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РЕСПУБЛИКИ КАЗАХСТАН

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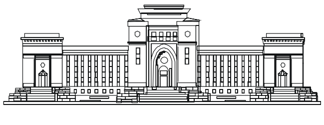

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STUDY OF FOAMS OBTAINED FROM *SOLANUM TUBEROSUM* PROTEIN EXTRACT: PROTEIN, GAS AND POLYSACCHARIDE INTERACTION

Abstract. The protein foams in food industry are often produced by animal protein, with consequent ethical (vegetarians) and health (allergies) problems,for these reasons proteins of plant origin are considered a new strategy in the improving of food safety.In this regard, the use of proteins of vegetable origin as the surface active compounds, specificallyfoaming characteristics were studied. These proteins were obtained from*Solanum tuberosum* (Agria variety), and from an extract of commercial patatin(Laffort). The topic was created by the need to recover the waste from the processing of potato starch, rich in proteins with interesting nutritional and technological properties but also cause of environmental issues.The extracts of potato were studied in different conditions of pH and in the presence of salt in order to better understand the optimal operating conditions for the production of stable foam. The results showed that the proteins of the potato give polydisperse foams, more or less stable and require the addition of polysaccharides to improve their performances. By analyzing the image of foam, some parameters regarding the size of the bubbles could forecast foam stability. For the obtaining a foam by the "sparging" method, with the addition of gas injected into the solution was used that could improve the characteristics of the foam.The result of this research confirm the good attitude of potato protein to produce stable foam.

Key words: foam, protein, potato, starch, surface active compounds, hydrocolloids.

Introduction

The proteins of potato tubers are of excellent nutritional quality and are superior to the protein of cereals and legumes [1]. Potato protein has highLysine content, which is present usually in a lesser amount in many other crops [2]. In addition, the potato protein digestibility as measured by PER (protein efficiency ratio) is exceptionally high and is considered very well in terms of biological quality among the major plant proteins [3]. Potato soluble proteins were identified and classified into 3 categories [4]: patatin, protease inhibitors and complex proteins at 22 kDa. Patatin is the most abundant fraction of potato juice and includes up to 30-40% of the protein; patatin is a family of glycoproteins of about 40 kDa determined by SDS-polyacrylamide electrophoresis (SDS-PAGE) [5]. The patatin fraction is a very promising foaming agent with foaming and stability similar to albumin powder [6]. Ralet et al. [6] studied the different foams of the different potato protein parts and found that the foams obtained by the patatin fraction are very stable, both in the presence and absence of NaCl and in different pH conditions. In addition, the foams of the potato protein are more stable than egg protein foams, with or without the addition of NaCl.

Stability is the most important property of a foam contained in a food: once the desired characteristic for the product has been obtained, the structure must be maintained at least until the product is consumed. Destabilization processes can be well distinguished when a foam is left to rest [7]: 1) the coalescence of gas bubbles; 2) disproportionate gas bubbles (polydispersion); 3) drainage liquid. Drainage involves the loss of liquid from the foam matrix, resulting in flow between the gas bubbles and the solution [8].

The increase in bubble size can be slowed by increasing the viscosity of the initial solution, before foaming. Coalescence is determined by the stability and attraction/repulsion forces between the thin liquid film forming the bubbles [9], while disproportionation (polydispersion) occurs through the diffusion of gas between bubbles of different sizes. The excess pressure inside the bubble, also known as Laplace pressure (LP), is inversely proportional to the diameter of the bubble itself. Thus, the concentration of dissolved gas at the interface of a small bubble will be greater than that at the interface of a large bubble. This favors the mass transfer and therefore a net loss of gas from the small to the largest bubble (Oswald ripening). The disproportion rate will depend on Laplace pressure, gas solubility, and other geometric factors [10].

Foam stability is most commonly monitored following its collapse (foam volume variation: SVS%) and increased volume of drainage liquids (SLS%). Both of them are macroscopic properties and can be easily measured, thus allowing a description of foam behavior over time [8].

The work purposeis to study the foaming properties of a potato proteins extract, *Solanum tuberosum*(Agria variety) [17], and compare them to those of a commercial potato extract. In particular, the presence of polysaccharides affects the viscoelastic properties of the solution and the foam with consequences on stability and expansion. For this reason, the two protein extracts will be studied in the presence of k-carrageenin, pectin and Arabic gum at different pHs. The "sparging" method, by adding gas to the bulk solution, can modify the foam characteristics. We propose to study the expansion of the foams obtained with CO_2 and N_2 from the two extracts at different pHs.

Materials and methods

Agria (0.5 Kg) tubers were shaken in presence of 100 ml of water mQ, 5 g of PVPP and 50 mM of DTT. After centrifugation, the supernatant was dialysed with 1 kDa membrane and lyophilized.

Foam production for "sparging" gas method

For the measurement of the foam by sparging gas, an instrument with a graduated cylindrical glass column in centimeters with a porous septum and faucet was used, connected to a CO_2 or N_2 at one atmosphere (Fig.1).

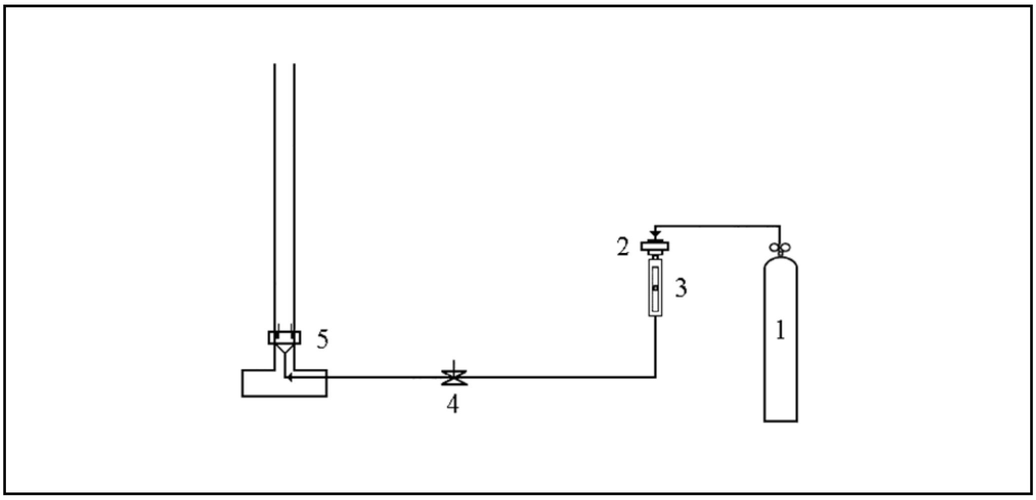


Figure 1 - Sparging column model:

1) CO_2 or N_2 cylinder,
2) Pressure regulating valve,
3) Gas injection hose,
4) Gas tap opening and closing valve,
5) Cylindrical glass column in centimeters with porous septum.

Samples were previously prepared in falcon: 20 ml of buffer (at different pHs, McIlveine buffer: pH 3 to 7) and 5 mg/ml of potato protein extract. Subsequently, falcons were mixed by vortex to allow the solubilization of the potato powder inside the buffer without foam formation. The sample were placed inside the cylinder and the tap was opened to allow the gas to be released (a CO_2 test was then followed by N_2) for a minute to be able to observe the foam expansion. The prepared samples were:

- 5 mg / ml of potato protein extract Agria variety + 20 ml of pH buffer 3 to 7,
- 5 mg / ml of Patatin Protein Extract + 20 ml of pH buffer 3 to 7.

Subsequently samples were prepared by adding 1% NaCl to observe the stability of the foam due to the ionic strength responsible for the interactions between proteins present in the foam film. The prepared samples were:

- 5 mg / ml of potato protein extract Agria + 200mg of NaCl + 20 ml of pH buffer 3 to 7,
- 5 mg / ml of Patatine Protein Extract + 200mg of NaCl +20 ml of pH buffer 3 to 7.

The following parameters were studied:

- %FE = (*ml foam volume*)/(ml *initial liquid volume*)x100
- *Sauter coefficient* or d_{32}

Sautercoefficient was calculated on the diameters of the bubbles analysed by stereo microscope SteREO ZEISS LUMAR software.

Images were also studied by optic microscope and electronic microscope SEM.

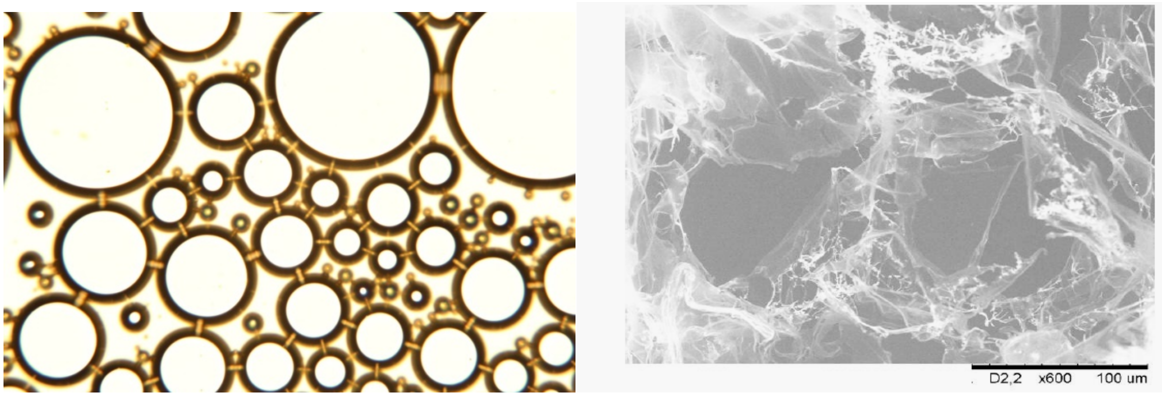
Polysaccharides

Prior to the foaming, polysaccharides were added at a concentration of 5 mg/ml to the buffered solutions in which the protein extract of Patatina and Agria was added. Polysaccharides are k-carrageenin, pectin and arabic gum (Sigma).

All experiments were carried out in triplicate.

Results and Discussion

The ionic strength, pH and the nature of the protein extract can affect the size of bubbles [11]. An image study on Agria foam was performed using a 60-magnification optical microscope, (Fig 2A) and the electronic microscope (Fig.2B) and a processing software allows the measurement of foam bubbles diameters.



A B

Figure 2. Potato protein foam observed at optic microscope (A) and electronic SEM microscope (B)

As shown in Table 2, the d_{32} is particularly high at pH 5 and 6, and this is due to the presence of high-density bubbles and low foam stability. In contrast in pH 7, the d_{32} has the lowest value, followed by the pH 3 and 4.

Fig. 3 reports descriptive data analysis in the form of box plot. As shown in the figure, at pH 4 there is a low variability, in fact, excluding the outlier values, the interquartile rank is lower than the others. The VC coefficient, as in all other cases, is quite high and equal to 48.4% (Table 1), while the median is close to the mean value of 101.85 µm (average diameter of the bubbles). On the contrary, the distribution with greater variability is at pH 5; in fact, as shown in Fig. 3, the interquartile rank is particularly wide.

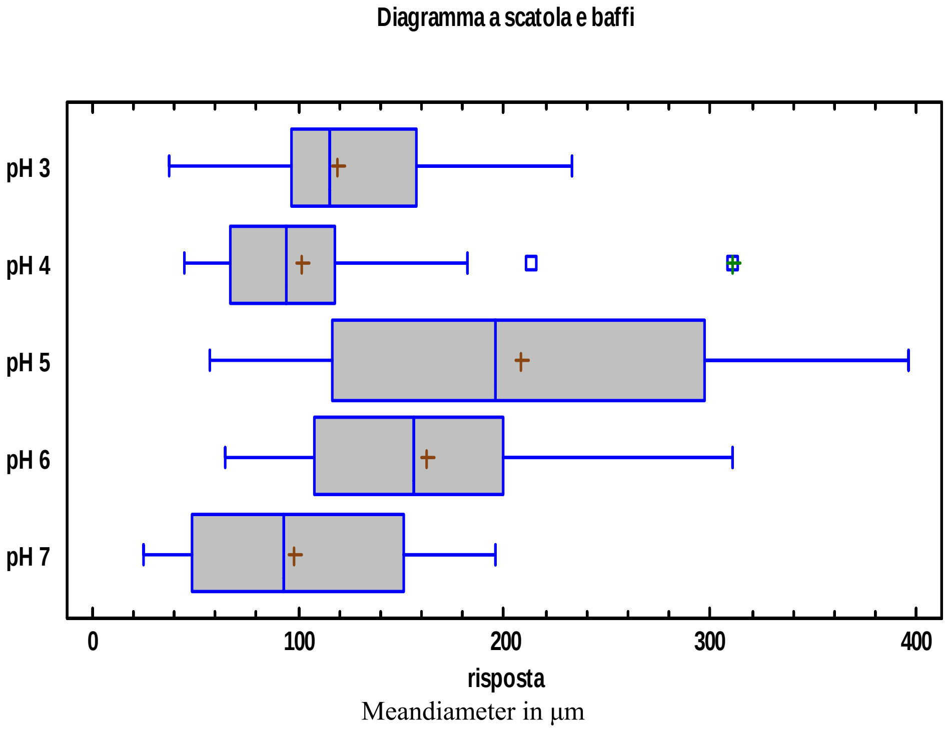


Figure 3 - Box Plot statistical analysis of the diameters of the foam bubbles, obtained at different pH

Observing the VC (Table 1), we notice that this parameter is greater than 40%, up to 54% at pH 7. By this analysis it could be stated that there is variability (polydispersion) expressed in terms of bubbles size, more or less accentuated depending on the pH of the solutions.

Table 1 - Statistical analysis carried out on the diameter of foam bubbles,obtained at different pH

	Medium	*Diversion standard*	*Coeff. of variation*	*Min.*	*Max.*	*Range*
pH 3	119,362	49,7126	41,6486%	37,18	233,11	195,93
pH 4	101,854	49,3063	48,4091%	45,36	310,41	265,05
pH 5	208,445	96,6788	46,381%	57,19	395,63	338,44
pH 6	162,122	69,27	42,727%	65,37	310,87	245,5
pH 7	97,8807	53,7626	54,9267%	24,78	195,2	170,42

Fig. 3 shows also the expansion of foams obtained by Agriaproteins and commercial patatin at different pHs and generated by N_2 by the sparging method. As shown in Fig. 3, the expansion of the potato foam seems to show values of the same order of magnitude of those obtained with CO_2. Even in this case there is a certain expansion at pH 5. The presence of salt, in the foam obtained with N_2, tends to reduce the FE% value with the exception of the foam at pH 7 which remains unchanged with respect to the sample without NaCl. Agria has an expansion of between 300% and 400% (as in the case of CO_2) and seems to be significantly affected by the presence of salt. Indeed, NaCl achieves in many cases 400% expansion (FE%).

Table 2 - $d_{32}$of diameters of the foam bubbles

pH	d_{32}
3	154,19
4	156,5982
5	282,2808
6	216,8933
7	144,8264

Expansion and stability of foam in presence of k-carrageenin, Arabic gum and pectin.

Generally, proteins alone are unable to form stable foams without the addition of other stabilizing agents, including, most commonly used polysaccharides. These are the most widely used in the food industry because they give texture characteristics as thickeners and gelling agents. Food macromolecules, such as proteins and polysaccharides, play a very important role in foam stabilization [12].

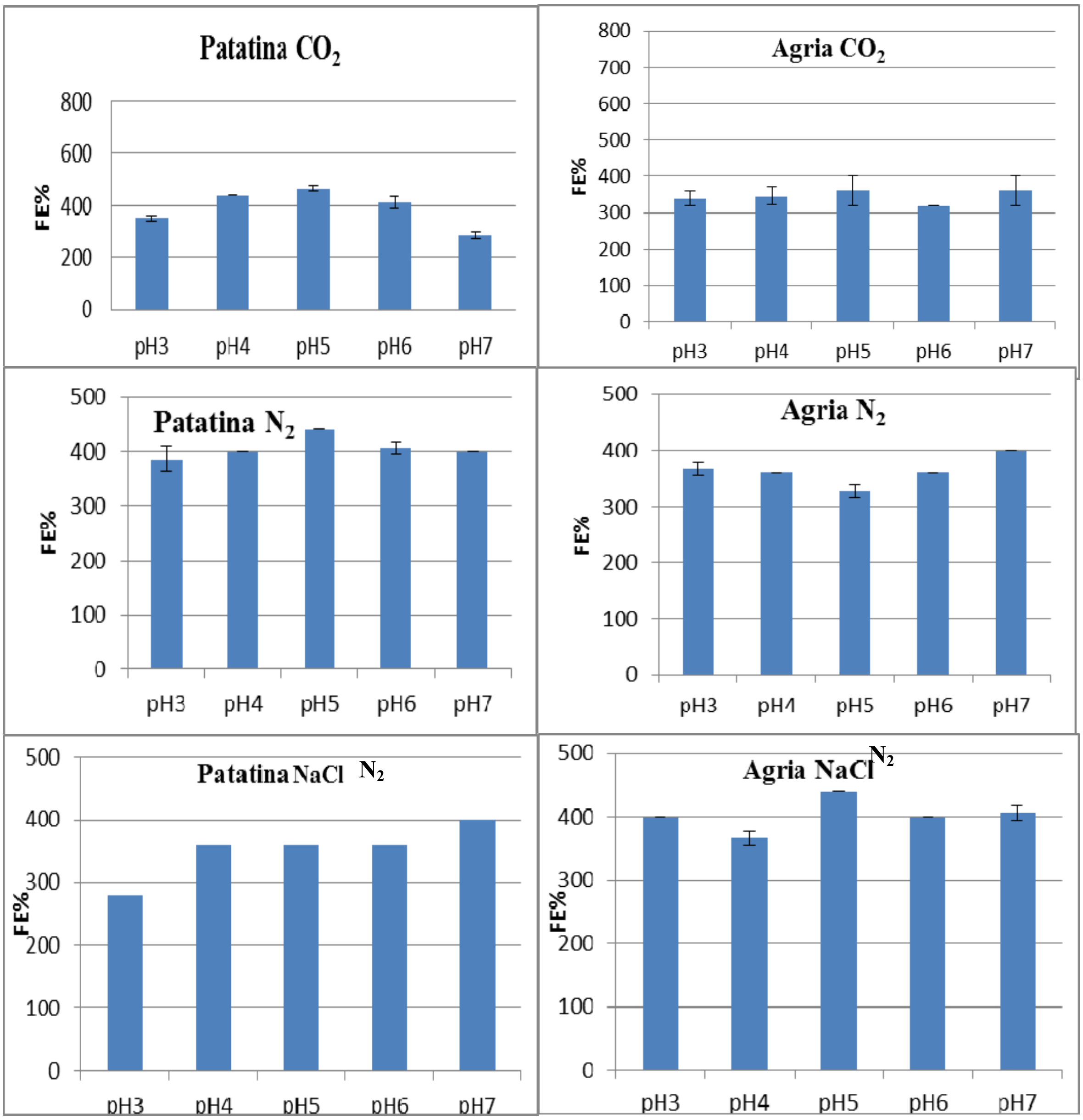


Figure 4 - Foam expansion (FE%) of the foams obtained at different pH, and in presence of CO_2, N_2, and NaCl

They act by delaying the drainage of the liquid and producing a viscoelastic film on the surface of the bubbles which protects them from breakage; all this prevents or delays the phenomenon that goes under the name of "Ostwald ripening" (larger bubbles grow at the expense of smaller ones).

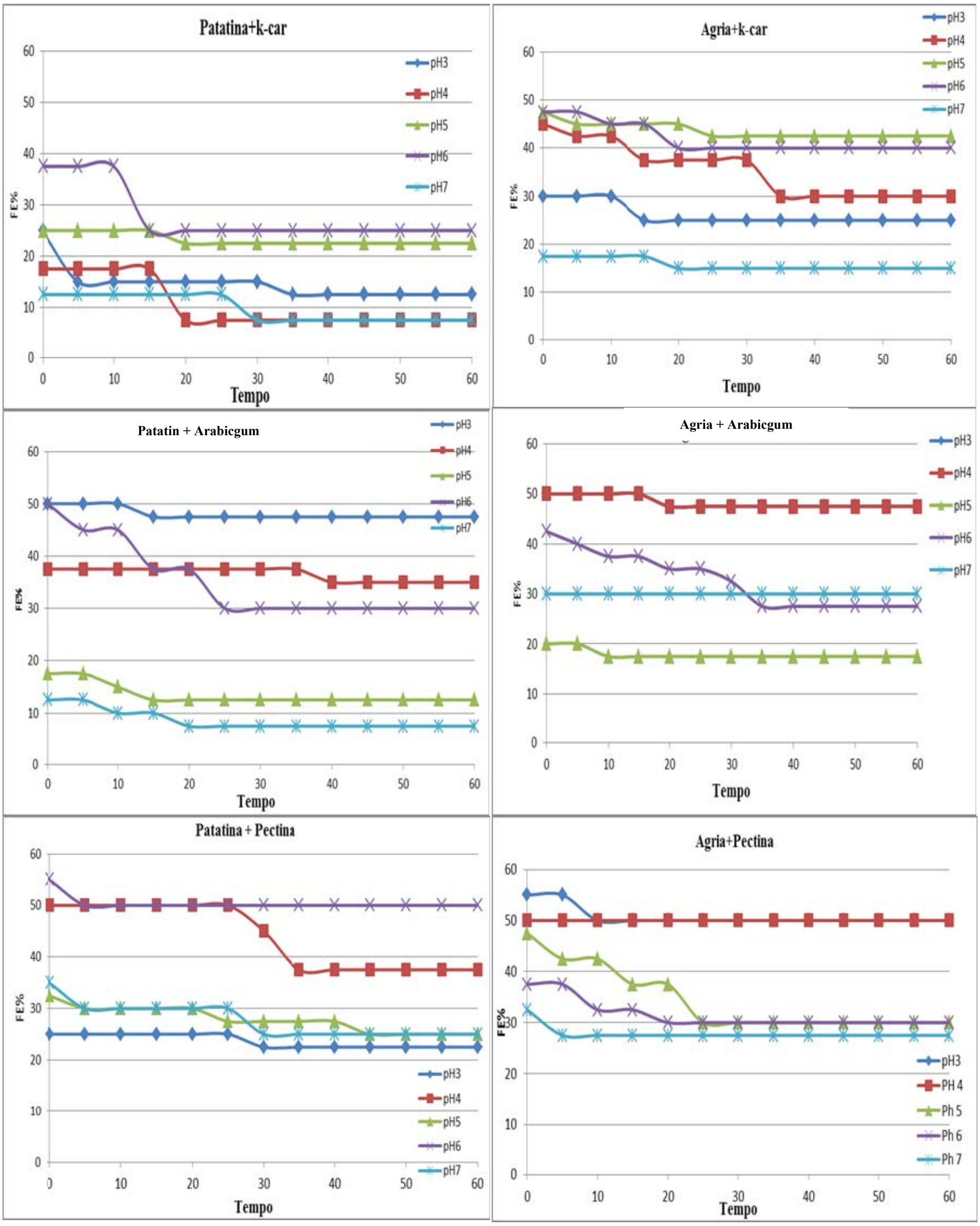


Figure 5 - Foam expansion (FE%) and stability over time of the foams obtained at different pH
and in presence of k-carragenin, Arabic gum and pectin

While proteins contribute to foam formation due to hydrophobicity and possible conformational rearrangement that allows quick adsorption to the air-water interface and leads to the formation of a coherent viscoelastic film [8], many hydrophilic polysaccharides do not adsorbe themselves to the interface. However, they can improve the stability of the protein foam by gelling the aqueous solution [13].

We analysed the behavior of the foam obtained from the proteins of Agria and the Patatin protein extract at different pHs and in the presence of k-carrageenin, Arabic gum and pectin. As shown in Fig. 4, the presence of polysaccharides such as k-carrageenine, Arabic gum and pectin decreases foam expansion as a result of the viscosity of the bulk solution.

As shown in Fig.5, the FE% parameter, monitored within 60 min does not undergo major variations. It seems that the presence of polysaccharides prevents the collapse of the foam. The presence of k-carrageenin results in an expanded foam, from 12 to 25% when present in pH 7, 5, 4 and 3 buffer. At pH 6 there is an expansion of 39% followed by a reduction (reaches 25%). In the presence of k-carrageenan, Agria's foam behaves differently from that of commercial patatin. In fact, 46% of initial FE% of foam is achieved in the presence of pH 4, 5 and 7. At pH 7 and 3, the FE% value is 29 and 30%, respectively, and, as in the case of patatin (Fig. 4), represents the lowest values. The presence of k-carrageenin in the protein solution can alter the behavior of proteins in the formation of foam. In fact, it has been reported that k-carrageenin-protein systems show a synergistic effect between the two polymers on gelling properties when the pH is above the pI of proteins [14].

In solution, the synergistic effect between the two polymers seems to be thermodynamically incompatible. At low pH values polysaccharides, characterized by the presence of S group, and the proteins interact by forming bonds. By increasing the pH beyond the proteins pI, the electrical charge of these proteins becomes negative. Consequently, repulsive electrostatic forces between protein molecules and polysaccharides increase. This leads to a mutual concentration of both polymers (polysaccharide and protein) in separate microfibers and promotes the gelatinization of polysaccharide [15]. In addition, the presence of a polysaccharide in the protein solution can modify the protein's foamy properties and impart desired product characteristics (eg puddings and mousse). Indeed, if the continuous phase of the proteins that form the foam gelates, interesting foam and texture stability features may emerge. It has been observed that adding k-carrageenin of soy proteins decreases foam expansion since the incorporation of the air is limited by the increased viscosity of the solution (gelating foam).

In this experiment, low concentrations of protein extract and k-carrageenin (5 mg k-carrageenine/ml solution) do not favor gel formation (in fact, the foam and liquid phase maintain a fluid consistency, data not shown). The presence of arabic gum (Fig. 5) determines: a FE% value (about 20%) at pH 5 very similar in Potatin and Agria and a value of 50% for both Patatin and Agria at pH 3. Both in the case of patatin and Agria the expansion (FE%) tends to decrease to pH 6 reaching about 30% after 60 min. At pH 7, the expansion of the Patatin foam is particularly low (equal to 10%) and tends to decrease as in the case of k-carrageenin. In the case of Agria, however, the expansion stands at 30% and remains constant.

The presence of pectin (Fig. 5) determines an expansion of 60% potatin foam at pH 6, and at pH 4 reaches 50% and then decreases to 38% after 30 minutes. On the contrary, FE% (50%) of Agria's foam at pH 4 remains constant over time. At pH 3, 5 and 7, the initial expansion of Patatin foam reaches 25-35% although the value decreases to stabilize around 23-25%. Agria foam at pH 5, 6 and 7, while achieving an initial FE% of 48, 38 and 32%, tends to stabilize at values close to 30%. In this case, at pH 3, Agria displays a FE% of 55% and stabilizes at 50%.

Goff et al. (2006) [16] proposed several mechanisms that describe the role of polysaccharides in controlling protein absorption at the gas-water interface. In the first case, only "free" proteins are available in the adsorption phenomenon at the gas-water interface and surface tension lowering. In the second case, adsorption to the interface involves the combination of protein-polysaccharide complex and free protein in the continuous phase. The rate of diffusion of newly formed complexes may be lower than that of free proteins and this results in a slower migration from the bulk solution to the interface.

This phenomenon involves an additional mechanism for association-dissociation of proteins from the complex with polysaccharide. This theory suggests that complexes close to the gas-water interface may undergo partial protein dissociation from the original complex and this depends on the strength of interactions with the biopolymer and the solvent conditions. The pH value thus becomes crucial in creating or not protein-polysaccharide complexion phenomena and affecting the viscosity of the bulk solution and the properties of the foam.

Conclusion

By the results obtained from this research it is possible to state that proteins extracted from Agria and Patatin form foams with stability and expansion characteristics that depend on the protein's nature of the sample, pH, presence of NaCl and polysaccharides. The image analysis of the bubbles, though only performed on the patatin sample, allowed to characterize the foams and to study the dimensions of the structures by linking them to the pH of the bulk solutions. By analysiing the bubbles and studying the frequency distributions, it can be deduced that the system is "polydispersed" and characterized by a certain heterogeneity in the dimensions due to pH.

This polydispersion is particularly evident at pH 5, close to pH of the potato protein. However, the presence of other proteins in the Agria extract, characterized by different pHs, makes complex understanding the foaming behavior of the sample. The presence of salt decreases the expansion of Agria's foam, while it increases that of Patatin. In all cases, stable foams are obtained over time, and this confirms the good foaming potentials of potato protein. The addition of polysaccharides limits foam expansion in all cases as a result of higher viscosity of the bulk solutions. However, protein-polysaccharide complexes or interactions give high foam stability, especially when the protein extract is represented by Agria. The polysaccharide that gives greater foam stability (expressed in SVS%) is pectin, both in the presence of Agria and Patatin. The sparging method allows to obtain very expanded foams than the mechanical stirring method, especially when CO_2 is used. The phenomenon is accentuated in the presence of NaCl. Even the N_2 allows a considerable expansion of the foam even if the "salt" effect is much lower.

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SOLANUM TUBEROSUM ПРОТЕИН ЭКСТРАКТІНІҢ КӨБІГІН ЗЕРТТЕУ: АҚУЫЗ, ГАЗ ЖӘНЕ ПОЛИСАХАРИДТЕР ӘРЕКЕТТЕСУ

Аннотация. Тамақ өнеркәсібіндегі ақуыз көбігі көбінесе жануарлар белоктарынан жасалады, содан кейін этикалық (вегетарианшылар үшін) проблемалар мен денсаулық проблемалары (аллергиялар) осы себептерге байланысты өсімдік белоктарының азық-түлік қауіпсіздігін жақсартудың жаңа стратегиясы болып саналады. Осыған байланысты өсімдік протеиндерін беттік-белсенді заттар, әсіресе көбіктендіретін қасиеттерін қолдану зерттелді. Бұл ақуыздар Solanum tuberosum (Agria сынып) және коммерциялық пататин сығындысынан (Laffort) алынған. Бұл зерттеу қызықты тамақтану және технологиялық қасиеттері бар ақуыздарға бай картоп крахмалы қалдықтарын өңдеу, сондай-ақ экологиялық проблемаларды шешу қажеттілігіне байланысты болды. Тұрақты көбік алу үшін оңтайлы жұмыс жағдайларын жақсы түсіну үшін әртүрлі pH жағдайында және тұз болған кезде картоп сығындылары зерттелген. Нәтижелер картоптың ақуыздары көп немесе аз тұрақты болып табылатын полидисперсті көбік түзеді және олардың сипаттамаларын жақсарту үшін полисахаридтерді қосуды талап етеді. Көбік кескіндерін талдау кезінде көпіршіктердің мөлшеріне қатысты кейбір параметрлер көбік тұрақтылығын болжауға мүмкіндік береді. Көбік алу үшін көбік сипаттамаларын жақсарта алатын ерітіндіге енгізілген газды қосу арқылы «бұршақпен» әдісі қолданылды. Осы зерттеудің нәтижесі тұрақты көбік алу үшін картоптың ақуызының жақсы жағдайын растайды.

Түйін сөздер: көбік, ақуыз, картоп, крахмал, беттік белсенді заттар, гидрохоллоидтер.

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ИССЛЕДОВАНИЕ ПЕНЫ, ПОЛУЧЕННОЙ ИЗ ЭКСТРАКТА ПРОТЕИНА *SOLANUM TUBEROSUM*: ВЗАИМОДЕЙСТВИЕБЕЛКА, ГАЗА И ПОЛИСАХАРИДОВ

Аннотация. Протеиновые пены в пищевой промышленности чаще всего вырабатываются из животного белка с последующими этическими (для вегетарианцев) проблемами и проблемами со здоровьем (аллергиями), по этим причинам белки растительного происхождения считаются новой стратегией улучшения безопасности пищевых продуктов. В связи с этим в работе было изучено использование белкорастительного происхождения в качестве поверхностно-активных соединений, в частности, вспенивающих свойств. Эти протеины были получены из *Solanumtuberosum* (сорт Agria) и из экстракта коммерческого пататина (*Laffort*). Настоящее исследование было вызвано необходимостью переработки отходов картофельного крахмала, богатых протеинами с интересными питательными и технологическими свойствами, а также вследствиерешения экологических проблем. Экстракты картофеля изучались в разных условиях pH и в присутствии соли, чтобы лучше понять оптимальные рабочие условия для производства стабильной пены. Результаты показали, что белки картофеля дают полидисперсные пены, более или менее стабильные и требуют добавления полисахаридов для улучшения их характеристик. Анализируя изображения пены, некоторые параметры, касающиеся размеров пузырьков, можно прогнозировать стабильность пены. Для получения пены был использован метод «барботирования» с добавлением газа, впрыскиваемого в раствор, что могло бы улучшить характеристики пены. Результат этого исследования подтверждает хорошее положение белка картофеля для получения стабильной пены.

Ключевые слова: пена, белок, картофель, крахмал, поверхностно-активные вещества, гидроколлоиды.

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