ORIGINAL RESEARCH PAPER

Stabilisation of Wet Protein Foams Using Starch Nano-Particles

Saeed Sadeghpour [1]*, ***Saghar Abedini*** [2]

[1] *Young Researchers and Elite Club, Robat Karim Branch, Islamic Azad University, Robat Karim, Iran*
[2] *Chemical Engineering Department, Amirkabir University of Technology, Tehran, Iran.*

Received: 2017-05-12 Accepted: 2017-09-14 Published: 2017-12-20

ABSTRACT
Protein isolate is used in the food industry in order to process and stabilise food foams. Therefore there has been a great deal of interest and research in order to understand the effect of processing parameters on the functional properties of the isolate. The major purpose of this research is to study the foamability of the different proteins - starch nano-particle system. The results from experiments revealed that the foam properties can vary significantly for certain protein solutions; however, the interfacial properties seem to be constant and the most important results from the experiments are A) in starches samples, adding protein had no significant effect on viscosity, consequently, there was no drainage limitation B) for starches sample surface tension is not limiting factor C) At same starch concentration Egg White protein (EWP) foams had a much higher drainage half-life time compared with pea protein (PPI) foams. The affected properties on foam stability, such as viscosity, surface tension and pH were compared to discover the best solution for foam stability.

Keywords: *Surface Tension, EWP, PPI, Nano-Particle, Overrun*

How to cite this article
Davari S, Shirkhanloo H, Motakef Kazemi N. Application of Nanoanalysis: Amine-Functionalized Mesoporous Silica Nanoparticles for Mercury Speciation in Human Samples. J. Nanoanalysis., 2017; 4(4): 265-271. DOI: 10.22034/jna.2017.543428.1034

INTRODUCTION

Foam is a multi-phase system consisting of a liquid or solid continuous phases in which dispersed gas bubbles are suspended [1]. As a colloidal system, foams are thermodynamically unsteady systems in which the gaseous material is briefly preserved as a separate dispersed phase in the liquid phase structure. In addition, the unique and extraordinary properties of foams result in several applications in various industries such as oil treatment and recovery, cosmetics, food, porous materials and fire protection.

Foam stability and the control of foam formation are the two important and very useful factors in food industry. Essentially, the high interface area of the gas-liquid interface and the various physical processes that take place in the foams cause their unstable nature and decrease the overall system's free energy [3]. In order to adjust the foam properties, protein isolates and particles are added to the mixture. Particles have more advantages for stabilizing compared with proteins. Particles can prevent against coalescence, drainage and especially disproportionation; proteins are not competent at preventing against disproportion. Drainage occurs when liquid flows out from the foam due to gravity and surface tension. Therefore, adding particles to the proteins is highly beneficial for stabilizing [2].

A number of researchers have recently studied the stabilization of food foams by the particles; in particular [7-10], has analyzed more the stabilization of foams and emulsion by solid particles in the food

* Corresponding Author Email: s.sadeghpour123@gmail.com
Tel: +98(21)56887901

field. EWP and PPI stabilized physicochemical properties of protein foams, which are subjected to the heat treatment under industrial conditions; however, there seems to be a lack of detail in the literature. Most literature has concentrated on foam ability and foam stability; unfortunately the texture and microstructure of foam has not been studied in depth and lacks research [11]. Lakkis and Villota (1990) said that the net charge of the molecules and the protein conformation explains the effect of pH on proteins. Negative protein charges are the result of low generally an increase of the pH in the protein solution away from the isoelectric point (pI), depending on the pI of the protein at different levels.

In this study the effect of nano-particle addition on foaming properties of two types of isolated proteins, EWP and PPI are considered. For this purpose, some properties of foams such as viscosity, drainage, overrun, surface tension are investigated. Also the effect of pH of foams around the isoelectric point is assayed.

MATERIAL AND METHODS

Material

Two proteins known as pea-protein from Kerry Food PLC and egg white protein, egg whites from chickens- from Sigma Aldrich, were used in this research. The eggs were obtained from a selective population of Leghorn hens with a routine diet [12].

Commercial starch nano-particles; starch sodium octenyl succinat from Ingredion (OSA=N-Creamer) was used in this research.

Table 1. Illustrates differences between nano-particles (starch), found from characterisation

Property	OSA
Viscosity (1 wt. %) [cP]	2.1
Surface tension (1 wt. %) [m.N/m]	60
Size [nm]	120

Methods

Preparation of the protein solutions

The protein solution was made from egg white and pea sources. For the preparation of the pea-protein solution, P-protein powder is dissolved in distilled water and the same instructions will apply to the preparation of the egg white solution. Then, the solutions need to be broken down in to two different concentrations as: 0.5 wt.%, 1 wt.%. All individual concentrations are characterized (viscosity, surface tension, stabilisation and pH), by the technique discussed in this section.

Preparation of the starch solutions

The same rules will apply while preparing starch solutions, and the same procedure will apply for the characterisation for each of them. Starch nano-particles with different concentrations including ,0.5 wt.%, 1 wt.% 3 wt.% and 5 wt.% were added to the solutions. Finally, the prepared starch solutions will be added to each one of the protein solutions and their different concentrations separately.

Foaming method

In order to obtain processed foam, a standard mixer (Hobart Mixing Machine-N50) was used with rotating beaters at 259 rpm planetary rotations with a beater rotation of 2.25 per planetary rotation to whip 200 mL of EWP for 5 minutes at room temperature.

Overrun measurement

After the proteins and starch solutions of 200 ml were prepared, the weight and pH level were measured. Then the obtained foam was transferred into a beaker with the volume of 200ml. The foam must then be weighed again. The overrun was characterized by this formula:

$$overrun\ (Vml\ solution)=((V(ml)wt.solution-V(ml)\ wt.foam)/(V(ml)wt.foam))\times 100$$

which V solution is weight of 200 ml of solution before foaming, and V foam is weight of 200 ml of solution after foaming procedure.

Foam characterisation and Foam stability

Foam half life

The beaker containing the prepared foam was placed in front of a camera, a Microsoft^{R} Life cam cinema^{TM}, which takes a picture every minute. As long as the foam reaches below the half line of the beaker, indicating the "half-life of the foam", then the half-life time of the foam can be calculated from the picture. The images will show the collapse of the foam during a time period and its half-life (half of the original height).

Drainage

The result derived from the foam stability illustrates the quantity of the liquid drained from the lamella of the foam structure. The moment that

the whipping stopped working, a specific volume of foam must be collected for weighting and kept at room temperature for a certain amount of time. The drainage liquid is then gently poured away and the remaining foam and its containing bowl will be weighed again [13].

Bulk phase

Rheology

When the solution preparation is done, it is needed to run the theological measurement test by utilizing the rheometer (type Kinexus pro (Malvern) using the double gap cell). The equipment must be adjusted to the desired characterization by setting the temperature at 25 ± 0.1°C and installing a serrated plate-plate geometry (40 mm of diameter for upper plate, 65 mm in diameter for lower plate and the gap height of 1.0 mm). All experiments were performed three times for each sample.

Surface tension

The next test is the measurement of the surface tension (s) of the egg white and pea-protein solutions with a tensiometer K100 (Kruss), Wilhelmy plate technique. The immersion depth used was 3mm and the time of measurement was 2000 seconds, which is related to foamability.

pH

In order to evaluate their stability and the effect that pH has on other foam properties, the solutions have been assessed at three different pH values: above, below and at the pI values.

A 0.5 mole of Sodium hydroxide (NaOH) and hydrochloric acid (HCL) was added in order to adjust the pH of these solutions and the SevenCompact™ S220 from Mettler Toledo was operated to accurately measure the pH. Firstly, the solution was assessed at neutral pH of 7, which is above the pI value. Secondly, the pH of the solution was altered so it is adjusted to the pI value of the proteins; which is pH 4.5 for EWP and 4.8 for PPI solution. Finally the pH was set to 3.5 for both proteins, which is below the isoelectric point (pI).

RESULT AND DISCUSSION

Bulk Phase Viscosity

In this study, the key preliminary objective was to measure viscosity before and after adding the starch (OSA-) into the proteins (EWP=Egg White protein and PPI=Pea protein). According to figure 1, viscosity of solutions prepared with native PPI and EWP were almost similar. By enhancing the concentration of these proteins, increase of viscosity was observed. By adding the OSA into the samples before foaming, viscosity was increased dramatically. By comparing results of figure 1, it could be concluded that effect of protein existence of solutions on viscosity has been low. Increase of viscosity should avoid the movement of liquid through the network of thin films and plateau borders, thereby slowing the drainage rate. Since viscosity remained approximately constant by adding proteins to OSA solutions according to figure 1, it could be concluded that drainage rate has been constant and there was no drainage limitation.

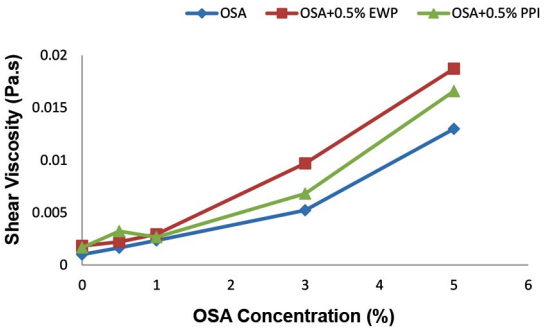

Fig. 1. Effect of OSA concentration and proteins on viscosity.

Surface Tension

The surface tension (σ) is an important property of foam. Generally it is known solutions to lower that it was not so much the absolute value of surface tension which is crucial, but rather the time dependence of σ. It means that dynamic surface tension during the foam formation is determining factor.

As it can be seen in figure 2, by increasing OSA concentration, surface tension has been decreased. Typically, when the bulk concentration ,approached zero, the surface tension of protein solution approached the value of the surface tension of water (72 mN/m).

By increasing the concentration of OSA in foams, overruns decreased according to figure 3.. Higher viscosity led to slowly foaming and consequently less overruns, and impeded incorporation of air bubbles. Increase of OSA concentration led to the sharp increase of viscosity. Conversely, variation of surface tension is low for OSA samples. Surface tension measurement is a method to study surfactant micellarization and possible interactions in solution. Before critical micellar concentration (CMC) point, surface tension, strongly has been decreased while surfactant concentration increased.

After reaching the CMC, surface tension remains constant. Therefore the CMC can be determined as concentration after which surface tension has not been changed. The CMC was determined by measuring the surface tension of different concentration of OSA. Low variation of surface tension for OSA was due to reaching CMC point. It could be concluded that foamability has been decreased in OSA because of insignificant variation of surface tension. According to above results it can be concluded that in case of OSA, surface tension is not a limiting factor.

According to figure 3, PPI had higher foam overrun than egg white protein at the same solution viscosity, suggesting a better foamability. The sample viscosity can influence molecular diffusion, and therefore reduce the adsorption rate of proteins. The difference between the two proteins might be due to the adsorption behavior of the proteins. Another important characteristic is the bubble size in foam. Smaller bubbles size corresponds to lower overrun. Therefore, the bubble size also led to different behavior between the two protein foams.

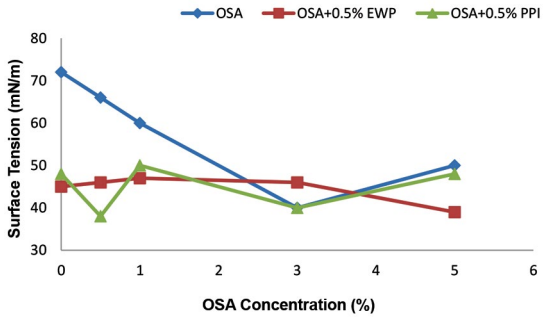

Fig. 2. Effect of OSA concentration and proteins on surface tension.

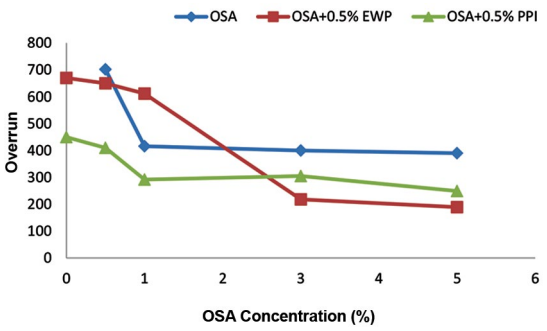

Fig. 3. Effect of OSA concentration and proteins on overrun.

The dynamic surface tension was also measured as a function of time, for studying the adsorption kinetics of proteins and nano-particles. Adsorption kinetics of proteins can be divided in multi steps: (1) a delay period, (2) the diffusion of the surfactant from the bulk onto the interface, (3) the adsorption and interfacial unfolding, and (4) rearrangement within the interface layer, multilayer formation and interfacial gelation [15-16].

The lag period is characterised by the plateau delay in surface tension curve. This time is only exists for the proteins. In the second step (diffusion of protein and nano-particles onto the interface), surface tension is related to the time by the exponent of 0.5. The first order law approach proposed by Graham and Phillips (1979) can be used to model both step 3 (adsorption/unfolding) and step 4 (rearrangement/multilayer formation and interfacial gelation phenomena). The constant rate of protein penetration and rearrangement can be obtained from the slope of the curve of surface tension versus time.

As a sample, the kinetic of 1% EWP at the air-water interface is shown in the figure 4. The lag time is the time required for the significant reduction in surface tension by adsorption of monomers at the interface. As reported by the researchers, the duration of lag time is correlated to the reveres bulk concentration of monomers. The absence of lag time in the figure 4 shows that the EWP is covered the interface instantaneously. This result is in contrast with those obtained by the authors [17-18], because the higher bulk protein concentration used in this study (1 %) compare to those of the literatures (about 0.01%).

The diffusion phenomenon is driven by the protein concentration gradient between the interface and the bulk aqueous phase. As mentioned above, the concentration of protein in this study was high. So, concentration gradient is decreased and as a result ,there is no limitation in diffusion of monomers at the interface.

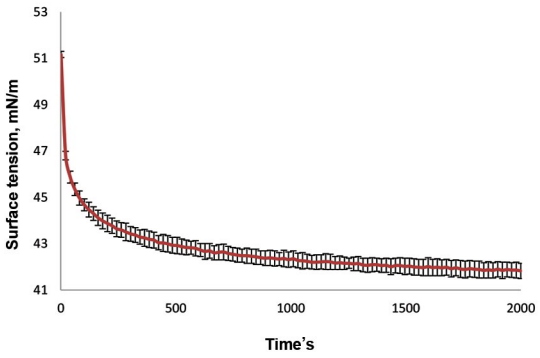

Fig. 4. The kinetics of 1% EWP at the air-water interface.

Foam stability

Foam half-life time

Proteins are the most widespread foaming agents used in foods and yet it appears that they may possess certain deficiencies if long-term foam stability is required. Different surface active particles (such as nanoparticles, starch, sucrose and so on) with different contents are added to the proteins to increase the stability of foams.

There is a competition between the protein and nano-particles for adsorption at the interface that lead to antagonistic or synergistic effects on stability of foam. Half –life time is an important parameter to evaluate the foam stability. Half-life analysis was performed by adding the various percent of the starch to the protein solutions, while recording the time in which 50% of the entrapped liquid in the foam was drained from the foam. Half-life time of foam including 0.5% EWP and 3% OSA is shown in Figure 5. As indicated in this figure, the time that foam volume is reduced to 50% of its initial condition is considered as half-life time.

Figu. 5. Half-life time of foam including 0.5% EWP and 3% OSA.

The results of the measured values for foaming agent are given in Figure 6. As can be seen in figure 6, solution viscosity of both proteins increased with OSA concentration; however, the foam drainage half-life of EWP increased exponentially while that of PPI increased linearly with increasing OSA content. Egg white protein foams had a much higher drainage half-life time than PPI foams at the same starch concentration. These results are in agreement with the observations of other researchers [5, 19].

It should be mentioned that the stability of pure EWP foams were more than PPI foams and also, protein contents exhibited positive effects on stability of foams (the results are not reported here). (Description: In all diagrams: Half time= Half life time).

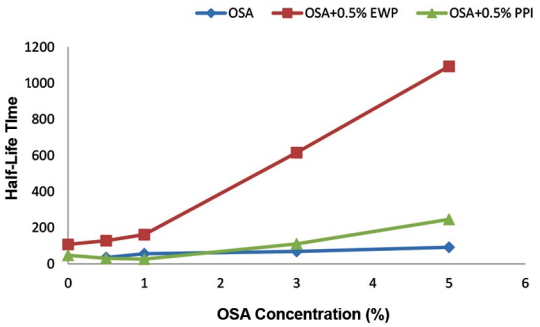

Fig. 6. Half -life time as a function of concentration of surface active nano-particles and proteins.

Effect of protein and starch

By reviewing the results of overrun and half-life time of EWP and PPI foams it could be concluded that there are two dissimilarities between them: 1) difference between the adsorption behavior of the proteins and 2) difference in size of bubbles in foams.

Viscosity is the affecting parameter on the adsorption rate of proteins. The viscosity of solution can influence on molecular diffusion of protein monomers. As mentioned above, viscosity of PPI solution was a little higher than EWP solution, so diffusion and consequently the adsorption rate of PPI protein is less than EWP. Because of insignificant differences in the viscosity of two types of protein foams, adsorption rate is not limited factor on dissimilarity between them.

Another difference is due to bubble size of the interface. Smaller bubble size leads to lower overrun. The air bubble size was determined by measuring sauter mean diameter ($d_{3,2}$) of air bubbles using a Mastersizer. The air bubble size for the EWP and PPI foams was 22.08 and 32.99μm, respectively. So it is predicted that overrun of native PPI foam should be higher than EWP foam. The latter is accordance with findings of overrun analysis.

The same approach could be performed for revealing the effect of starch on the foam characteristics. The viscosity of OSA solution was much higher pure solution so molecular diffusion and adsorption rate of OSA-1 solutions was less than pure solution. on the other hand, average bubble size produced in OSA foam was 120.2. In contrast to proteins, the adsorption rate is dominant factor affecting foamability of starch solution. The results indicated that overrun for OSA-1 should be less than without it. The difference in foamability of OSA and pure solution is dramatically enhanced

by increasing the concentration of starch, which corresponded to a sharp increase in viscosity of OSA solution.

Drainage

Foams are thermodynamically unstable, and their stability is affected by factors such as drainage, disproportionation, and coalescence. Drainage of liquid (including the gravitational drainage and marginal regeneration) consists of the liquid flow from the lamellae to the plateau borders.

Viscosity of the fluid continuous phase has an effect on Protein foam stability [20]. A high viscosity should avoid the movement of liquid through the network of thin films and plateau borders, thereby slowing the drainage rate [21-22]. Generally, increase of OSA could decrease foam overrun and increase foam stability. The increase in foam stability is based on a decreased drainage rate; usually associated with an increase in continuous phase viscosity (Figure 7).

In case of OSA and EWP protein solutions, Enhancement of OSA concentration led to decrease of drainage. This result is in agreement with our expectation. Since the viscosity of solutions increased, film drainage would decrease and foam stability would increase.

But, in case of OSA and PPI protein solutions, enhancement of OSA concentration led to decrease of drainage. This result indicated that in despite of viscosity increase, because of weak interaction between OSA and PPI, increase of drainage has been occurred. Interaction of PPI with OSA was not strong and therefore, foam stability would decrease and drainage would be increased. Finally, it could be concluded that PPI protein could not interact with OSA well. So PPI protein foams would be unstable. Conversely, decrease in drainage rate with increase of starch in case of EWP, indicated good interaction between EWP and starch.

Effect of pH

The effect of pH on the foam properties of EWP, PPI and OSA was determined by measuring the foamability, life time of foam and drainage in the pH range 3.5 to 7 at an initial protein concentration of 0.5%. Generally, OSA, which are typically surface active with appropriate hydrophobicity, can co-adsorb to the air-water interface by the formation of soluble complexes with a protein. Changing the pH and ionic strength of solution around the isoelectric point of protein has influence on degree of complexation.

Effect of pH was studied in 3 points with positive, negative and neutral charges. In pH=7 which was above the isoelectric point (pI), proteins carried negative charge. In PI, pH=4.8 for EWP and pH=4.5 for PPI, they were neutral and in pH=3.5, below the PI, they carried positive charge.

According to table 2, in case of OSA+EWP, in isoelectric point (pH=4.8) there was an extremum point. In PI, due to enhancement of protein adsorption, overrun and half time of EWP foams has been increased and therefore drainage has been decreased. In case of PPI with OSA, change in pH has a little effect on overrun, but the effect of pH variation has been seen in PPI+OSA half time and drainage. Half time has been in maximum amount and drainage has been in minimum amount in pI point due to increase of protein adsorption of at the air-water interface.

Table 2. Analytical results of spike of Hg^{+2} and R-Hg concentration in human blood samples

Sample	Added (μg L^{-1})		Found[a](μg L^{-1})		Recovery (%)	
	Hg (II)	R-Hg	Hg^{+2}	R-Hg	Hg^{2+}	R-Hg
Blood A	-----	-----	1.25 ± 0.04	0.65 ± 0.02	-----	
	1.0	-----	2.27 ± 0.11	0.62 ± 0.03	102	
	-----	1.0	1.22 ± 0.06	1.63 ± 0.05	-----	
Blood B	-----	-----	0.82 ± 0.03	0.43 ± 0.02	-----	-----
	0.2	-----	1.01 ± 0.05	0.45 ± 0.03	95	-----
		0.2	0.84 ± 0.04	0.64 ± 0.03	-----	105
Blood C	-----	-----	5.65 ± 0.22	2.24 ± 0.09	-----	-----
	2.0	-----	7.58 ± 0.34	2.19 ± 0.11	97	-----
		2.0	5.61± 0.26	4.26 ± 0.18	-----	101

[a] Mean of three determinations ± cconfidence interval (P=0.95, n=5).

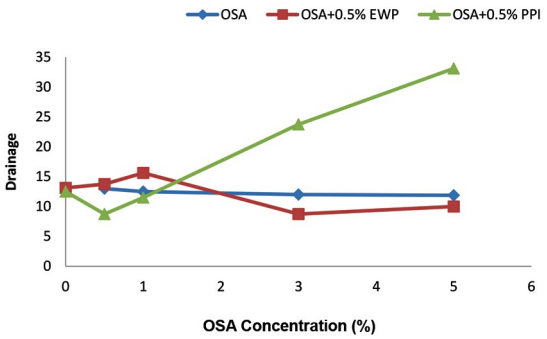

Fig. 7. Effect of adding OSA and protein on drainage.

CONCLUSION

In conclusion, as a result of an increasing concentration of starch, foam stability increases. Moreover, the effect of starch on egg white proteins is greater than on pea proteins. Adding starch to the proteins had very little effect on viscosity and the advantages of using starch as a nano-particle is firstly, starch is an organic nano-particle and secondly, it will be able to increase the stability without a decrease of foamability. In an isoelectronic point (pI), due to improvement of protein adsorption, overrun and the half-life time of egg white protein foams treated by starch have been increased and therefore drainage has been decreased. Moreover, egg white protein is more stable compared with pea proteins. There is the most important conclusion for all the results, which were given from the experiment:

In OSA samples, adding protein had very low effect on viscosity and therefore, there was no drainage limitation.

In OSA, surface tension is not a limiting factor.

Egg white protein foams had a much higher drainage half-life time than PPI foams at the same starch concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Physical chemistry of surfaces, A.W. Adamson and A.P. Gast, 1997, Wiley, 1st ed. New York.
2. P.A. Wierenga and E.S. Basheva, Colloids Surf., A, 344, 72 (2009).
3. X. Du, L. Zhao and H. Chen, Colloids Surf., A, 436, 599 (2013).
4. J.M. Rodriguez Patino, M. Dolores Naranjo Delgado and J.A. Linares Fernfindez, Colloids Surf., A, 99, 65 (1995).
5. X. Yang and E.A. Foegeding, Food Hydrocolloid, 24, 227 (2010).
6. P.J. Wilde, Curr. Opin. Colloid Interface Sci., 5, 176 (2000).
7. A. Timgren, M. Rayner and P. Dejmek, Food Sci. 1, 157 (2013).
8. P.A. Wierenga and H. Gruppen, Curr. Opin. Colloid Interface Sci., 15, 365 (2010).
9. Handbook of Food Proteins, T. Strixner and U. Kulozik, 2011, Cambridge, UK: Woodhead Publishing Limit.
10. J.M. Rodriguez Patino, C.C. Sanchez and M. Rosario Rodriguez Nino, Adv. Colloid Interface Sci., 140, 95 (2008).
11. I. Nicorescu, C. Vial and E. Talansier, Food Hydrocoll, 25, 797 (2011).
12. M. Kuropatwa, A. Tolkach and U. Kulozik, Food Hydrocoll, 23, 2174 (2009).
13. E. Talansier, C. Loisel and D. Dellavalle, LWT-Food Sci. Technol., 42, 496 (2009).
14. MA. Bos and T. Van Vliet, Adv. Colloid Interface Sci., 91, 437 (2001).
15. M. Juan, R. Patino, C. Carrera Sánchez and Ma. Rosario Rodríguez Niño, 140, 95 (2008).
16. R. Miller, V.B. Faineman, A.V. Makievski, J. Kra¨gel, D.O. Grigoriev and V.N. Kazakov, Adv. Colloid Interface Sci., 86, 39 (2000).
17. C.J. Beverung, C. J. Radke and H.W. Blanch, Biophys. Chem., 81, 59 (1999).
18. O.E. Perez, C.C. Sanchez, A.M.R. Pilosof and J.M.R. Patino, Food Hydrocoll, 22, 387 (2008).
19. T.K. Berry, X. Yang and E.A. Foegeding, J. Food Sci., 74, 269 (2009).
20. P.J. Halling, Crit. Rev. Food Sci. Nutr., 15, 155 (1981).
21. E. Xin Yang and A. Foegeding, Food Hydrocoll, 24, 227 (2010).
22. D. Oboroceanu, L. Wang and E. Magner, J. Food Eng., 121, 102 (2014).