Research Article

Role of Polysaccharides in Complex Mixtures with Soy Protein Hydrolysate on Foaming Properties Studied by Response Surface Methodology

Karina D. Martínez and Ana M. R. Pilosof

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina

Correspondence should be addressed to Karina D. Martínez; karinadafnem@yahoo.com.ar

The complex mixture studied, a hydrolyzed soy protein (HSP), κ-carrageenan (κC), and an hydroxypropyl methylcellulose (HPMC), could be used as a foaming agent under refrigeration or heating conditions because of the presence of one polysaccharide (HPMC) that gels on heating and another (κC) that gels on cooling. The objective of this work was to study the role of these polysaccharides on foaming properties by whipping methods at heating conditions. For this purpose, response surface methodology was used to optimize the mixed product in foamed food systems. The obtained results showed that the combination of E4M, κC, and HSP is an adequate strategy to generate good foam capacity and stability at heating conditions. The huge stability increase of foams at heating conditions was ascribed to combined effect of polysaccharides: gelling property of E4M and the viscozieng character imparted by κC to continuous phase of foaming.

1. Introduction

Soybean proteins are widely used in many foods as functional and nutritional ingredients [1].

Native soy protein, because of its compact tertiary structure, has limited foaming [2–5] and emulsifying [6–8] properties. Structural modifications allowing for greater conformational flexibility of protein may improve their ability to stabilize foams and emulsions. Many studies have demonstrated that the enzymatic hydrolysis of soy proteins improves their functional properties, including solubility, emulsifying, and foaming characteristics [9–11]. Nevertheless, it has been reported that limited hydrolysis may improve foaming capacity but decrease foam stability [12–15]. Therefore, their use would require the addition of polysaccharides as stabilizers. Most high-molecular weight polysaccharides, being hydrophilic, do not have much of tendency for adsorption at the air-water interface, but they can strongly enhance the stability of protein foams by acting as thickening or gelling agents [16]. However, these gelling properties would

change as temperature increases or decreases conforming cooking process. As a result, an adequate hydrolyzed soy protein-polysaccharides mixed system in an appropriate concentration would be useful to achieve acceptable foaming properties. So, a HPMC with gelling capacity on heating conditions and κC with gelling properties on cooling ones were selected. Thus, the mixed system could be produced and used on heating or cooling treatments.

In a previous work, we study the rheology, through elastic component (G') and relative viscoelasticity ($\tan \delta$) and thermal transitions of continuous phase of the same systems to determine their behavior at 70°C. It was concluded that E4M was the principal component which determines the elastic and viscoelastic characteristics of complex mixture at 70°C, due to the gelled structure produced at heating. However, the presence of high hydrolyzed soy protein and/or κC concentration would inhibit the E4M gel formation which resulted in a low G' and high $\tan \delta$ values. On the basis of these results, we intend to associate them with the foaming properties at 70°C.

The system studied in the current work involves these biopolymers, with changes of concentration according to the Doehlert matrix [17] used as experimental design. Hot temperature was selected (70°C) to simulate heating treatments and study the behavior of the system foamed.

Accordingly, an optimizing method was used to study the role of these polysaccharides on foaming properties by whipping methods at heating conditions.

2. Materials and Methods

2.1. Materials. A commercial soy protein isolate (SP) (90% protein) from Sanbra, Brazil, was used as substrate for the hydrolysis with fungal protease from *Aspergillus oryzae* with endopeptidase activity, provided by Quest International. The protein isolate was denatured as detected by differential scanning calorimetry. The following polysaccharides (PS), from Sanofi Bio-Industries, Argentina, were used without further purification: κ-carrageenan κC and a hydroxypropyl methylcellulose (HPMC) called E4M from Dow Chemical Co.

2.2. Enzymatic Hydrolysis. SP isolate (72 g in 1.200 mL of water) was hydrolyzed according to Zylberman and Pilosof [18] batchwise by treatment with fungal protease at pH 7, 50°C for 1 h, with enzyme/substrate (E/S) ratio: 2/100. Hydrolysis was stopped by heating at 80°C for 10 min.

The variation in pH was very small (maximum decrease 0.3 pH units) and was adjusted back to the original value with diluted NaOH. Hydrolysates were lyophilized.

The degree of hydrolysis (DH), defined as the percentage of peptide bonds cleaved, was calculated from the determination of free amino groups by reaction with *o*-phthaldialdehyde (OPA) according to Church et al. [19]. Protein hydrolysate with 4% DH (HSP) was obtained.

2.3. Foaming Properties

2.3.1. Foam Formation. 20 mL of solutions was foamed at 60°C, where κC was not gelled [20], in a graduated tube (3 cm diameter) for 3 min with Griffin & George stirrer at 2500 rpm. Overrun was calculated as follows:

$$FO\ (\%) = \left[\frac{\text{foam volume} - 20}{20} \right] 100. \tag{1}$$

The data reported are means of at least two replicates. The error was less than 10%.

2.3.2. Foam Drainage and Collapse. After foaming production, the stability of foams simulating cooking process was studied by increasing the temperature at 70°C. The stability against liquid drainage and collapse of height of foams at these conditions was recorded.

The liquid drainage was evaluated as the time to start the foam drainage (lag time to drainage, min) and the time required to drainage 20%. This drainage rate (k_{dr}, min^{-1}) was calculated as 1/time.

The foam collapse was calculated as the time required to start the collapse (lag time to collapse, min) and the time required to decrease in 2 mL the height of the initial foam (collapse time 2 mL, min).

The data reported are means of at least two replicates. The relative error for every measure was less than 10%.

2.4. Experimental Design. The combined effect of a hydrolyzed soy protein (4% DH), a hydroxyprpyl methylcellulose called E4M and κ-carrageenan concentrations, on foaming properties was evaluated by response surface methodology. A Dohelert design was selected to elaborate the experiment, which is associated with second-order models detecting optimum values and interactions [17]. This experiment involved 3 factors as independent variables where $x_1 =$ HSP, $x_2 = E4\,M$ and $x_3 = \kappa C$ with 7, 5, and 3 levels of concentrations, respectively. The concentrations used were 2 to 10% wt/wt for HSP, 0.2 to 1.8% wt/wt for E4M, and 0.2 to 1.8% wt/wt for κC.

The responses or dependent variables evaluated were the foam overrun at 60°C (FO), the foaming stability properties at heating conditions as liquid drainage: lag time to start the foam drainage *(lag time to drainage)* and their rate (K_{dr}), and collapse foams properties: lag time to start foam collapse *(lag time to collapse)* and the time for the collapse of 2 mL of their initial height *(collapse time 2 mL)*.

A second-degree polynomial model was fitted for each dependent variable as follows:

$$\begin{aligned} Y = {} & bo + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_1^2 + b_5 x_2^2 + b_6 x_3^2 \\ & + b_7 x_1 x_2 + b_8 x_1 x_3 + b_9 x_2 x_3, \end{aligned} \tag{2}$$

where Y is the corresponding dependent variable; *bo*, *bii*, and *bij* are regression coefficients, and *xi* denotes the coded independent variables, linearly related to HSP, E4M, and κC levels.

The real and coded levels of the independent variables used in the experiment design are shown in Table 1.

2.5. Statistical Analysis. The model goodness-of-fit was evaluated by the coefficient of determination (R^2) and the analysis of variance (ANOVA). The response surface and contour plots were developed using the polynomial equations, obtained by holding one of the independent variables at a constant value (at the central point) and changing the levels of the other two variables using Statgraphics Plus 3.0. software.

3. Results and Discussion

3.1. Foaming Properties at Heating Conditions. Table 2 shows the results corresponding to foaming properties obtained for all combinations studied at heating (70°C). It can be seen that FO value was inversely related to κC concentration in all EP (see also Table 1).

κC addition limited the air incorporation due to the high viscosity imparted during the foam formation. A similar behavior for foams of soy proteins in the presence of xanthan gum has been reported [21].

TABLE 1: Experimental matrix: real and coded (in brackets) values for the studied variables.

EP	HSP (wt/wt)	E4M (wt/wt)	κC (wt/wt)
1	6 (0)	1 (0)	1 (0)
2	6 (0)	1.8 (1)	1 (0)
3	6 (0)	0.2 (−1)	1 (0)
4	10 (0.866)	1. 4 (0.5)	1 (0)
5	2 (−0.866)	0.6 (−0.5)	1 (0)
6	2 (−0.866)	1.4 (0.5)	1 (0)
7	10 (0.866)	0.6 (−0.5)	1 (0)
8	7.33 (0.283)	1.4 (0.5)	1.8 (0.8165)
9	4.67 (−0.283)	0.6 (−0.5)	0.2 (−0.8165)
10	4.67 (−0.283)	1.4 (0.5)	0.2 (−0.8165)
11	8.67 (0.567)	1 (0)	0.2 (−0.8165)
12	7.33 (0.283)	0.6 (−0.5)	1.8 (0.8165)
13	3.33 (−0.567)	1 (0)	1.8 (0.8165)
14	6 (0)	1 (0)	1 (0)
15	6 (0)	1 (0)	1 (0)

EP: experimental point; HSP: hydrolyzed soy protein (4% DH), E4M: hydroxypropyl methylcellulose; κC: kappa carrageenan.

TABLE 2: Experimental points (EP) and the corresponding results obtained for foaming properties at heating conditions (70°C).

EP	FO (%)[*]	Lag time drain. (min)[*]	K_{dr} (min^{-1})[*]	Lag time to collapse (min)[*]	Collapse time 2 mL (min)[*]
1	150	14.0	2.78×10^{-2}	17.5	125
2	55	8.0	2.22×10^{-2}	63.5	220
3	55	11.5	4.26×10^{-2}	27.5	157
4	90	94.0	0.37×10^{-2}	58.5	235
5	140	9.0	5.71×10^{-2}	57.5	110
6	150	1.3	4.88×10^{-2}	41.8	60
7	60	16.7	1.56×10^{-2}	11.5	101
8	70	52.0	0.91×10^{-2}	10.8	45
9	250	8.5	7.27×10^{-2}	100.0	150
10	260	15.5	2.94×10^{-2}	111.0	135
11	200	13.0	3.85×10^{-2}	146.5	155
12	85	20.6	1.67×10^{-2}	115.2	162
13	150	15.0	3.28×10^{-2}	26.5	107
14	120	15.5	2.76×10^{-2}	15.5	123
15	110	16.0	3.00×10^{-2}	17.0	115

[*]Mean ± SD % less of 10% for foam overrun and drainage stability parameters of at least two replicates.
FO (%): foam overrun
Lag time drain.: time to start the foam drainage.
K_{dr}: 1/time required to drainage 20% of total possible.
Lag time to collapse: time required to start the collapse
Collapse time 2 mL: time required to decrease in 2 mL the height of the initial foam.

Authors of [22] have found similar results by using this polysaccharide on foams made from β-lactoglobulin, native and denatured soy protein. It was shown that κC addition decreased foam expansion, because air incorporation was limited by increasing the viscosity of the solutions.

Multiple regression analysis has been done for foaming parameters. The regression coefficients obtained are showed in Table 3.

In most cases R^2 resulted with a good fit and the "lack of fit" resulted nonsignificant which means that the model could explain the 80% of observed responses. In other cases, "lack of fit" resulted significant which means that the order of the regression was not secondary (the model may not include all appropriate functions of independent variables or the experimental region may be too large for a quadratic model that was used). However, when a large amount of data

TABLE 3: Model coefficients estimated by multiple linear regression for foaming properties.

	FO	lag time to drainage	K_{dr}	lag time to collapse	Collapse time 2 mL
Constants	125.278	13.177	0.0305	13.415	116.956
Linear					
HSP	−51.658**	27.212*	(−0.0034)	13.297*	34.321*
E4M	(5.997)	11.433*	(−0.0035)	−3.692*	11.561**
κC	−81.669*	11.802*	(−0.0001)	−39.449*	−22.629*
Quadratic					
HSP^2	(6.233)	19.088*	(0.0011)	12.016*	(−9.472)
E4M^2	(−69.783)	(−2.718)	(0.0017)	33.244*	72.986*
κC^2	83.312*	9.666*	0.0235*	99.748*	(1.663)
Interactions					
(HSP) × (E4M)	(3.109)	53.219*	0.0356*	56.760*	94.831*
(HSP) × (κC)	(9.192)	14.356*	0.0247**	−23.927*	−56.965*
(E4M) × (κC)	(−13.323)	(0.884)	0.0593*	−83.168*	−86.715*
R^2	0.964	0.852	0.8965	0.839	0.872
Lack of fit	NS	*	NS	*	*

HSP: hydrolyzed soy protein at 4% DH; E4M: hydroxypropyl methylcellulose; κC: kappa-carrageenan.
*Significant value at $P < 0.05$; **significant value at $P < 0.01$; ( ) nonsignificant value.
Reduced equations for foaming properties:
FO = 125.278 − 51.685 HSP − 81.669 κC + 83.312 κC^2
Lag time to drainage = 13.177 + 27.212 HSP + 11.433 E4M + 11.802κC + 19.088 HSP^2 + 9.666 κC^2 + 53.219 HSP × E4M + 14.356 HSP × κC
K_{dr} = 0.0305 + 0.0235 κC^2 + 0.0356 HSP × E4M + 0.0247 HSP × κC + 0.0593 E4M × κC
Lag time to collapse = 13.415 + 13.297 HSP − 3.692 E4M − 39.449 κC + 12.016 HSP^2 + 33.244 E4M^2 + 99.748 κC^2 + 56.760 HSP × E4M − 23.927 HSP × κC − 83.168 E4M × κC
Collapse time 2 mL= 116.956 + 34.321 HSP + 11.561 E4M − 22.629 κC + 72.986 E4M^2 + 94.831 HSP × E4M − 56.965 HSP × κC − 86.715 E4M × κC.

was included in the analysis, a model with significant lack of fit could still be used [23].

For FO, a significant linear variable for κC was observed. The negative regression coefficient points out that the κC addition to foam measured at 60°C conduces to a FO decrease, as was seen before Table 2. The largest value indicated that it was the most important variable influencing FO. The positive value of the quadratic coefficient for κC points out that FO presents a minimum in the response.

A significant negative linear coefficient corresponding to HSP was also observed, which indicates a decrease of FO when HSP was present.

It was also demonstrated that a decrease in foam expansion at high concentrations is believed to correspond to reduced solubility of protein solutions [24]. In the other way, interaction effects between variables have been detected. Concerning lag time for foam drainage, a significant influence of every component at heating conditions was observed.

The positive regression coefficients for HSP, E4M, and κC indicate that, in increasing concentrations of these components, the response increases and HSP presented a major influence. The positive quadratic coefficients of HSP and κC indicate a minimum presence in the response.

Synergistic interaction effects between HSP × E4M and HSP × κC were also observed, stronger in the first case. Tolstoguzov [25] has studied different effects on the interactions between proteins and polysaccharides. Under these experimental conditions of pH and temperatures, thermodynamic

incompatibility would be favored and the effect of increasing the effective polysaccharide concentration would be the main explanation for the apparently increased foam stability of the mixed system. E4M gel formation would promote more stable foams against drainage, and HSP × κC would promote the stability by viscosity increase of the continuous phase.

The K_{dr} response did not present significant linear effects ($P < 0.05$). The positive quadratic term, resulting as a consequence of the addition of κC, indicates a minimum presence. Moreover, HSP × E4M, E4M × κC ($P < 0.05$), and HSP × κC ($P < 0.01$) interactions were significant, which conduce to a slightly antagonist effect by increasing the K_{dr}.

In relation to the lag time for foam collapse, HSP showed a positive linear effect, increasing it by HSP addition, whereas the presence of E4M and κC decreased strongly this response.

The positive quadratic coefficients of three components indicate a minimum presence as a consequence of their addition.

A synergistic interaction between HSP × E4M which induces a lag time for foam collapse increase at heating can be also observed. At the same time, HSP × κC and E4M × κC resulted as antagonist interactions by decreasing this response. Moreover, it can be observed that κC may provoke an increment of lag time to collapse. This indicates an unfavorable effect of κC on this parameter in its molten state at 70°C, whereas HSP tended to favor the lag time to collapse by increasing it strongly. These results confirm that the types of interactions at heating conditions that conduce to
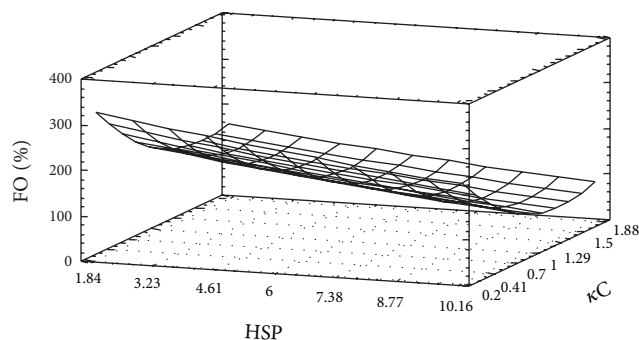
FIGURE 1: Foam overrun (FO) of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt). HSP: hydrolyzed soy protein at 4% DH; E4M: hydroxypropyl methylcellulose; κC: kappa-carrageenan.
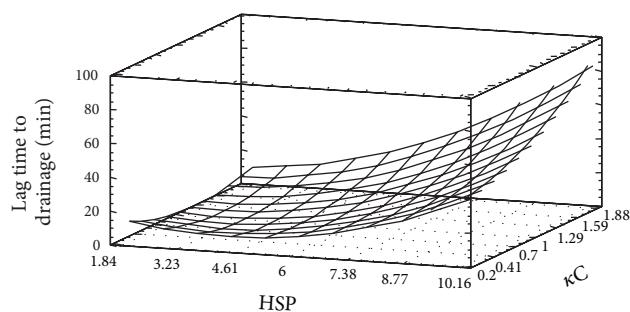

FIGURE 2: Lag time to drainage of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt). HSP: hydrolyzed soy protein at 4% DH; E4M: hydroxypropyl methylcellulose; κC: kappa-carrageenan.

FIGURE 3: Drainage rate (K_{dr}) of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt). HSP: hydrolyzed soy protein at 4% DH; E4M: hydroxypropyl methylcellulose; κC: kappa-carrageenan.

drainage or collapse process are very different [2]. Evidently, molten κC presence would not favor the E4M gelation, which may be essential for collapse process of foams.

With reference to the time for collapse 2 mL of foams, it was found that HSP and κC showed linear effects on this response at heating ($P < 0.05$). The negative and positive coefficients values for HSP and κC indicate an increase and a decrease of this response by addition of each one respectively. A positive linear effect ($P < 0.01$) due to E4M addition, which means that this polysaccharide produces an increase of this response can be also observed.

E4M showed as well a positive quadratic effect, indicating a minimum presence. Interaction effects between all components studied were observed. HSP × E4M interaction showed synergistic effects (positive value), whereas HSP × κC and E4M × κC presented antagonist interactions, decreasing the collapse time at heating with their combinations. At difference of the lag time to drainage and the K_{dr} (where different results were obtained with the polysaccharide addition), the lag time for foam collapse and collapse for 2 mL had the same tendency.

E4M was kept constant at the central point value to generate the responses surfaces and contour lines plots for all responses studied.

Figure 1 shows the response surface plot for FO and indicates that the highest FO was obtained when HSP and κC were in low concentrations. This behavior points out the great significance of κC in the decrease of FO as stated before. However, comparing with HSP alone (2% (wt), FO: 200%) it can be seen that the components concentrations could be extent to higher values to obtain foams with a reasonable FO with these mixed systems.

Figure 2 shows the response surface for *lag time to drainage* of foams at heating conditions. This response was significantly influenced by HSP and κC simultaneously.

Figure 3 shows the response surface plot for drainage rate of foams, K_{dr}. Drainage rate is minima in a high range of HSP and κC combinations. On the basis on their regressions coefficients, κC is a higher significant variable than HSP on the K_{dr}. However, this parameter did not show a relevant
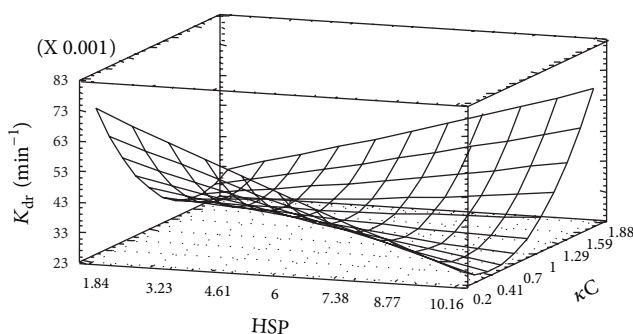
significance on foaming behavior as its *lag time to drainage* showed (Figure 2). The response showed strong variations with concentrations increase, becoming consequently in a more appropriate drainage index of foams at 70°C. That conclusion was made on the basis of that 80 minutes for *lag time to drainage* may be enough to heat the mixed system without the drainage taking place. As a result, the K_{dr} would not be so relevant in this context.

Figure 4 shows the response surface plot for the *lag time to collapse* of foams at heating. Principally, HSP increased this response and κC decreased it. The response strongly decreases when κC is in increasing concentration value and it intensifies at high HSP concentrations.

Since the *lag time to collapse* of the HSP alone (2% (wt)) was 1.3 minutes, it would be said that the polysaccharides addition would always produce stabilization against the collapse start, which is very important for systems that go through heating process.

Figure 5 shows the response surface plot for the *collapse time* 2 mL of the initial foams at heating. It can be seen that the effect of the addition of each component depends on the others concentrations, denoting a big kind of interactions between them. Thus, at low HSP concentration,
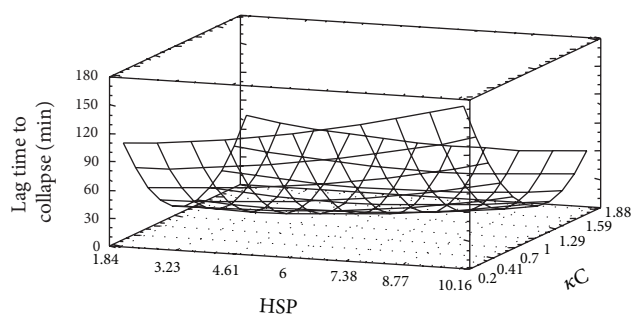
FIGURE 4: Lag time to collapse of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt). HSP: hydrolyzed soy protein at 4% DH; E4M: hydroxypropyl methylcellulose; κC: kappa-carrageenan.
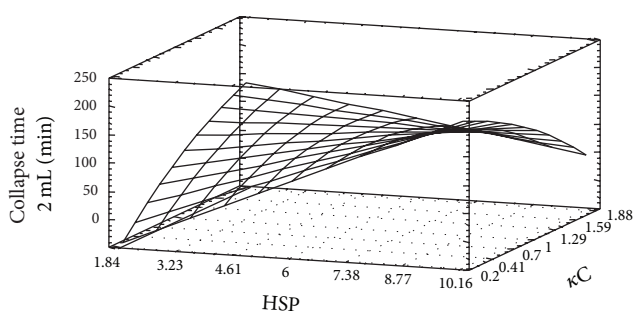

FIGURE 5: Collapse time to 2 mL of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt). HSP: hydrolyzed soy protein at 4% DH; E4M: hydroxypropyl methylcellulose; κC: kappa-carrageenan.

the κC increasing concentration produces an increment of the collapse time, whereas, in high HSP concentration, the increasing κC concentration promotes a decrease of this response.

As well as for the *lag time to drainage*, the results obtained showed that the times attained were enough to an eventual thermal treatment of the foam without the start of the foam collapse.

Therefore, the best combination for an increase of the collapse time resulted in a low κC and high HSP concentrations.

4. Conclusions

It can be concluded that the role of polysaccharides in a combined way with a limited hydrolyzed soy proteins is fundamental to generate foams with a considerable stability against the liquid drainage and collapse of the foam over heating conditions, keeping a good foam capacity.

The huge stability increase at high temperatures was ascribed to a combined effect between the gelling character of E4M at 70°C and the interaction with κC, which provides viscosity to the system. In a parallel work [26], we demonstrated that E4M is the principal component which determines the elastic and viscoelastic characteristics of continuous phase

of complex mixture at 70°C, due to the gelled structure produced at heating process. Moreover, κC is not gelled at this temperature and would impart viscosity to the system at 70°C.

Synergistic interactions between protein and polysaccharides influence the foaming properties at heating. From the study, two important interactions were observed: HSP × E4M and HSP × κC. In the first case, under these conditions, thermodynamic incompatibility conduced to effective concentration of polysaccharide increas which would be the principal factor of foam stability. However, as it was stated, lag times to drainage and collapse of foams resulted in decisive parameters for foaming properties in these conditions. As a result, HSP × κC interactions would establish the foaming performance.

From these analyses it can be determined which concentrations would be used to optimize the foaming properties. The most significant components were HSP and κC. The HSP concentration should be high (>7% (wt)) and κC should be in a low concentration (lower than 0.7% (wt)) to formulate a foam with enough lag times of drainage and collapse that allow heat process without foam overrun decrease.

Conflict of Interests

There is no conflict of interests of any kind regarding the publication of this article.
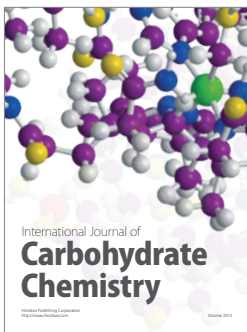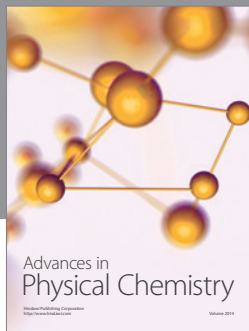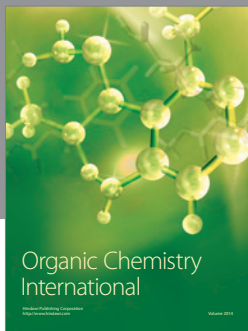
Acknowledgments

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