of protein foams was recorded at different times of 0, 1, 5, 10, 20, 30, 40, 50 and 60 min in three repetitions. The initial volume at 0 min is the foam capacity of the protein solution, while the ratio of 10-min foam volume to the initial foam volume represents the mid-range stability of the foams.

2.2.7. Bubble size characterization

To determine the foam bubble size distribution, optical microscopy images of the foams were taken using a Keyence VHX microscope at 200X magnification. The solutions were prepared and agitated similarly to the foam volume test, and then transferred into a petri dish to be imaged at different time intervals. Images of different parts of the foams were taken to obtain a statistical representation of the bubbles. The images were then analyzed with the ImageJ image processing software to find the average size, number, and distribution of bubbles in solutions for each specific protein.

3. Results and discussion

3.1. FTIR of proteins

Interpreting band position, intensity and width characteristics of an FTIR spectrum can give an insight into the protein molecular structures [51]. Most analyses are performed on the mid-infrared region which consists of 9 sub-regions: Amide I to VII, and amide A and B. Each specific property of the proteins resides in specific regions, for example, secondary structure of proteins is primarily present in amide I and II region [46]. For instance, in the amide I region α-helices are situated at around 1650 cm^{-1} and β-sheets at 1623–1641 cm^{-1} and 1674–1695 cm^{-1}. Secondary structure analyses of proteins have been performed extensively in the literature, on proteins such as human serum albumin, soy glycinin, whey, peanut, rice, Trypsin and others [52–55].

The change in the secondary structure as a result of pH has been studied by Usoltsev et al. [52]. As the human serum albumin (I) unfolds to some degree in the alkaline solution, the α-helix content decreases by 10 %, and the β-sheet content increases by 8 %. In general, for HSA the alkaline solution results in conversion of α-helix to β-sheets. This mechanism is unique to each protein and type of denaturant and may be completely different for another protein [52]. Similar results were observed by Qi et al. [56] for β-casein, in which an increase of pH from 6.75 to 10.5 resulted in the reduction of α-helix structures and increase of other structures such as β-structures.

The secondary structure of the 13 proteins studies in this investigation in both DI and SPS was analyzed and the compositions were determined through the second-derivative deconvolution of the amide I peak. The spectra for select proteins and the second-derivative peak fitting for NFMP in SPS are presented in Fig. 1a and 1b, respectively. As can be seen from the spectra, small shifts in the height and band center of the peaks in the amide I/II/III region caused by the pH increase changes the corresponding secondary structures present in the protein. Although Amide I has the strongest signal, it also suffers from the interference of O—H groups of the water which might make it harder to distinguish between the effect of the two. On the other hand, Amide III region does not have any O—H vibrations, but has a significantly weaker signal compared to the other regions [57]. The main mode of change from DI to SPS is the decreasing contribution of ordered structures like α-helices and β-sheets, and an increase in the turns and random coils of the secondary structure. Prior studies showed that the pH-dependent denaturation of most proteins reduces the composition of ordered strands and increases the composition of coils and random structures

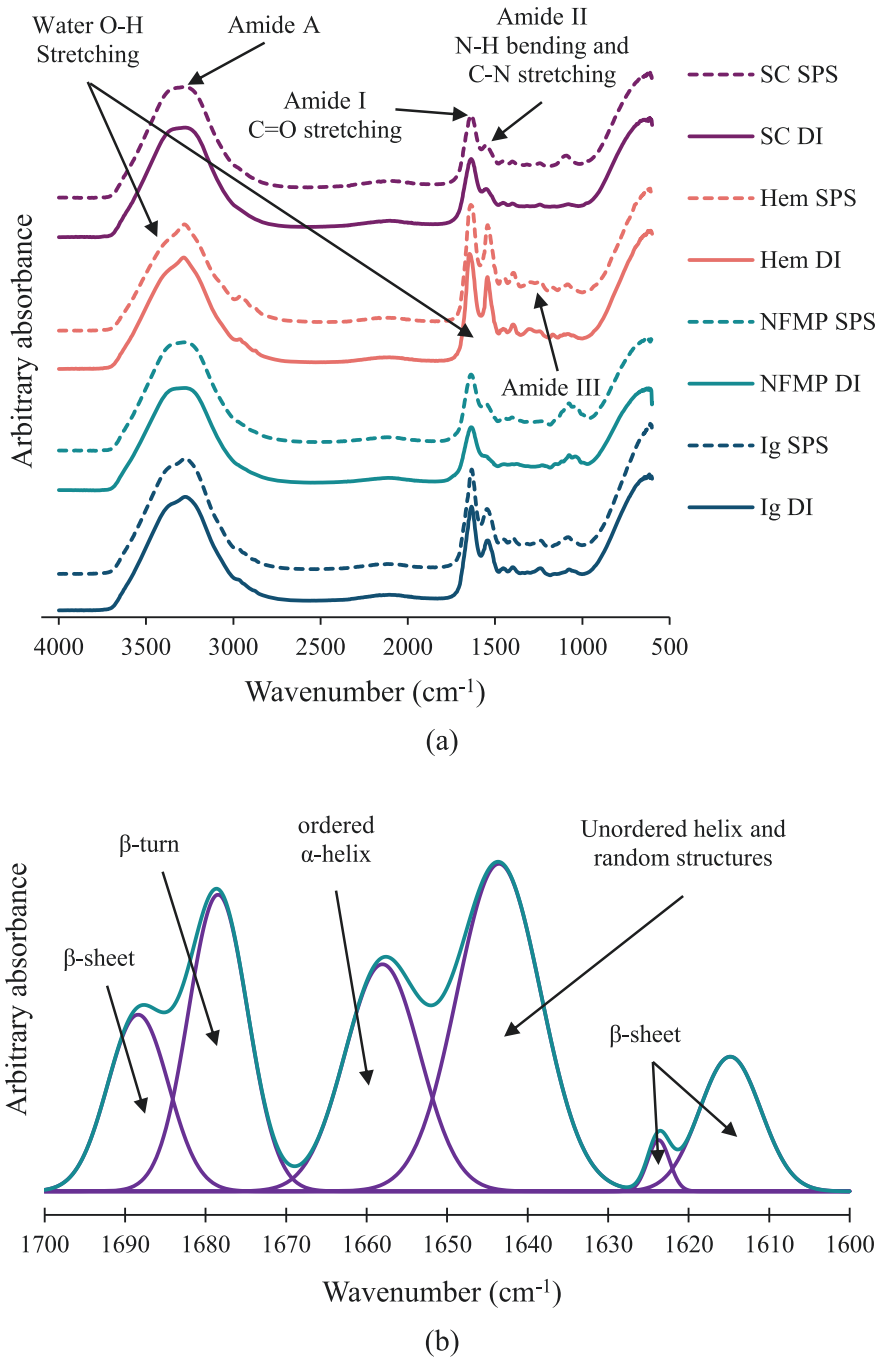


Fig. 1. (a) FTIR absorbance spectra of select proteins in DI and SPS and (b) second-derivative deconvolution and peak assignment of NFMP in SPS.

[56]. The modes of change in the amide I to III sub-regions from DI to SPS are also delineated in the absorbance spectrum of Fig. 1. For example, in SC, the peaks of amide I in 1500–1700 cm^{-1} do not show any significant alterations, which is consistent with its lower sensitivity to pH and is also reflected in the insignificant change in secondary structure. NFMP on the other hand, shows the development of a major peak from DI to SPS, coinciding with the peaks associated with β-turns and random coils, and as such increases the contribution of these structures. This protein conformation change is mostly attributed to the high pH of the SPS which results in the denaturation of protein secondary structures, but it can also be in part because of the presence of ions such as Ca^{2+} in the SPS. These conformational changes in the protein secondary structure occurs through interactions of Ca^{2+} with the C═O, COO^{-} and N—H groups of the protein, as shown in the study by Alhazmi [58], which can shift the band corresponding to α-helix to a higher frequency –in order of a few wavenumbers- and reduce α-helix content as much as 40 % [58].

3.2. Charge

The surface charge of the proteins is affected by the pH of their solutions. Generally, as the net difference between pH and isoelectric point (pI) of the proteins increases, so does their zeta potential. Although very dependent on proteins, pIs usually lie between 4 and 9, such as 5–7 for WP [59], 4.5 for Alb [60] and 4–5.5 for SPI [61]. Lys has a higher pI of 10.5 [62]. Therefore, when proteins are dissolved in a highly alkaline

solution above their pI, their negative surface charge increases.

Fig. 2 displays zeta potential in DI and in an alkaline solution with a pH around 13 but without the ions of SPS. Except for SC, all proteins showed a higher negative charge in the alkaline solution, compared to in DI. The increase in charge in proteins with increased pH is attributed to the deprotonation of functional groups of the amino acids in proteins [63]. In addition, at very high pH, proteins undergo molecular changes including unfolding and bond breakage, which could expose more functional groups previously hidden in the interior of proteins structure leading to higher negative charge. The increase in negative charge of the proteins enhances the repulsive force among the proteins leading to improved solubility of the proteins [64]. This is expected to aid in better adsorption of proteins onto the foam film, which can influence foaming characteristics of the proteins. The unchanged surface charge of SC could be because of its resistance to denaturation due to its already unstructured molecular structure [65]. Although it seems that this resistance to denaturation is also very dependent on the protein extraction and purification process, as SC could not resist denaturation in other studies [66]. This preparation method specifically affects gelatin type proteins like BG that undergo minimal change in surface charge, and in which the processing method can significantly affect the structural properties such as pI. That is, gelatins can have a pI of 5 with one processing method, and a pI of 9 with another [67,68]. It is possible that the BG protein used in this study is of the latter processing method and not very sensitive to the high alkaline pH.

3.3. Hydrodynamic size and protein chain weight

Protein size can affect foaming stability of the solution through differences in adsorption to the foam bubble interface film, since it is harder for larger protein particles to adsorb to the thin film interface of bubbles [69]. Some degrees of dissociation happen in DI depending on the protein type, but DI is not generally very effective in breaking the bonds between coagulated proteins [70]. High pH of 13, on the other hand, promotes unfolding and breakage of the molecular bonds in proteins [71]. The increase in negative charge and breakage of bonds provide the necessary driving force for the dissociation of these coagulated particles into smaller subunits [36,70]. It is seen from Fig. 3a that an increase in pH reduced the hydrodynamic size of the protein, especially those that showed large aggregation in DI. Unlike these groups of proteins, those such as CP, Lys, Hem, Alb and BG that showed a smaller hydrodynamic size in DI, demonstrated a less pronounced change in size at high pH. The increase in hydrodynamic size of WP at high pH could be due to the re-aggregation of WP subunits caused by alterations occurring in the molecular structure of WP. Understanding the exact mechanisms underlying this behavior is beyond the scope of this study. The relatively unchanged hydrodynamic size of SC appears to be in line with its surface charge behavior, which stems from insensitivity of its molecular structure to pH change.

The electrophoretic results of SDS-PAGE for 9 proteins of the study are shown in Fig. 3b along with the weight marker. The other proteins were not suitable for SDS-PAGE running and despite the experimentation with different solvents, they did not show good separation. The SC molecular structure is composed of chains of casein with usual weights around 20 to 35 kDa, where the big blot of proteins is seen. β-Lactoglobulin and α-Lactalbumin chains of WP on the other hand, are 15–20 kDa and are showing up as bold spots in the gel. NFMP, which is composed of proteins of SC and WP and other less prevalent residues possesses almost the same bands as the two [72,73]. Ig seems to have protein chains from 25 to 150 kDa, most of which are situated around 50–75 kDa. Alb has the largest band near 40 kDa, close to the weights associated with Ovalbumin. Lys is mostly clear and only shows one band near the very bottom of the gel close to weights around 10–15 kDa, that correspond to its protein size [74]. The chains that constitute the hemoglobin protein have a weight of 15–16 kDa, very close to what band weights are showing for Hem in the gel [75]. Try shows prominent bands near the 20–35 kDa range, close to the weights of the Trypsin protein. The Pepsin protein has a weight of approximately 35 kDa and a tentative band shows its weight around that value for Pep in the gel [76], although another smaller band near 15 kDa is also seen.

3.4. Surface tension

Fig. 4 shows the surface tension of the protein solutions at different concentrations of 0.625 %, 1.25 % and 2.5 % in DI and SPS. The surface tension of the proteins did not change noticeably with concentration for most proteins in DI and SPS as is evident in Fig. 4. This seems to suggest that these concentrations are more than the critical micelle concentration of the proteins in DI and SPS.

The effect of increased alkalinity and presence of ions in SPS on the surface tension of the solution can be seen by comparing Fig. 4a and 4b. These effects are very specific to each protein's properties, and it is not viable to issue a broad verdict for all of them. It is noted that while some proteins exhibited an increase in surface tension in SPS compared to DI, some other proteins showed an opposite behavior. One reason for decreased surface tension of the proteins in SPS compared to DI could be related to the increased solubility of proteins in SPS. Solubility of protein solutions is dependent upon the charge, which is regulated by the pH level and the intrinsic pI of the protein itself [64,77,78]. The farther a protein solution's pH is from its pI, the higher the solubility of that protein usually is [64,78]. Since many of the used proteins in this study have pIs situated around 4 to 9, an increase of pH to 13.6, a value far

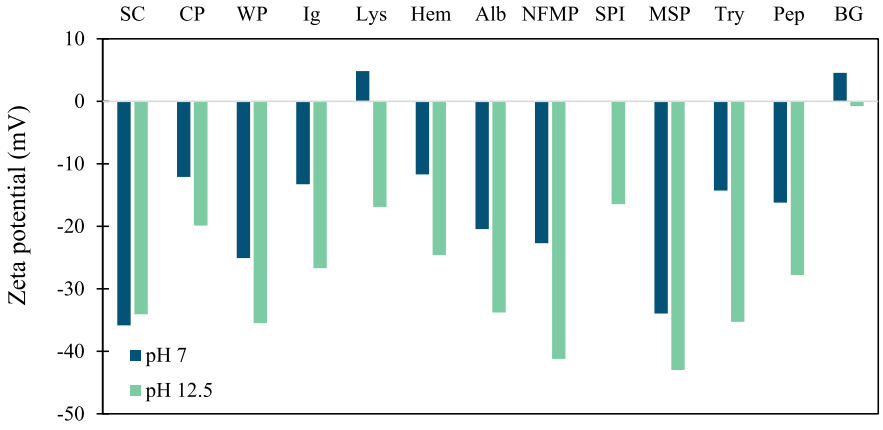
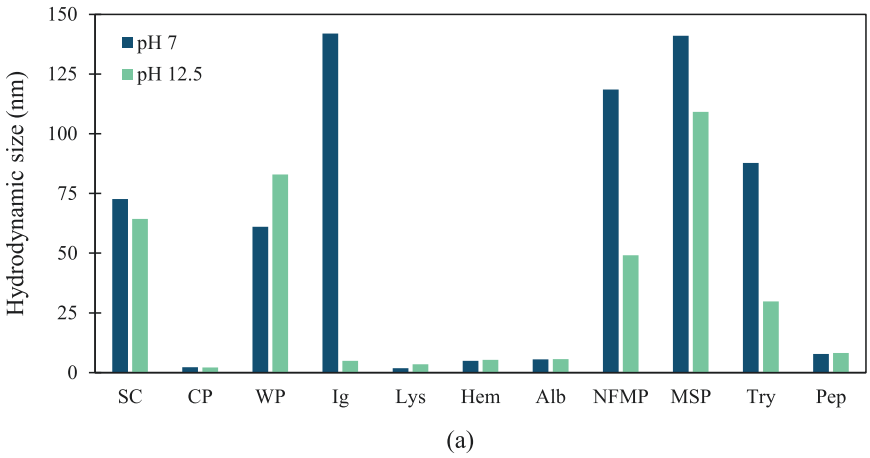
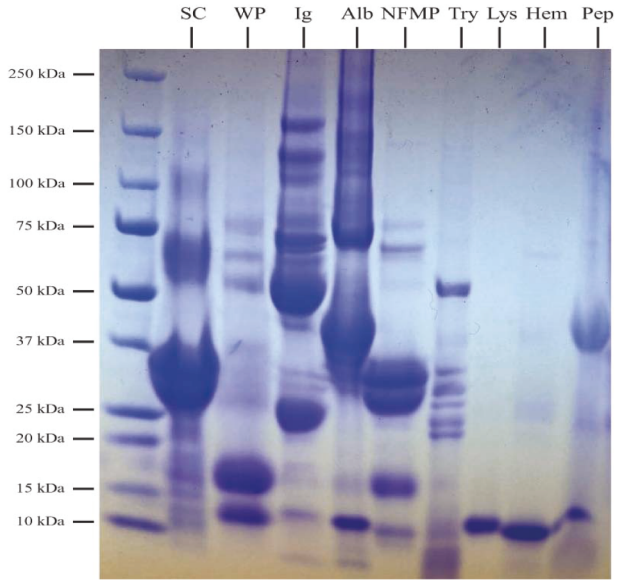


Fig. 2. Effect of pH on protein zeta potential.



(a)



(b)

Fig. 3. Determination of protein size. (a) Effect of pH on the protein hydrodynamic size and (b) SDS-PAGE analysis of the proteins.

from their pI can increase the charge and solubility of the proteins. The implication of increased solubility is that there are available dissolved proteins in the solution that can adsorb on the interface and increase the packing density of the proteins on the film interface. This higher solubility of the solution is one of the main reasons for lower surface tension of some proteins in SPS compared to DI. SPI is one of the cases that shows the effect of solubility on the surface tension. The solubility of SPI is so low in DI that a stable solution could hardly be made to measure surface tension or other properties such as foaming. Whereas in SPS, SPI became very soluble and reduced the surface tension of the solution.

Another reason for decreased surface tension of some proteins can be attributed to the unfolding of the proteins when they are exposed to the high pH of SPS, and subsequently, when they adsorb onto air–liquid interfaces. This time-dependent unfolding exposes the hydrophobic parts of the proteins that are hidden in the inner layers in the native state of the proteins, which typically takes on a globular morphology, and affects the surface tension of the air–liquid interface. While in DI this effect is minimal, in SPS proteins have already undergone some amount of unfolding and irreversible denaturation because of high pH of the solution [79,80]. The unfolding of proteins exposes the hydrophobic groups in the molecular structure of the proteins lending them increased surface hydrophobicity, which is expected to reduce surface tension of the solution and result in higher foam volume [81,82].

This is not the case for all proteins though. Since proteins don't have an exclusive hydrophobic tail as some surfactants do and instead have patches of hydrophobic/hydrophilic amino acids on their surface, the change in their hydropathicity is dependent upon the conformational changes in the 3D structure and the exposure of these amino acids. Therefore, with increased denaturation, some hydrophilic amino acids can become exposed as well and potentially increase the surface tension. While in some proteins hydrophobicity can increase in high pH [83], in others it decreased and the protein solution became more hydrophilic [84]. The effect of this increased hydrophilicity can potentially be an increase in surface tension, as is seen for some proteins in this study. Although measuring hydrophobicity of proteins can be a bit tricky, as some studies have shown that different experimental methods can give contradictory results in high pH environments [85].

Also, there are various cations in SPS including Ca^{2+}, Na^{+}, and K^{+}, which could interact with negatively charged proteins. The presence of these cations can reduce the solubility of the protein solutions due to the charge shielding or screening effect and complex formation with proteins in the case of Ca^{2+}, which is expected to decrease protein efficacy

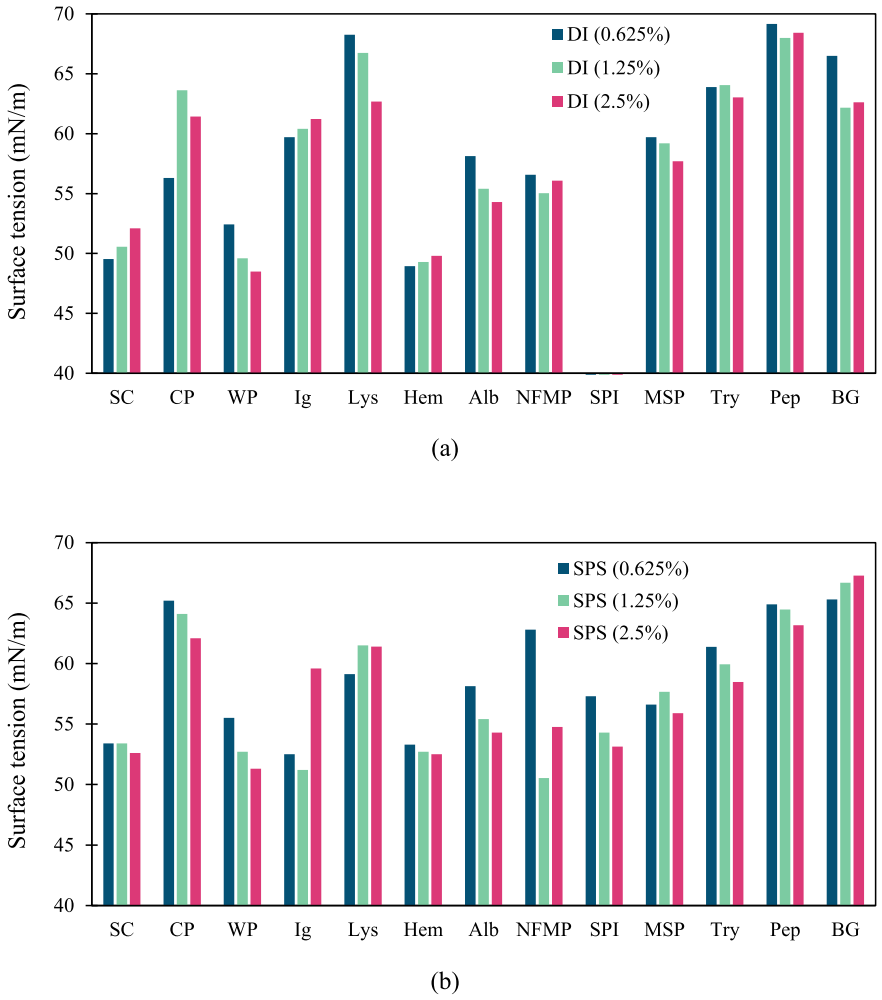


Fig. 4. Surface tension of proteins at three concentrations in (a) DI and (b) SPS.

on reducing the surface tension of the solution [86].

The effect of salts on foaming is a complicated process. Prior studies have indicated that these effects may exhibit themselves as changes in solubility, protein-proteins interactions in the interface, and disjoining pressure between the two sides of the film [80]. It has been shown that Ca^{2+} can adsorb onto the film interface and move between the protein mesh and change the shear rheology and protein interactions on the bubble film. This can induce an increase in the shear viscosity modulus of the solution [87]. The presence of ions in the solutions could promote accumulation of proteins at air–water interface due to the reduction of repulsive forces between the proteins.

It is stipulated that the observed surface tension behavior of the proteins in SPS and DI is governed by a combination of multiple mechanisms with somewhat opposing effects [1]. The contribution of each of these mechanisms varies among the proteins as it is a function of their specific molecular structure. This explains why the proteins showed different surface tension behaviors in DI and SPS.

3.5. Viscosity

Viscosity of the bulk solution is influential on the foaming properties of surfactants. While a very high or very low viscosity is generally considered to affect the foaming properties negatively - resulting in low foamability and low stability, respectively – the effect of viscosity is also highly dependent on the type of surfactant [88]. The unfolding of the protein structure can result in the exposure of previously buried hydrophilic amino acids, which could lead to better binding affinity to water, and as a result increased viscosity and higher stability of the foams [69]. The exposure of buried groups can also lead to higher contact area that would affect viscosity. This means that the increase in pH would make the solution more viscous.

Although the bulk viscosity and air–water interface viscosity can be different, it can be assumed that they are correlated in a simplified system. As such, the viscoelastic monolayer of bubbles can reduce destabilizing effects that reduce the stability of bubbles such as drainage, gas diffusion and coalescence [89]. Despite this positive effect on bubble stability, a very high viscosity is thought to also be harmful, as it might reduce the incorporation of air into the solution in the first place and reduce the foamability. Therefore, instead of very low or high viscosities, a medium range of viscosity is expected to be effective for foaming [89,90].

In almost all cases shown in Fig. 5, the kinematic viscosity increased with the increased concentration of the protein solutions in SPS. Increased concentration of proteins generally translates into a more saturated solution and a harder environment for the flow of the solution. Especially if the particles are large, as some of the proteins in this study are, this effect is more accentuated.

A summary of all the experimental data related to the physiochemical properties of the proteins is presented in Table 2.

3.6. Foaming of proteins

3.6.1. Stabilizing/Destabilizing forces in bubbles

Prior studies have examined the foaming of surfactants and related

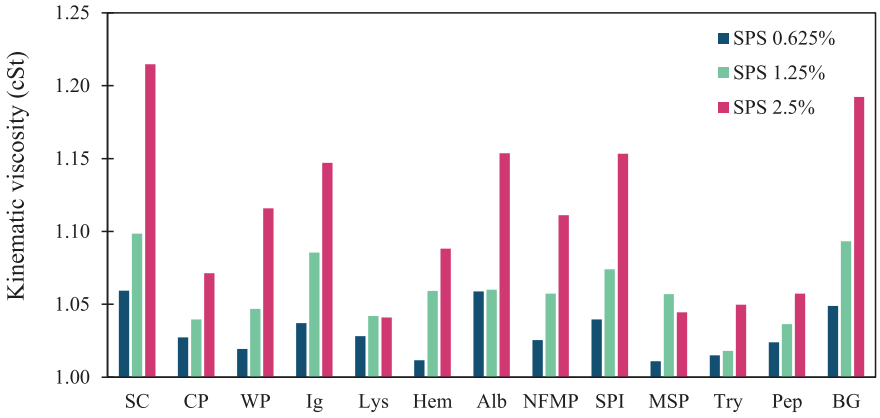


Fig. 5. Effect of protein concentration on the kinematic viscosity of proteins in SPS, measured using an Ubbelohde viscometer.

Table 2
Summary of the experimental physiochemical results of the proteins used in this study.

Protein	Surface tension (mN/m)						Surface charge (mV)		Hydrodynamic size (nm)		Kinematic viscosity (cSt)		
	DI			SPS			DI	High pH	DI	High pH	SPS		
	0.625 %	1.25 %	2.50 %	0.625 %	1.25 %	2.50 %					0.625 %	1.25 %	2.50 %
SC	49.53	50.57	52.10	53.40	53.40	52.60	−35.87	−34.10	72.70	64.30	1.06	1.10	1.21
CP	56.30	63.63	61.43	65.20	64.10	62.10	−12.10	−19.90	2.24	2.17	1.03	1.04	1.07
WP	52.43	49.60	48.50	55.50	52.70	51.30	−25.07	−35.50	61.09	82.97	1.02	1.05	1.12
Ig	59.70	60.40	61.23	52.50	51.20	59.60	−13.27	−26.70	142.00	4.94	1.04	1.09	1.15
Lys	68.25	66.75	62.68	59.13	61.50	61.40	+4.84	−16.90	1.83	3.46	1.03	1.04	1.04
Hem	48.93	49.30	49.80	53.30	52.70	52.50	−11.70	−24.60	4.88	5.36	1.01	1.06	1.09
Alb	58.13	55.40	54.30	58.13	55.40	54.30	−20.47	−33.80	5.55	5.62	1.06	1.06	1.15
NFMP	56.57	55.03	56.07	62.80	50.53	54.76	−22.70	−41.23	118.49	49.12	1.03	1.06	1.11
SPI	–	–	–	57.30	54.30	53.13	–	−16.47	–	–	1.04	1.07	1.15
MSP	59.70	59.20	57.70	56.60	57.66	55.90	−33.97	−43.00	16.54	17.34	1.01	1.06	1.04
Try	63.90	64.06	63.03	61.37	59.93	58.47	−14.27	−35.30	141.06	109.14	1.01	1.02	1.05
Pep	69.17	68.00	68.43	64.90	64.47	63.17	−16.23	−27.80	87.78	29.76	1.02	1.04	1.06
BG	66.50	62.17	62.63	65.30	66.67	67.27	+4.56	−0.75	–	–	1.05	1.09	1.19

bubble stability mechanisms, in both solution and cement mixture [1,2,5,6,8,48,89]. These studies focused on surfactants and air-entraining admixtures (AEA) which can be different from proteins in both molecular structure and physicochemical properties. An important interaction of surfactants in the solution is that they create a viscous film in the air–water interface, and form compounds in the interface based on the ionic nature of the solution. In the case of the anionic type, hydrophobic groups form calcium salts with the calcium ions, whereas the cationic type form hydroxides from their hydrophilic groups. The nonionic ones are incapable of forming ionic compounds [4]. The insoluble films formed by anionic types are preferred, as they can maintain their integrity and increase the stability of the air bubbles. As shown in the previous sections, proteins gained an increased negative charge in the high alkaline environment, so similar behavior to anionic surfactants can be expected. These ionic films can also increase the stability of bubbles by repulsing adjacent bubbles and preventing gas diffusion. This is postulated to be the reason for generally smaller bubbles in anionic surfactant systems [4].

The integrity of the film of the bubbles plays an important role in the foaming and air-entrainment characteristics of the AEAs, especially in the ones that surface tension reduction is small and the stability of foams relies on the film strength [1]. The strength of the film is responsible for the foam stability against adverse effects, including gas diffusion, bubble coalescence, rupture and drainage [1,4,89]. Characteristics such as film thickness or AEA adsorption on the film interface are good indicators for determining how effective this protective layer is [12]. The bubble film is affected by the properties of the solution and the surfactant. For example, higher ionic strength has been shown to correlate with a thinner film, because it leads to the inner and outer sides of the bubble film consisting of adsorbed surfactants and dissolved ions repulsing each other less effectively [2,12]. This mechanism was indicated in a study by Shan et al. [91], where it was shown that the introduction of a nonionic surfactant and its adsorption onto the interface can lower this ionic repulsion and create a more stable film. Gas diffusion (disproportionation or Ostwald ripening) between bubbles due to pressure difference, bubble coalescence because of weak interfacial film and the drainage of liquid from the interfacial film are the major destabilizing causes in a foam system in concrete [8].

3.6.2. *Foam capacity*

The initial and 10-min foam volume of all proteins in DI and SPS at different concentrations are depicted in Fig. 6a and 6b and Fig. 7a and 7b, respectively. Significant differences in foaming performance exist between different proteins. A good foam performance requires both high initial volume of air in the solution and satisfactory stability of the generated bubbles over time. While some proteins such as Ig and Hem showed good foamability and stability of the foam in both DI and SPS, others such as Alb and BG lacked one of the two or both. It is noted that the proteins generally exhibited an increase in foaming capacity and stability in SPS compared to in DI. There are a few proteins including CP and BG that did not show much foaming ability in either DI or SPS. The main factor contributing to increased foaming capacity of the proteins in SPS compared to DI is the improvement of protein solubility and stability in SPS due to molecular changes and increased electrostatic

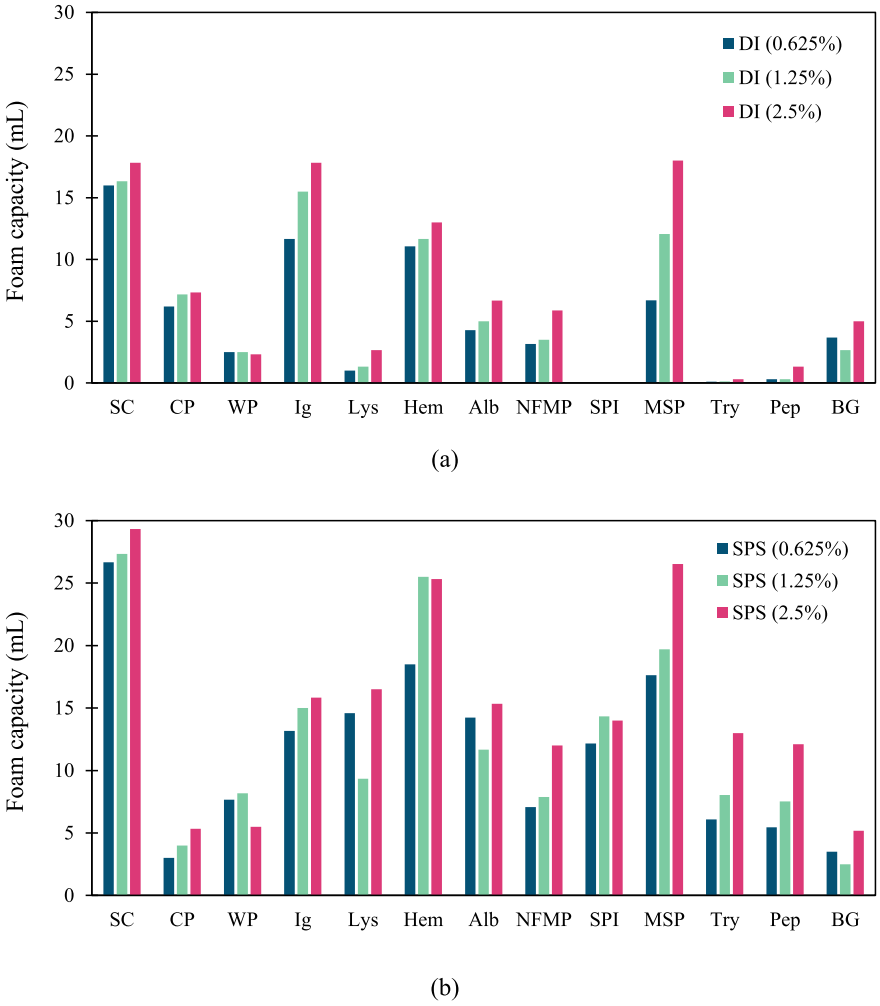


Fig. 6. Foam capacity of proteins at different concentrations in (a) DI and (b) SPS.

repulsive forces among proteins. This effect is especially more evident in the case of proteins including SPI, Try, and Pep, that showed virtually no foaming in DI because of low solubility; the degree of increase in foam capacity is specific to each protein. In a previous study, it has been shown that for the rice bran protein, the high alkaline environment of above pH = 10 (far from its pI of 4.5) resulted in high solubility and improved foaming capacity [77]. Ruíz-Henestrosa et al. [92] measured solubility for soy protein globulins up to a pH = 12 and observed that the highest solubility occurred at these highly alkaline regions. The corresponding foaming properties were also improved in higher solubility regions.

Stability, which is indicated by the 10-min foam volume, is also affected by the protein solubility, as a result of higher presence of proteins in the solution and their consequent adsorption on the bubble interface, as was shown by Rodríguez Niño and Patino for bovine serum albumin [93]. This improvement in stability with higher packing density of proteins on the bubble interface correlated with a tougher bubble and improved foam strength [89]. In the case of soy protein for instance, a higher foaming performance was observed when the adsorption on the interface was higher [92]. Similar behavior has been observed for various other proteins [94].

The presence of cations in SPS could affect the foam stability by adsorbing on the interface. At bubble interface, they can reduce the repulsive forces between the proteins or potentially form bonds with the protein leading to increased stability of the mesh network structure of the bubble interface [1,89].

The enhanced interface viscosity and elasticity, in turn, protects the bubbles against destabilizing interactions such as gas diffusion, coalescence, and drainage and, as a result, increases stability. On the other hand, the presence of cations can reduce the repulsive forces between the bubble films promoting thinning, coalescence and other destabilizing mechanisms of the foam [1]. For instance, high concentrations of NaCl can result in the depression of solubility and the subsequent reduced foam stability in β-lactoglobulin [95,96] and pigeon pea protein [97].

It seems that the combined effect of the complex mechanisms described above favors improving the foaming characteristics of the proteins in SPS compared to in DI. Fig. 8 combines the initial vs 10-min foam volume of all proteins in DI and SPS. The straight line delineates perfect stability of the foams and points near it possess high stability, although they may have very low initial foaming to begin with. Proteins such as Hem SPS with high initial capacity and stability are generally placed near the top right part of the graph. On the other hand, SC SPS has moderate stability despite possessing the highest amount of initial foam. It is seen that the data corresponding to DI is concentrated in the lower left part of the graph indicating increased foam performance of the proteins in SPS compared to in DI.

3.6.3. *Foam bubble size and distribution*

Fig. 9 shows the average foam bubble diameter in DI and SPS for the 1.25 % protein concentration. The size of each bubble was measured using the ImageJ software by analyzing the area of foams gathered in a

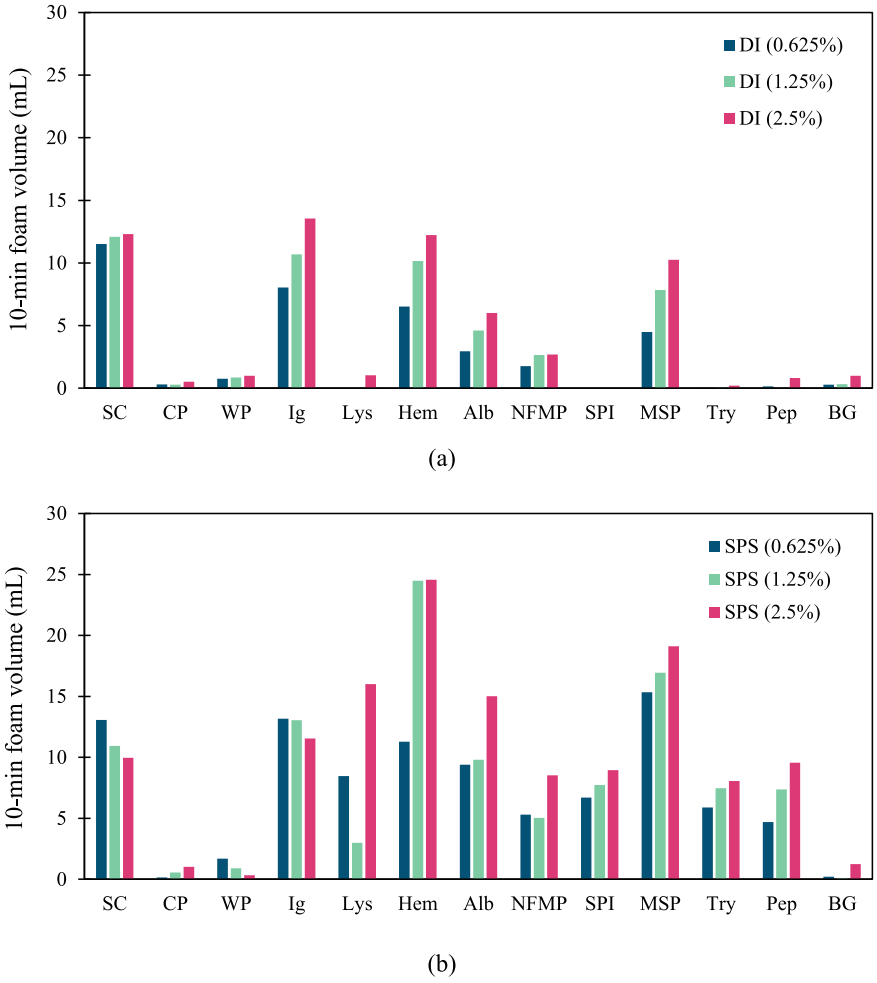


Fig. 7. Foam volume at 10 min of proteins at different concentrations in (a) DI and (b) SPS.

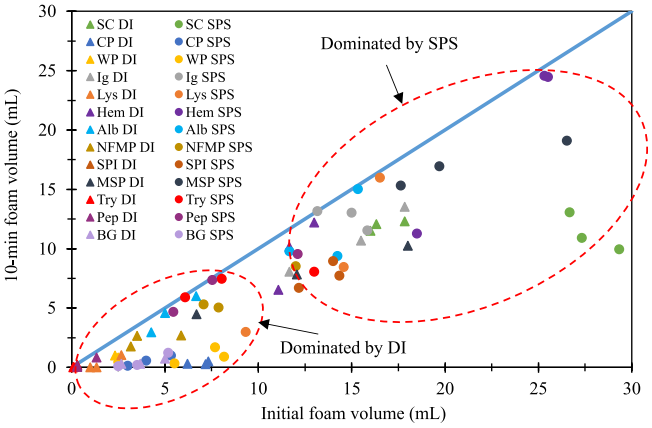


Fig. 8. Effectiveness of various proteins in foam creation and stabilization in DI and SPS. Points inside each group denote different concentrations of the proteins (0.625%, 1.25% and 2.5%).

petri dish, which were then used to find the diameter of each bubble, assuming a near ideal circular shape for the bubbles. It is noticed that the foam in DI showed a wider bubble size distribution compared to that in SPS. Generally, while proteins with smaller bubbles in DI such as Lys and BG exhibited an increase in bubble size in SPS, the proteins with larger bubbles in DI, such as MSP and Alb, had a significant reduction of size in SPS, and this reduction in size is more pronounced in the case of MSP. Most bubbles in SPS seem to be more stable in a diameter vicinity of 150 μm.

The relation between bubble size and stability depends on the initial size of bubbles, surface tension of the bubble film, the Laplace pressure - pressure difference between inside and outside of the bubble [89]. If the initial bubble size is very small, the high Laplace pressure makes it unstable and inclined to expand in size or coalesce with other larger bubbles. Therefore, the bubbles have to expand to some degree before surfactant mesh can be formed in the bubble film to enhance the film stability. On the other hand, very large bubbles cannot form strong films and are very unstable against outside disturbances. Therefore, the decrease in bubble diameter due to the inward pressure of the thin film can result in a more stable form [89,98].

Fig. 10 exhibits the size distribution of the bubble diameter in DI and SPS. Only the results of a select number of proteins that showed the overall patterns are included in this figure. Some proteins such as Lys and WP showed a narrow size distribution with the average size being lower than 100 μm. There are also proteins like MSP that demonstrated a wide bubble size distribution with an average larger than 200 μm. Most of the remaining proteins showed a relatively moderate distribution with the average being centered around 100–200 μm.

3.6.4. *Bubble structure*

Fig. 11 shows the optical micrographs of the bubbles of select proteins in DI and SPS with a concentration of 2.5 %. Only the images of select proteins are included to illustrate the wide range of foaming

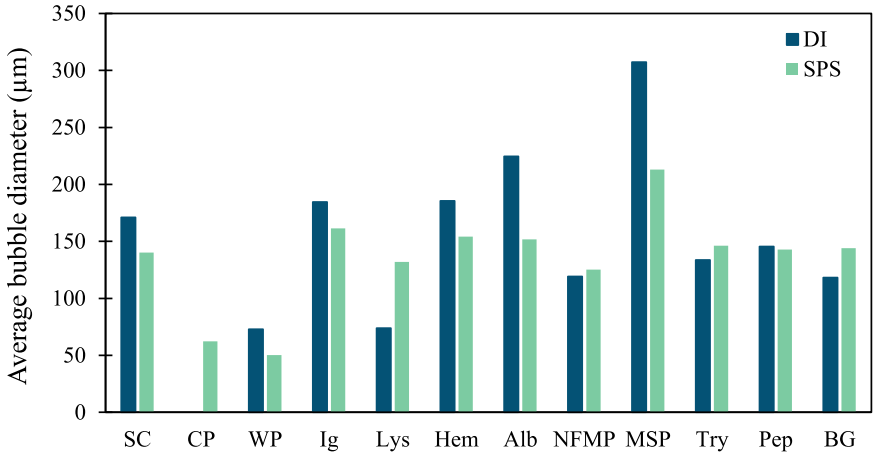


Fig. 9. Average foam bubble diameter for the 1.25% protein concentration in DI and SPS.

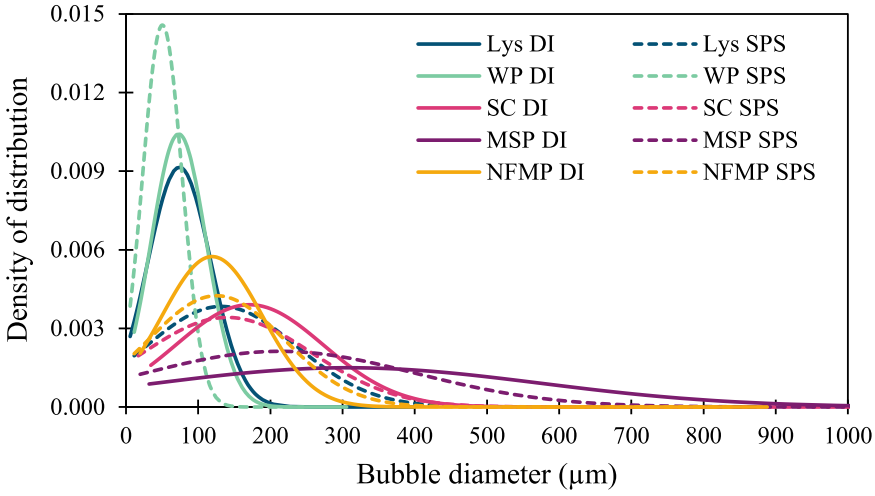


Fig. 10. Bubble size distributions of select proteins at the concentration of 1.25% in DI and SPS.

characteristics of the proteins. While in the case of WP, the structure of bubbles did not change in DI and SPS, in the case of Try and Lys, the difference between the bubble structure in DI and SPS is evident and this difference is more pronounced in the case of Lys.

The properties of the bubble film are important in their stability. A thin film is formed over time as the adsorption of proteins on the bubble film continues. As the adsorbed proteins on the film approach a saturation level, a mesh network of proteins is formed on the film, which improves its resistance to destabilizing phenomena, such as drainage and coalescence, by enhancing the overall stability of the foams.

Microscopic images in Fig. 12 display this phenomenon to some extent for Lys and Hem. It is interesting to note that the bubble film of Lys in DI appeared to be smoother and thinner compared to that in SPS as seen from Fig. 12(a) and 12(b). This could be due to the formation of a mesh network of proteins on the bubble film resulting in a stable bubble film.

This observation seems to be in agreement with the foam capacity and stability results of Lys in DI and SPS as discussed in the previous sections. Since the stability of the interfacial film is low in DI, the bubbles are susceptible to destabilizing factors, such as coalescence, which is seen in Fig. 12a where two smaller bubbles are merging into a larger bubble. On the other hand, as can be seen in Fig. 12b, although bubble surfaces are touching each other, coalescence is not observed. This explains the observation that Lys in SPS possesses much better foam stability compared to in DI. It is also seen that the bubble film in the case of Hem seemed to be thicker in both DI and SPS. This observation is in line with high foam stability of Hem in both DI and SPS, as demonstrated in Figs. 6 and 7.

The microscopic images of the bubbles over time corresponding to Alb and BG at the concentration of 2.5 % in SPS is presented in Fig. 13. In the case of Alb, little to no change can be seen, which is consistent with its very high stability of almost 1 in the 2.5 % concentration. The foam was also calm and no movement of the foam was observed. On the other hand, BG exhibited unstable bubbles and its bubble structure changed more noticeably at the initial stage and then to a lesser extent at later time.

3.7. Relationship between foaming characteristics and physicochemical properties

3.7.1. Effect of surface tension

Surface tension is one of the major physicochemical properties of the protein solutions that directly affects their foaming characteristics. The surface tension vs 10-min foam volume for each concentration of the proteins is shown in Fig. 14. As expected, there is a correlation between the 10-min foam volume and surface tension in both DI and SPS. The

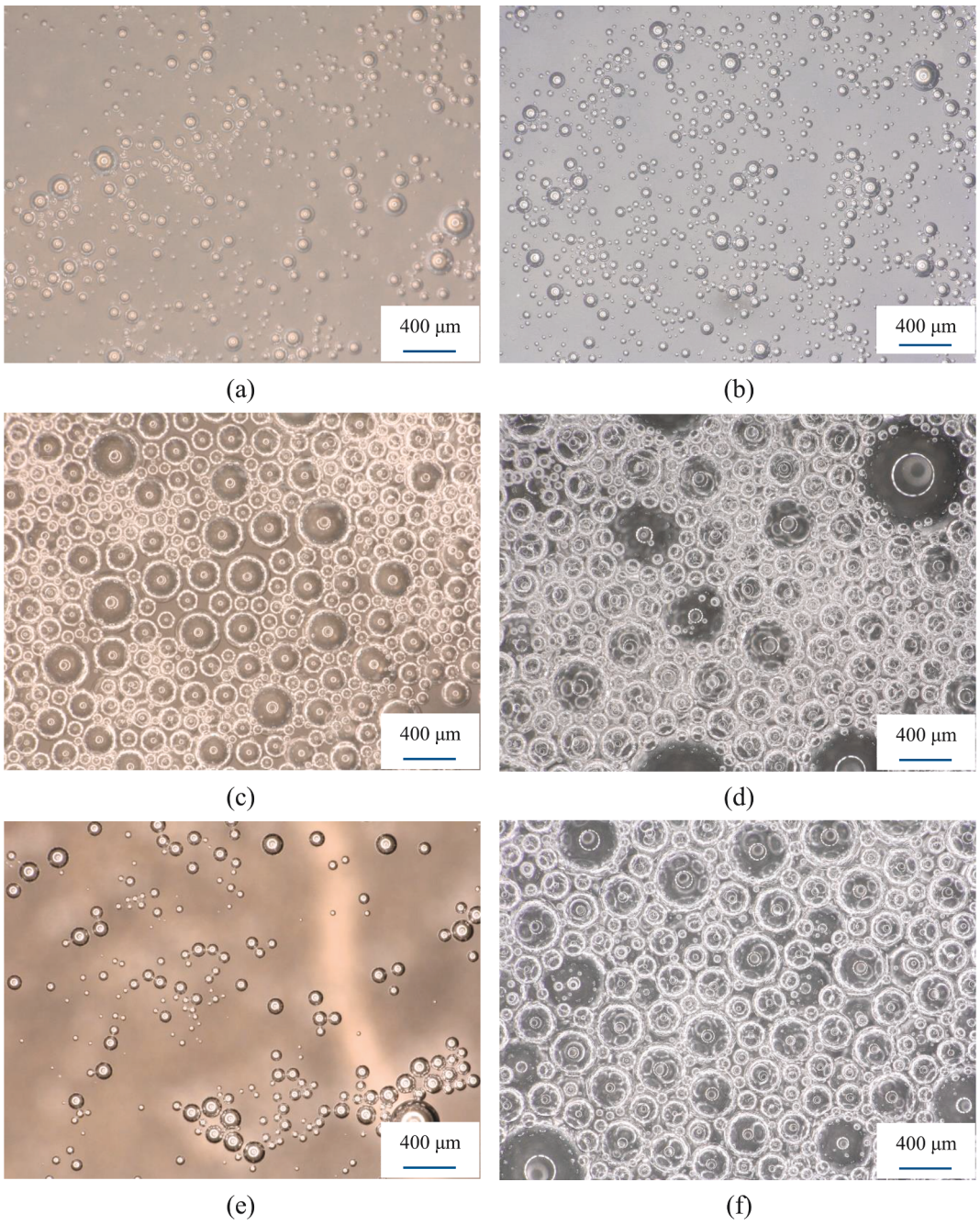


Fig. 11. Microscopic images of the initial foam bubbles at a concentration of 2.5% corresponding to (a, b) WP in DI and SPS, (c, d) Try in DI and SPS, and (e, f) Lys in DI and SPS.

correlation is stronger for the 0.625 % concentration in both DI and SPS, with the R^2 values of 0.38 and 0.56 respectively, but the correlation declines in higher concentrations of 1.25 % and 2.5 %. This behavior is a key point that shows the declining impact of surface tension in high concentrations. As discussed in section 3.4, the increase in concentration did not significantly change surface tension, however, some proteins experienced an improvement in foaming. Therefore, it is postulated that the change in the foaming of some proteins is not caused by a change in surface tension, but rather the change in other parameters such as viscosity, self-aggregation of proteins, and higher saturation of the solution with anionic proteins. It should be noted that while surface tension is an important parameter affecting the foaming behavior, the effect of the above-mentioned parameters becomes more pronounced, particularly when surface tension remains unchanged. This can explain why the correlation between 10 min-foam volume and surface tension seems to weaken when the concentration increases as the influence of other properties of the solution on foaming increases.

It is also interesting to note that this correlation is stronger in SPS than in DI. As seen in Fig. 14a, many proteins in DI with surface tension between 70 and 50 mN/m had near-zero 10-min foaming volumes. In DI, these proteins were unable to produce enough early foam (low foaming capacity) or were unable to maintain them for the duration of 10 min (low stability), which resulted in a low correlation compared to the proteins in SPS. On the other hand, the protein data points in SPS figure demonstrated a wider range of 10-min foaming volume because of better foamability and foaming stability, resulting in a better correlation. The reasons for the enhanced foaming were discussed in the previous sections, where the better solubility and higher negative charge of the

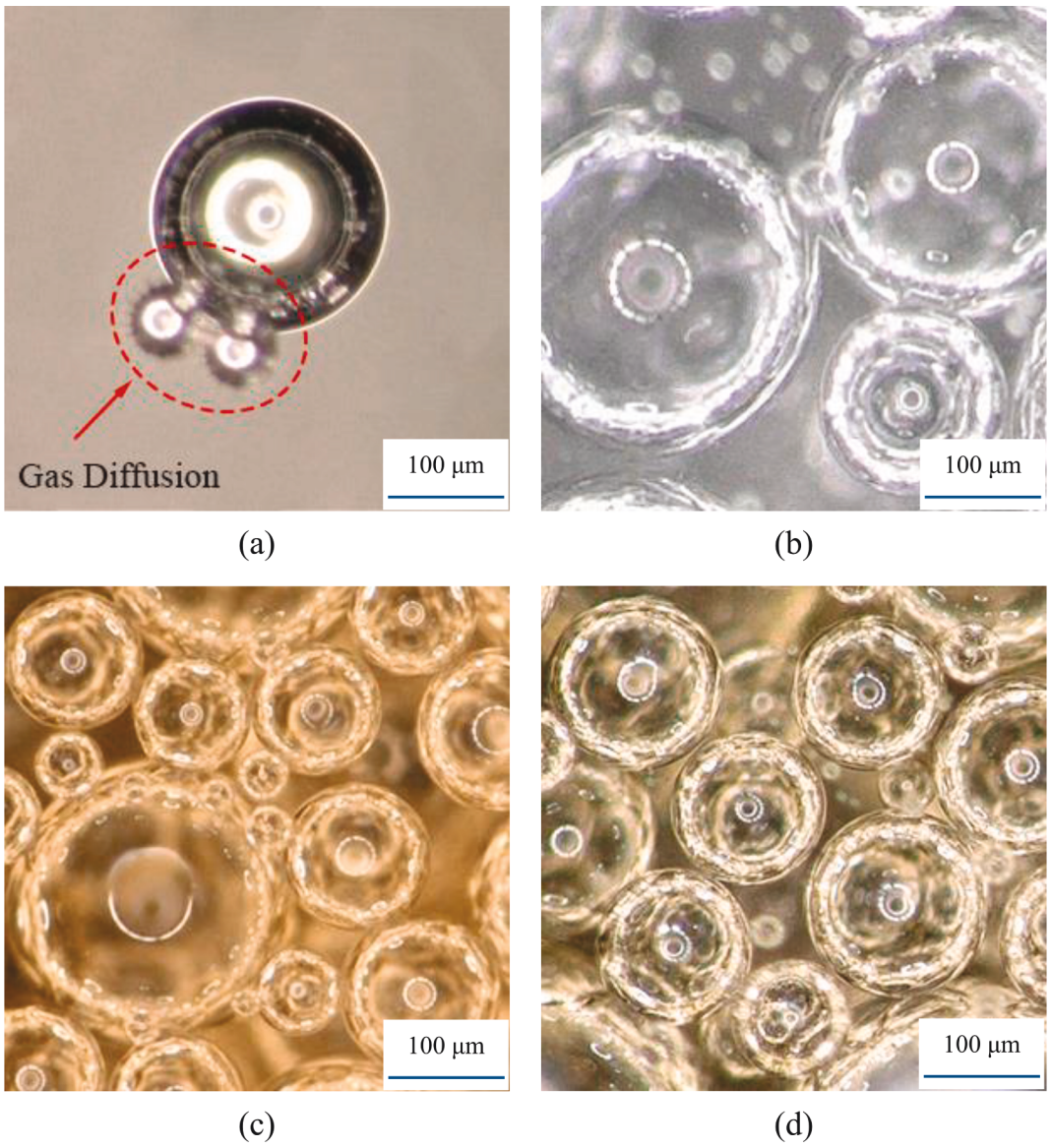


Fig. 12. Microscopic images of bubble film at a protein concentration of 2.5% corresponding to (a, b) Lys in DI and SPS and (c, d) Hem in DI and SPS. While gas diffusion is obvious in (a), the bubbles in (b) are more stable and can resist coalescence and gas diffusion because of their more stable film.

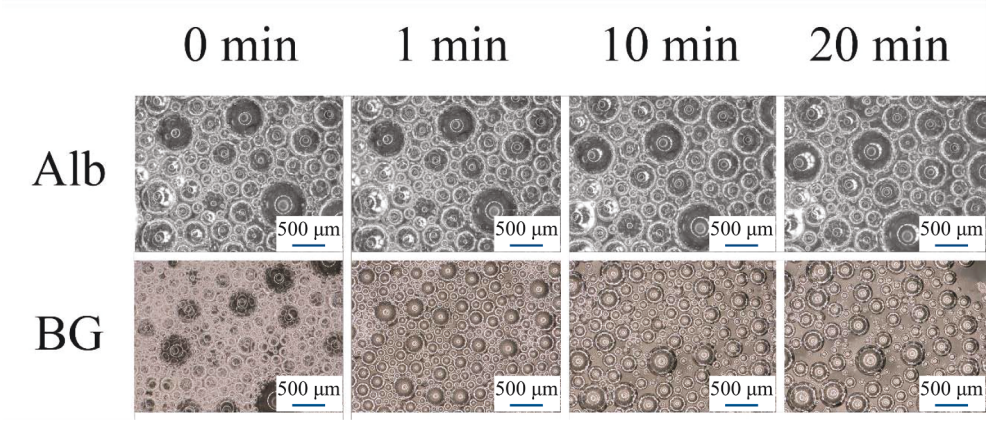


Fig. 13. Microscopic images showing the change in foam bubble size and distribution with time, of Alb and BG at a concentration of 2.5% in SPS.

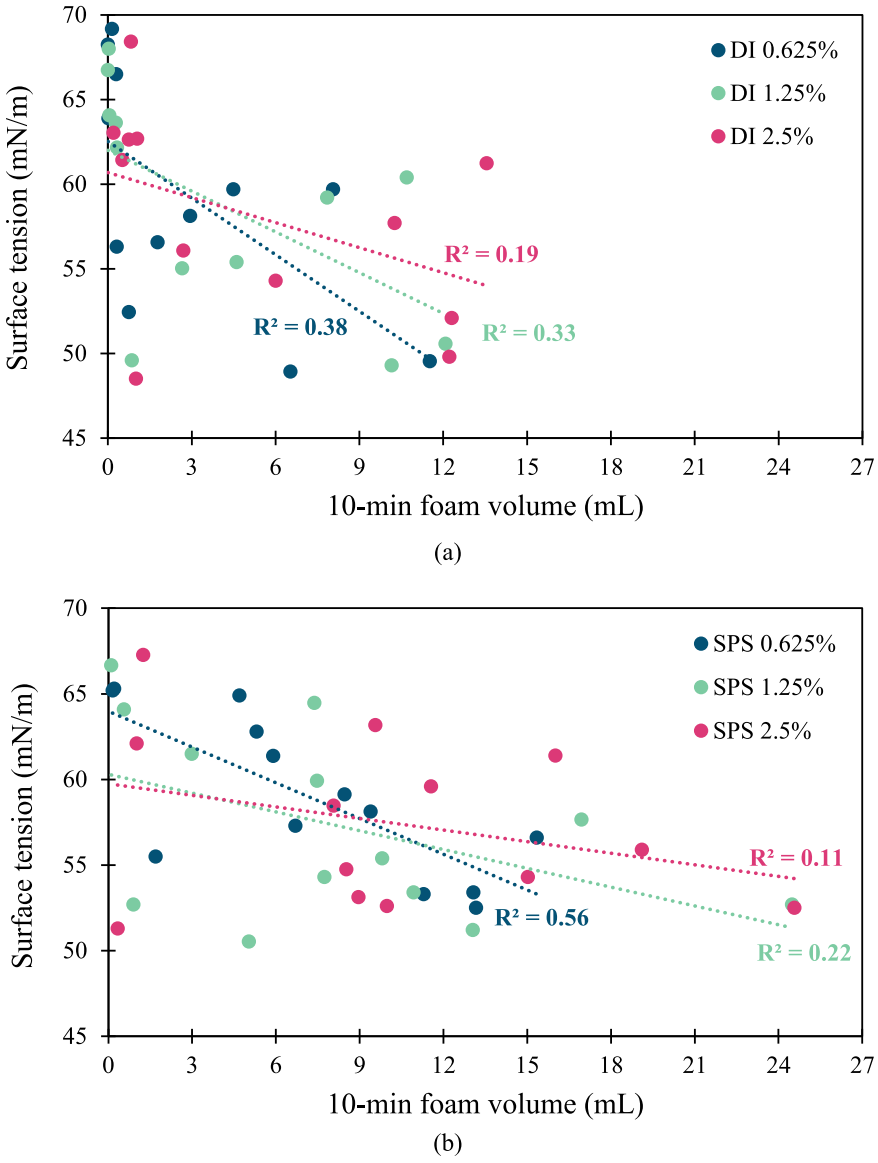


Fig. 14. Foam volume as a function of surface tension for all proteins at different concentrations in (a) DI and (b) SPS.

proteins were cited as the dominant factors.

3.7.2. Combined effect of physiochemical interactions

In order to examine the combined effect of the physicochemical properties on foaming, contour plots in Fig. 15a and 15b are presented to study the combined influence of surface tension and viscosity on 10-min foam volume of all proteins in DI and SPS, respectively.

It is seen that in DI, lower surface tension alone is not adequate in producing large foam volumes, and even surface tensions as low as 52 mN/m have low amounts of 10-min foam volume in low viscosities. This is mainly because these proteins in DI don't produce enough foam in the first place because of a low solubility in DI or have low stability because of a weaker film interface of bubbles. The increase in viscosity is expected to improve the viscoelastic liquid film of the bubbles and lower the amount of drainage that affects the foam volume over time [89]. That is why in relatively higher viscosities we can see an increase in 10-min foam volume even in higher surface tensions.

On the other hand, in SPS, intermediate foaming is seen in almost all viscosities even at much higher surface tension values. The highest foaming is observed in the relatively lowest surface tension and medium viscosity range. The blue band seen on lowest surface tension range is due to the lack of enough data points in this region and should not be taken as an actual characteristic. This improvement is due to other physicochemical factors such as improved solubility in SPS, higher bubble stability because of charged film interface and other bubble stability mechanisms. Viscoelastic properties of the film interface can lower many destabilizing mechanisms such as gas diffusion, drainage and bubble repulsion [89].

4. Conclusions

In this paper, the physicochemical characteristics of 13 proteins and their foaming behavior in DI and SPS were examined to help in understanding the air-entraining mechanisms of proteins in cementitious mixtures. The following conclusions can be drawn from the results of this investigation:

- The pH of the pore solution strongly influenced the proteins and their physiochemical properties compared to that in DI. At high pH, the surface charge of the proteins gains more negative charge resulting

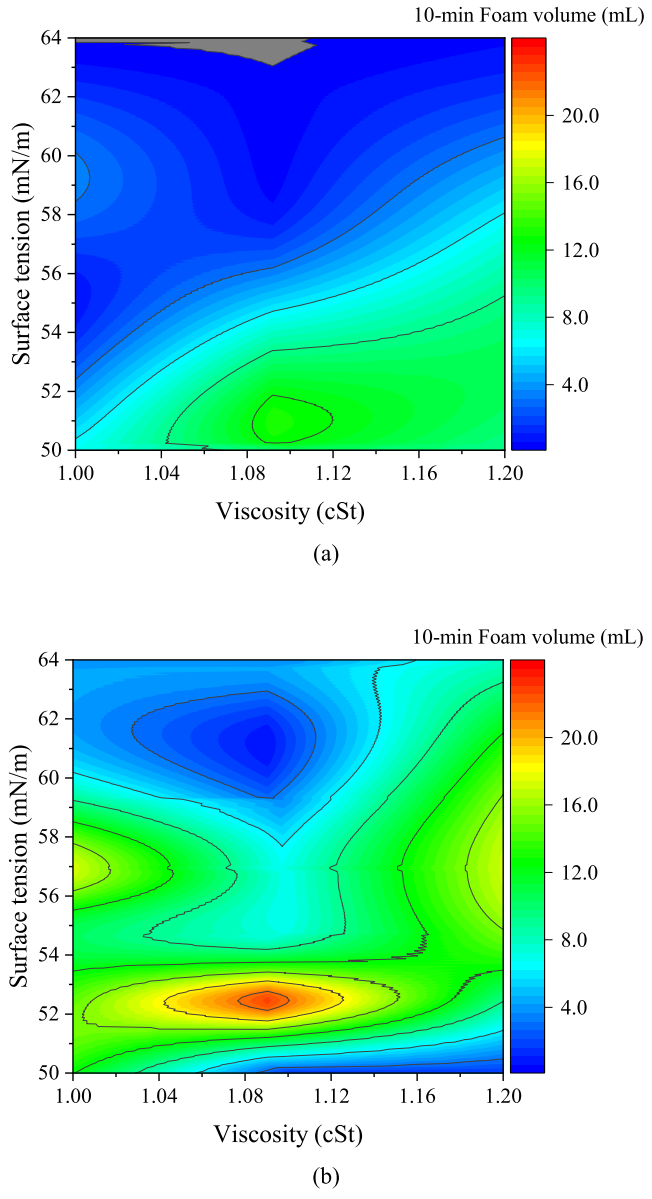


Fig. 15. The combined effect of surface tension and viscosity on 10-min foam volume of all proteins at different concentrations in (a) DI and (b) SPS.

in better solubility and stability of the proteins compared to in DI. The hydrodynamic size of the proteins was shown to generally reduce at high pH compared to their natural state due to the dissociation of protein aggregates.

- The effect of pH and ionic strength of SPS on the surface tension of protein solutions was found to be protein-dependent; some proteins showed an increase and some showed a decrease in surface tension in SPS compared to in DI and this was attributed to their molecular structure.
- The physiochemical changes of the proteins in SPS proved beneficial to their foaming properties, and higher foam capacity and 10-min stability were observed. Increased protein solubility and enhanced stability of the bubble film in SPS contributed to the improved foaming properties of the proteins in SPS compared to in DI.
- Generally, the proteins with large bubble sizes in DI, demonstrated a noticeable reduction in bubble size in SPS; however, the proteins with small bubble sizes in DI showed a slight increase in size in SPS. Optical microscopy showed increased roughness and thickness in the bubble film of the foam in SPS compared to in DI, which could be evidence of increased accumulation of proteins on the bubble films and enhanced foaming behavior of the proteins in SPS compared to in DI.
- It was found that surface tension plays an important role in foaming properties of proteins in both DI and SPS; however, in SPS, the effect of surface tension on foaming becomes less important than in DI. This could be due to increased effect of other physicochemical properties on foaming in SPS. The effect of surface tension on foaming behavior diminished at high concentrations.

CRediT authorship contribution statement

Mohammad Sadegh Tale Masoule: Methodology, Data curation, Writing – original draft, Validation. **Elvis Baffoe:** Methodology, Data curation. **Ali Ghahremaninezhad:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing 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.

Data availability

Data will be made available upon reasonable request.

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