Food Bioscience 3 (2013) 19–28

Available online at www.sciencedirect.com

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Rheology and thermal transitions of enzymatically modified soy protein and polysaccharides mixtures, of potential use as foaming agent determined by response surface methodology

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ARTICLE INFO

Article history:
Received 25 July 2012
Received in revised form
21 March 2013
Accepted 29 April 2013

Keywords:
Soy protein
Hydrolysates
Polysaccharides
Gelation
Response surface methodology

ABSTRACT

The complex mixture we studied could be used as a foaming agent under refrigeration or heating conditions because of the presence of one polysaccharide that gels on heating, a hydroxypropylmethylcellulose called E4M, and another that gels on cooling, κ-carrageenan (κC), together with hydrolyzed soy protein.

The concentration effect of each biopolymer on its rheological behavior at 70 °C and thermal behavior of the mixture was studied. For this purpose, a Doehlert design and a response surface methodology were used to design the experiment and analyze it respectively.

The rheology of mixed systems on heating was mainly determined by E4M because this polysaccharide gels on heating. However, a high protein or κC concentration E4M gelation was prevented.

The statistical analysis showed that E4M exhibited the best performance for both the variables studied.

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1. Introduction

The structural modifications of soy proteins may improve its surface behavior and functionality changing the conformational flexibility of proteins (Carp, Wagner, Bartholomai, & Pilosof, 1997; Kim & Kinsella, 1987a,b; Wagner & Guéguen, 1999). The hydrolysis of native soy protein leads to increase the foaming and emulsifying properties (Kinsella, 1979; Liu, Lee, & Damodaran, 1999; Yu & Damodaran, 1991).

Only limited enzymatic treatment (0–10%) with proteases can substantially enhance the foaming properties of proteins. In the same way, extensive hydrolysis is detrimental to the emulsifying and stabilizing properties of protein hydrolysates due to the production of many short peptides (Agboola, Singh, & Munro, 1998; Ye, Hemar, & Singh, 2004).

It has been reported that limited hydrolysis may improve foaming capacity but decrease foam stability (Bernardi, Pilosof, & Bartholomai, 1991; Bombara, Añon, & Pilosof, 1997; Chobert, Sitohy, & Whitaker, 1988; Vioque, Sanchez-Vioque, Clemente, Pedroche, & Millan, 2000). This could be due to the exposure of hydrophobic areas and increased molecular flexibility of polypeptides that increases the affinity for the interface and the adsorption rate (Ipsen et al., 2001). However, the decrease in molecular size resulting from

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http://dx.doi.org/10.1016/j.fbio.2013.04.008

hydrolysis can be expected to decrease the ability of the polypeptides at the interface to interact so that less viscoelastic films will cause a decrease in foam stability. In reference, we have determined a limit of hydrolysis degree of soy proteins for foaming and interfacial properties improvement (Martínez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009). In that work commercial soy protein isolate and hydrolysates from 0.4% to 5.35% degree of hydrolysis were used.The impact of the hydrolysis on foaming and interfacial properties was studied and the relationship between them was analyzed. We observed that the hydrolysis of soy proteins increased the surface activity at bulk concentrations and was related with improvement of foaming properties. However, at 5.35% degree of hydrolysis the relative viscoelasticity of films decreased, and was related with a higher collapse of foams. Thus, a low degree of hydrolysis (2–5%) would be enough to improve interfacial properties maintaining acceptable foaming properties.

Therefore, because of the decreased foam stability of hydrolyzed proteins, their use would require the addition of polysaccharides as stabilizers. Most high-molecular weight polysaccharides, being hydrophilic, do not have much tendency to adsorb at the air–water interface, but they can strongly enhance the stability of protein foams by acting as thickening or gelling agents (Dickinson, 2003). However, there are some recent works in conditions of thermodynamic incompatibility between the protein and polysaccharide (i.e. above the protein isoelectric point) that support the evidence of interactions between proteins and polysaccharides at fluid interfaces (Baeza, Carrera Sanchez, Pilosof, & Patino, 2004; Baeza, Carrera Sanchez, Pilosof, & Patino , 2005) which has been related to enhancement of functionality of adsorbed proteins (Baeza, Carrera Sanchez, Rodríguez Patino, & Pilosof, 2005; Carp, Bartholomai, Relkin, & Pilosof, 2001).

Hydroxypropylmethylcellulose (HPMC) is a polysaccharide used in the food industry for different applications. One of the more important characteristics is the capacity to form thermal gels that melt upon cooling.

There are several interpretations about the gelation mechanism of aqueous solutions of methylcellulose and HPMC in the literature. Most work agrees with a two-step mechanism for the gelation process. The first called *pregel-regime* involves hydrophobic interactions that lead to cluster formation. Kato, Yokoyama, and Takahashi (1978) have proposed that the *pregel-regime* is mainly determined by the aggregation of the most hydrophobic domains of HPMC chains, i.e. trimethylglucose units that act as “cross-linking loci” on heating. This association results in the increase of the size of hydrophobic aggregates.

The second stage or *gel-regime* corresponds to the gelation (i.e. network formation) that occurs at higher temperatures and it is commonly associated to phase separation (Kobayashi, Huang, & Lodge, 1999). According to Kato et al. (1978) the second stage involves hydrophobic association of less hydrophobic domains, i.e. di- and mono-methylglucose. *Pregel* to *gel-regime* transition has been observed around 50 °C by Kobayashi et al. (1999).

κ-Carrageenan (κC) is an anionic sulphated polysaccharide which forms thermo-reversible gels used in refrigerated foamed products. Its gelling properties may be affected by protein addition (Ould Eleya & Turgeon, 2000; Tziboula & Horne, 1998,1999).

By heating aqueous dispersions of κC at temperatures above 60 °C, the polysaccharide hydrates and adopts a random coil conformation. Gelation occurs on cooling at a critical temperature (T_{gel}) and has been attributed to a two-stage reaction involving a coil–helix transition followed by aggregation of helices. The hysteresis between setting and melting temperatures for κC gels is attributed to aggregation of carrageenan helices that strengthens the gel network. Experimental evidence suggests that at T_{gel}, carrageenan helices start to form and these helices aggregate during aging at low temperature so that melting of the gel would occur at a higher temperature than T_{gel}. Melting and gelling temperatures of κC are influenced by salt concentration and the presence of proteins that influences the thermal hysteresis, which could affect the stability and sensorial properties of foam products formulated with κC. Thermal hysteresis was increased and shifted to higher temperatures in the presence of different proteins (Baeza, Carp, Pérez, & Pilosof, 2002; Lundin & Hermansson, 1998). Protein denaturation also has a large effect on κ-carrageenan gelation and on its gel properties (Baeza et al., 2002).

The rheological properties of continuous phase of foams and the relative concentrations of components could determine their stability.

In a previous publication, the same systems involving an aqueous solution of soy protein hydrolysate (HSP), and the same two polysaccharides E4M and κC, with changes of concentration according to the Doehlert matrix (Doehlert, 1970) used as experimental design were studied (Martínez & Pilosof, 2012). Relative viscoelasticity of HSP and polysaccharides mixtures at cooling conditions was analyzed at 10 °C to gain knowledge on dispersed systems applications on refrigerated food products.

It was concluded that E4M–HSP and E4M–κC interactions mainly controlled a high relative viscoelasticity of mixed systems at 10 °C. As a result, the use of hydroxypropylmethylcelluloses in combination with hydrolyzed soy proteins and κC could determine the relative viscoelasticity of continuous phase of dispersed systems in those conditions.

The system studied in the present work involves HSP, E4M and κC as well and the same experimental technique and statistical methodology were applied. In this case, high temperatures to simulate heating process and study the behavior of the system were selected. Thus, rheological properties of mixed system were also studied.

Moreover, in these particular conditions studied, E4M gels in heating conditions and melts in cooling ones, whereas κC gels in cooling conditions and melts in heating ones. To cover the minimal state where both polysaccharides were in a molten state in a mixing system that could lead eventually to destabilizing effects of any disperse system, the relation of concentrations together with hydrolyzed protein interactions should be studied. For this reason, the lowest temperature of E4M melting and the highest for κC gelling process were found out to reach these required conditions. Therefore, the thermal transitions of polysaccharides were studied such as E4M melting temperature and κC gelling temperature. Accordingly, the response surface methodology was used to

analyze the concentration effect of each component of the mixed system.

Response surface methodology has been used with success to analyze the effects of water and gums in objective and sensorial optimization of bread formulas (Collar, Andreu, Martínez, & Armero, 1999; Toufeili et al. 1994) dough baking process egg white proteins (Kobylasnki, Perez, & Pilosof, 2004), κC–whey proteins mixed systems (Tziboula & Horne, 1998), caseins (Lundin & Hermansson, 1998) and β-lactoglobulin–PGAs systems (Baeza et al., 2002) among others.

The derived equations can describe how test variables affect the response and the interaction among variables, so they are useful to predict the performance of complex systems and to optimize formulations.

The aim of the present study was to determine, by dynamic rheological studies, the rheological and thermal behavior of hydrolyzed soy protein–polysaccharides mixtures analyzing the concentration effect of these components.

2. Materials and methods

2.1. Materials

A commercial soy protein isolate (SP) (90% protein) from Sanbra, Brazil, was used as a substrate for hydrolysis with fungal protease from *Aspergillus oryzae* with endopeptidase activity of 400,000 HUT/g, optimum pH and temperature activity of 5–7 and 55 °C respectively, provided by Quest International. The protein isolate was denatured as detected by differential scanning calorimetry with pH of 6.9 and depreciable ionic strength. The following polysaccharides (PS), from Sanofi Bioindustries, Argentina, were used without further purification: κ-carrageenan (κC), and a hydroxypropylmethylcellulose (HPMC) called E4M from Dow Chemical Co.

2.2. Enzymatic hydrolysis

SP isolate at 6% w/w in distilled water was hydrolyzed according to Zylberman and Pilosof (2002) batch-wise by treatment with fungal protease at pH 7, 50 °C for 1 h, with enzyme/substrate (E/S) ratio: 2/100. The 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, Swaisgood, Porter, and Catignani (1983). Protein hydrolysate with 4% DH (HSP) was obtained.

2.3. Rheology and thermal studies of enzymatically modified mixed system

Dynamic rheological properties were determined with a Paar Physica MCR 300 (Gaz, Austria), equipped with parallel plate geometry. The measures were performed in the linear region at 0.01% strain and 1 Hz frequency. The temperature at the bottom plate was controlled with a Peltier System Viscotherm VT2, Paar Physica (Gaz, Austria), and liquid paraffin was applied to the sample exposed surfaces to prevent evaporation. Samples were heated from 60 °C to 70 °C at a rate of 10 °C/min and kept at 70 °C for 15 min, then cooled to 10 °C at 10 °C/min. The storage module (G'), loss module (G'') and relative viscoelasticity ($\tan\delta$) reached at the end of the heating period were evaluated from the dynamic measurements.

The thermal transitions of polysaccharides were also studied such as E4M melting temperature (T_mE4M) and κC gelling temperature (T_gκC) by interceptions of G' and G'' or where $\tan\delta$ becomes maximum or minimum respectively.

All parameters reported are means of at least two replicates and the error was less than 10%.

2.4. Experimental design

The combined effect of hydrolyzed soy protein, E4M and κ-carrageenan concentrations was evaluated by response surface methodology. A Doehlert design was selected to elaborate the experiment, which is associated with the second order models detecting optimum values and interactions (Doehlert, 1970). This experiment involved 3 factors as independent variables where x_1=HSP; x_2=E4M; x_3=κC with 7, 5 and 3 levels of concentrations respectively. The concentrations used were 2–10% (wt/wt) for HSP; 0.2–1.8% (wt/wt) for E4M and 0.2–1.8% (wt/wt) for κC.

Therefore, the complete design involved a total of 15 experimental data points (EP) with a replica of central point (1, 14 and 15 EP).

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

The responses or dependent variables evaluated were the elastic module (G') and the relative viscoelasticity ($\tan\delta$) at the end of heating phase experiment, and the thermal transitions of polysaccharides at this temperature range: E4M melting temperature (T_mE4M) and κC gelling temperature (T_gκC) of the mixed systems.

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

$$Y = bo + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2^2 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3$$

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

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 plots were developed using significant parameters ($P<0.05$) of 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

3.1. *Rheology of mixed enzymatically modified system*

Fig. 1 shows the elastic and viscous components (G' and G'' respectively) and relative viscoelasticity (tan δ) evolution as a function of time for an example. It can be seen that always $G' > G''$ and a tan δ of approximately 0.35 is observed pointing out that at 60 °C, E4M is gelled. Pérez, Wargon, and Pilosof (2006) have used different experimental techniques to characterize the E4M gelling on heating. The dynamic rheological technique allows to determine the gel point, about 60 °C. It was observed that elastic component G' increased and remained constant near 75 °C, where the gel structure formation of E4M was maximized.

Table 1 – Experimental matrix: real and coded (in brackets) values for the studied variables. EP: experimental point; HSP: hydrolyzed soy protein (4% DH); E4M: hydroxypropylmethylcellulose; κC: kappa carrageenan.

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)

During the cooling stage in the present work, two thermal transitions (in some cases, superposed) were observed. The first corresponds to E4M melting temperature where the elastic and viscous components overlap, and tan δ becomes maximum. The second thermal transition corresponds to κC gelling temperature where the elastic component increases quickly and tan δ decreases tending to 0.1 values.

Table 2 shows the elastic, viscous components and tan δ at the end of heating (70 °C). It can be seen that some EP (5, 9, 11 and 12) presented low values of elastic module. Their corresponding dynamic rheological diagrams obtained were different from the rest of the EP. A large oscillation of G'' values and consequently, of tan δ, indicates that these EP did not establish a defined gelled structure (data not shown).

Multiple regression analysis has been done for G' and tan δ responses at the end of heating step (70 °C). The regression coefficients obtained are showed in Table 3.

In these cases "lack of fit" resulted significant for both responses, which means that the order of the regression was not secondary (the model may have not included all appropriate functions of independent variables or the experimental region may be too large for the quadratic model used). However, when a large amount of data was included in the analysis, a model with significant lack of fit could still be used (Box & Drapper, 1987). Thus, we considered the high coefficients R^2 as evidence of the applicability of the regression model between the ranges of variables included.

All correlations coefficients resulted significant at $P<0.05$ for G'. The significant linear terms were for HSP and E4M. The positive value of regression coefficient for E4M points out that an increase of their concentration increases G'. However, due to the existence of a significant quadratic term, this increase was not linear. E4M is the determinant component and the highest linear coefficient value determined the solid behavior of mixed system at 70 °C. On the other hand, HSP resulted in a non-linear reduction (see quadratic term) of G' when HSP concentration was increased. κC showed a significant

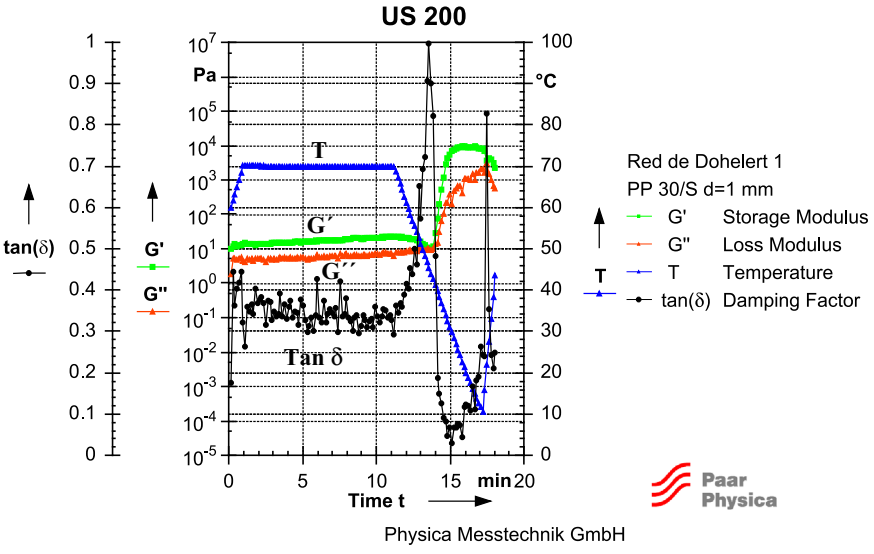

Fig. 1 – Evolution of elastic component (G'), viscous (G'') and relative viscoelasticity (tan δ) as a function of time for an experimental point as example (EP no. 6). The program involves a heating phase (from 60 to 70 °C), an isothermal phase (70 °C) and a last phase of cooling (from 70 to 10 °C).

Table 2 – Experimental points (EP) and the corresponding results obtained for viscoelastic properties (G′, G″ and tan δ) at the end of heating phase (70 °C).

EP	G′ (70 °C)*	G″ (70 °C)*	tan δ (70 °C)*
1	20.25	5.86	0.297
2	109.17	27.50	0.298
3	10.90	5.00	0.378
4	37.18	9.40	0.264
5	3.00	0.90	0.300
6	12.80	5.54	0.337
7	9.50	3.55	0.336
8	17.60	7.17	0.408
9	3.07	1.36	0.443
10	34.70	6.78	0.222
11	4.60	2.30	0.500
12	2.84	1.42	0.500
13	38.35	12.34	0.302
14	18.20	6.02	0.331
15	17.80	6.14	0.318

* Mean ± SD % less than 10% for G′, G″ and tan δ parameters of at least two replicates.

Table 3 – Model coefficients estimated by multiple linear regression for the viscoelastic properties (G′ and tan δ) at the end of heating phase (70 °C). EP: experimental point.

	G′ (70 °C)	tan δ (70 °C)
Constants	22.6990	0.3130
Linear		
HSP	−3.3711*	0.0893*
E4M	37.3697*	−0.0931*
κC	(0.4462)	(0.0108)
Quadratic		
HSP^2	−12.4348*	−0.0943*
$E4M^2$	35.9278*	(0.0257)
$κC^2$	−19.1065*	0.1428*
Interactions		
(HSP) × (E4M)	(2.2988)	0.0723**
(HSP) × (κC)	−11.5847*	(−0.0620)
(E4M) × (κC)	−19.8397*	(0.0588)
R^2	0.8481	0.8676
Lack of fit	*	*

Reduced equations for viscoelastic properties: G′ (70 °C) = 22.6990 − 3.3711HSP + 37.3697E4M − 12.4348HSP^2 + 35.9278$E4M^2$ − 19.1065$κC^2$ − 11.5847HSP × κC − 19.8397E4M × κC tan δ (70 °C) = 0.3130 + 0.0893HSP − 0.0931 E4M − 0.0943HSP^2 + 0.1428$κC^2$ + 0.0723HSP × E4M. ( ) Non-significant value.

* Significant value at $P < 0.05$.
** Significant value at $P < 0.01$.

negative quadratic term indicating a maximum presence when κC concentration increases. The significant terms of interactions (HSP × κC and E4M × κC) presented negative values which indicate an antagonistic effect due to a decrease of solid character of the system studied. In reference to HSP × κC mixed system, Baeza et al. (2002) studied the gelation and melting processes of κC in the presence of native and denatured soy protein isolates by dynamic rheological techniques. In the presence of proteins, large increase in storage module of the mixed gels compared to single κC gel was observed. However, the increase was higher when soy protein was denatured, by comparing with native soy protein isolate. Thus, they concluded that the different behaviors observed with the proteins would be related to their water absorption capacity, molecular size, flexibility and superficial charge provoked by the protein molecular structure. Therefore, in the present work, the interactions between κC and the hydrolyzed soy protein could produce a similar result.

Concerning E4M–κC interaction, an unfavorable effect can be seen, by reducing the elastic module at heating conditions. In a parallel work (results not published), we observed the unfavorable E4M and κC interaction through the same experimental design and analysis methodology on foam stability at the same conditions. Those results showed that these polysaccharides conduced to velocity drainage and foam collapse increases (stability decrease of foams) with the E4M–κC interaction effect. Therefore, the greater significant coefficient of the dynamic rheological behavior of continuous phase of mixed systems would influence the foam stability at these conditions.

The significant lack of fit for tan δ indicates the same issue as the former analysis that the model was not completely adequate. Nevertheless, the tan δ fluctuation for PE 5, 9, 11 and 12 can be responsible for this result.

In an analogous way, as G′ resulted, significant linear terms for HSP and E4M were found. In this case the positive value of the regression coefficient for HSP points out that an increase of concentration produces an increase (not linear, a non-linear term coefficient was observed) of tan δ, which indicates a less viscoelastic system. This result corresponds to a lower G′ value, which reveals a lower solid character with its addition, whereas an increase of E4M concentration produces a linear decrease of tan δ which corresponds to a higher relative viscoelasticity. κC only presented a significant quadratic term and the single significant interaction term corresponded to HSP × E4M ($P < 0.01$).

Figs. 2a, b and 3a, b show that the response surface plots corresponded to the studied responses as a function of significant factors of each case holding the central point as a constant value.

When G′ response was studied as a function of HSP and E4M concentrations (Fig. 2a), the highest elastic module values were obtained in a high concentration of E4M in the mixed system with a little influence of HSP. However, when the response was studied as a function of E4M and κC concentrations (Fig. 2b), at high E4M concentration the response depended on the κC concentration, seeming an antagonistic effect on the elastic module when κC was in the highest concentration.

When relative viscoelasticity at 70 °C response was studied as a function of HSP and E4M concentrations (Fig. 3a), two regions in the plot with the lowest tan δ are possible to obtain. One of them was at lower HSP and higher E4M concentrations and the other, at higher HSP and lower E4M concentrations at the same time. It seems that the HSP–E4M biopolymers should be in different ranges of concentrations (one in high and the other in low) to obtain a system with a

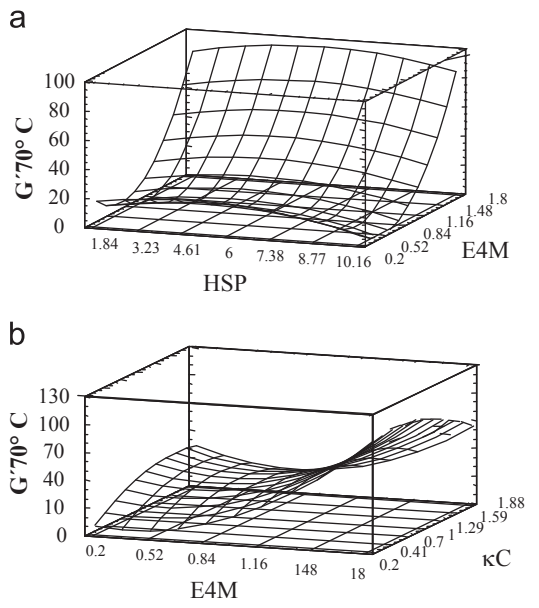

Fig. 2 – G′ 70 °C corresponding to (a) response surface plot as a function of concentrations of HSP and E4M in a concentration of κC at central point replication (1% wt/wt) and (b) response surface plot as a function of concentrations of E4M and κC, in a concentration of HSP at central point replication (6% wt/wt).

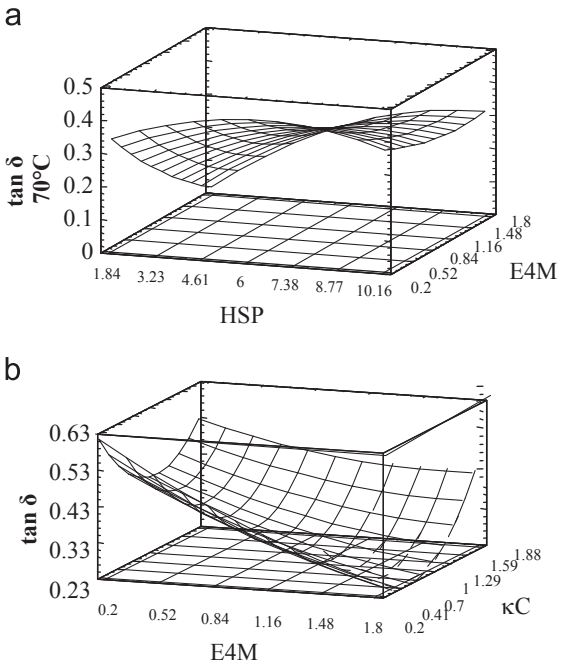

Fig. 3 – Tan δ corresponding to (a) response surface plot as a function of concentrations of HSP and E4M, in a concentration of κC at the central point replication (1% wt/wt) and (b) response surface plot as a function of concentrations of E4M and κC, in a concentration of HSP at central point replication (6% wt/wt).

high relative viscoelasticity at heating conditions. However, these results are determined by the temperature studied. In a previous work, a contrary tendency was observed at 10 °C

Table 4 – Experimental points (EP) and the corresponding results obtained for melting and gelling temperatures of E4M and κC respectively.

EP	T_mE4M (°C)*	T_gκC (°C)*
1	41.15	38.30
2	49.70	49.00
3	46.80	42.60
4	41.15	39.00
5	64.20	32.60
6	45.13	44.70
7	42.60	36.15
8	41.14	39.00
9	49.70	21.40
10	38.30	31.19
11	41.10	21.40
12	62.70	40.40
13	51.00	44.70
14	41.10	36.86
15	41.10	36.86

* Mean ± SD % less than 10% for T_mE4M and T_gκC of at least two replicates.

(Martinez & Pilosof., 2012). In that work, it could be seen that when relative viscoelasticity response was studied as a function of HSP and E4M concentrations, two regions in the plot with the lowest tan δ are possible to obtain. One of them was at lower HSP and E4M concentrations, and the other, at higher HSP and E4M concentrations. As a result, we concluded that the HSP–E4M interaction, which denoted a beneficial effect by increasing the relative viscoelasticity, should be in similar range concentrations at cooling conditions as a difference of the current work.

When relative viscoelasticity response was studied as a function of E4M and κC concentrations (Fig. 3b), a dissimilar tendency was found. It can be seen that there exists just a range where the relative viscoelasticity was lower. It was detected at higher E4M concentrations and relatively low κC concentrations. As a result, in spite of the gelled state of E4M at 70 °C, the improved viscoelasticity of continuous phase in combination with κC viscosity can be reached only when this gelled polysaccharide was in a high concentration due to the polysaccharide interactions at this condition.

3.2. *Thermal transitions of enzymatically modified mixed system*

The thermal behavior of polysaccharides E4M and κC from rheological measures as interceptions of G' and G'' and changes of tan δ of the mixed system during the dynamic rheological study were evaluated as was mentioned before.

Table 4 shows the responses: E4M melting temperature (T_mE4M), and κC gelling temperature (T_gκC) of the experimental design.

In the case of T_mE4M, a similar tendency between E4M melting temperature and E4M concentration of mixed system was not observed (Table 1). However, when T_gκC was analyzed, a similar tendency between this response and the κC concentration in the experimental design was observed.

Table 5 – Model coefficients estimated by multiple linear regression for melting and gelling temperatures of E4M and κC respectively.

	T_mE4M (°C)	T_gκC (°C)
Constants	41.513	37.416
Linear		
HSP	−5.695*	(−1.364)
E4M	−6.070*	4.220*
κC	4.980*	10.172*
Quadratic		
HSP^2	6.798*	(−2.597)
$E4M^2$	6.595*	8.356*
$κC^2$	5.019*	−8.152*
Interactions		
(HSP) × (E4M)	10.952*	(−3.915)
(HSP) × (κC)	4.240*	(−3.686)
(E4M) × (κC)	−10.892*	−5.664*
R^2	0.896	0.961
Lack of fit	*	NS

Reduced equations for thermal transitions: T_mE4M (°C) = 41.513 − 5.695HSP − 6.070E4M + 4.980κC + 6.798HSP^2 + 6.595$E4M^2$ + 5.019$κC^2$ + 10.952HSP × E4M + 4.240HSP × κC − 10.892E4M × κC T_gκC (°C) = 37.416 + 4.220E4M + 10.172κC + 8.356$E4M^2$ − 8.152$κC^2$ − 5.664E4M × κC. ( ) Non-significant value.

* Significant value at $P < 0.05$.

Multiple regression analysis has been done for T_mE4M and T_gκC. The regression coefficients obtained are showed in Table 5.

The T_mE4M "lack of fit" significant result means that the proposed model would not be adequate. However, a reasonable R^2 was obtained which implies a tolerable correlation.

All regression coefficients resulted significant for T_mE4M. It means, this response is strongly determined by linear, quadratic and interaction effects between components. Along with linear effects HSP and E4M decreased the T_mE4M (negative coefficient), whereas κC increased this response (positive coefficients). All components presented positive quadratic coefficients indicating minimum presence. The interaction effects showed that HSP and E4M increased T_mE4M (positive coefficient); it was also seen between HSP and κC with a lower coefficient value. However, the interaction between E4M and κC decreased the T_mE4M (negative coefficient). Thus, the interaction between polysaccharides as well as HSP and E4M, in a linear way, would promote the best performance after taking into account that a lower melting temperature of continuous phase at 70 °C would favor the structure stability.

The gelling temperature coefficient of regression can be analyzed in the same table.

E4M and κC produced positive effects on T_gκC with a higher coefficient value for κC. This response increased with the E4M and κC concentration increase. E4M and κC showed also significant quadratic coefficients, positive for E4M and negative for κC which implies a minimum and a maximum presence when E4M and κC concentrations increase respectively. Gelling temperature of κC depends on concentration. In the presence of adequate ions, the gelling phenomenon would occur at higher temperatures when the polysaccharide concentration is higher.

An interaction effect between E4M and κC was found. Its negative regression coefficient obtained points out a decrease of T_gκC.

That is, taking into account that a higher gelling temperature of continuous phase at 70 °C could favor the structure stability, it can be concluded that interaction of E4M and κC in a linear way would promote a good performance.

Fig. 4a–c shows that the response surfaces correspond to T_mE4M as a function of all components.

When T_mE4M response was studied as a function of HSP and E4M concentrations (Fig. 4a), it can be generally seen that

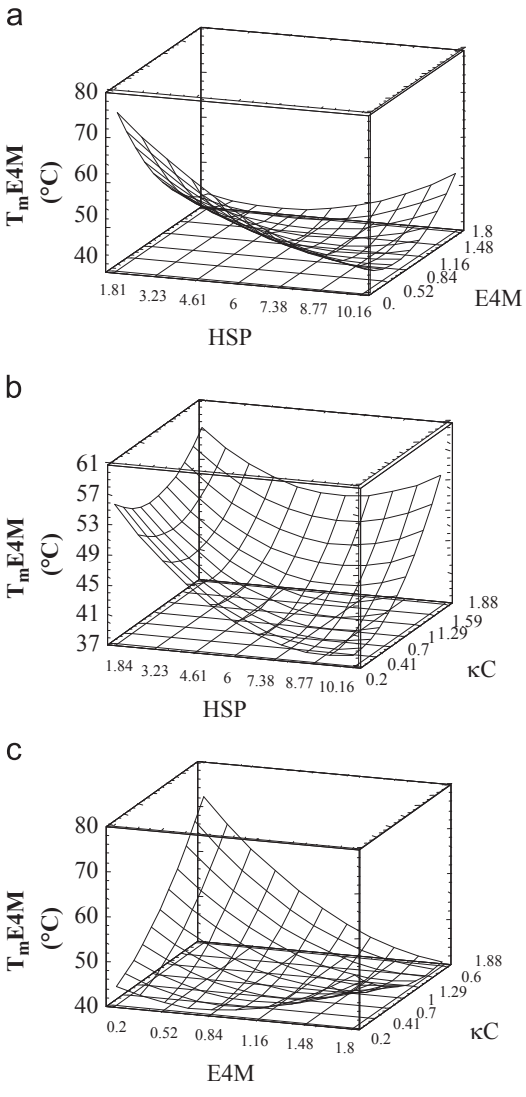

Fig. 4 – Response surface plots of melting temperature of E4M as a function of (a) HSP and E4M concentration, in a concentration of κC at central point replication (1% wt/wt), (b) HSP and κC concentration, in a concentration of E4M at central point replication (1% wt/wt) and (c) E4M and κC concentration, in a concentration of HSP at central point replication (6% wt/wt).

a wide favorable range of the variable (where it resulted to be lower) at higher HSP concentration and at all E4M concentration range was obtained. However, the variable studied becomes the lowest at two regions in the plot: one region at lower HSP and higher E4M concentrations and the other, at higher HSP and lower E4M concentrations. As resulted for the relative viscoelasticity, it seems that the HSP–E4M biopolymers should be in different ranges of concentrations (one in high and the other at low) to obtain a system with a potential improved stability at 70 °C.

While T_mE4M response was analyzed as a function of HSP and κC concentrations (Fig. 4b), a defined range with the lowest value response is possible to obtain. This range corresponds to HSP at high concentrations and to κC at low concentrations. It seems that HSP has to be in high concentration to prevent the κC effect when E4M was in the central point (1% wt/wt).

When T_mE4M response was studied as a function of E4M and κC concentrations (Fig. 4c), two regions in the plot with the lowest value are possible to obtain. One of them was at lower E4M and κC concentrations and the other, at higher E4M and κC concentrations. Thus, biopolymers should be in a similar range of concentrations to obtain a system with an improvement system stability at 70 °C.

Fig. 5a shows the corresponding response surface plot for T_gκC at HSP in the central point (6% wt/wt).

It was observed that the highest T_gκC was obtained when κC was about 1.4% (wt/wt), and a little E4M influence is seen in these conditions. However, when the κC concentration was lower, the E4M presence produced an increase of T_gκC. This increase can be attributed to a thermodynamic incompatibility phenomenon at neutral pH which determined an increase of effective concentration of κC due to exclusion phenomenon (Tolstoguzov, 1997). Baeza et al. (2002) studied the gelling and melting transitions of κC at neutral pH in the presence of β-lactoglobulin, native and denatured soy protein by rheological and calorimetric techniques. It was observed that in the presence of proteins, the κC gelling temperature and elastic moduli increased compared with the polysaccharide alone.

Preceding observations on κC-protein systems showed synergistic effects between biopolymers (Kampf & Nussinovitch, 1997; Mleko, Li-Chan, & Pikus, 1997; Neiser, Draget, & Smidsrod, 2000; Ould Eleya & Turgeon, 2000; Schorsch, Jones, & Norton, 2000). The principal cause would be the thermodynamic incompatibility between biopolymers in the solution. This phenomenon conduces to a mutual

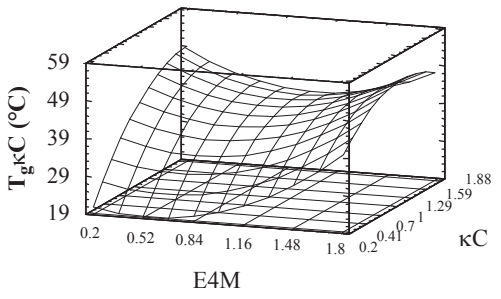

Fig. 5 – Response surface plot of gelling temperature of κC as a function of E4M and κC concentrations, in a concentration of HSP at central point replication (6% wt/wt).

increase of concentration at separated phases that favor the gel formation of hydrocolloid. In a previous publication (Martınez, Carrera Sanchez, Pizones Ruız-Henestrosa, Rodrıguez Patino, & Pilosof, 2007), where the effect of limited hydrolysis of soy protein at the interactions with polysaccharides on interfacial properties was evaluated, a synergistic behavior could be seen for soy protein hydrolysates and λC (other carrageenan polysaccharide type). The existence of a limited thermodynamic compatibility between protein and λC may account for the observed surface pressure increase of the mixed system. Even if the polysaccharide does not participate in the interface, the protein and polysaccharide in the vicinity of the air–water interface could lead to concentration of adsorbed protein by a depletion mechanism (Baeza, Carrera Sanchez, Pilosof et al., 2005a).

In reference to protein–polysaccharide interactions, thermodynamic incompatibility is the main event in solutions, depending on some factors, such as pH, ionic strength and relative concentration of molecules (Zhang & Foegeding, 2003; Zhang, Foegeding, & Hardin, 2004). Therefore, it would be useful to correlate formulation, physicochemical properties of modified protein and polysaccharides, to study process conditions to obtain new foamed products.

4. Discussion

It would be very valuable to obtain a new ingredient made from hydrolyzed soy protein and polysaccharides and use it as a foaming agent on heating process. However, the rheological properties of continuous phase of dispersed system and the relative concentrations of components could determine their foaming properties.

For this purpose, E4M and κC in the mixed system with 4% hydrolysate of soy protein were selected. Therefore, an experimental design was necessary to project the required combinations of these components. Thus, a statistical method was used to determine i.e. which relation of concentration leads to a lower T_mE4M and a higher T_gκC at the same time, which eventually conduces to the most stable cooked disperse system formation at cooling storage.

It can be concluded that E4M is the principal component which determines the elastic and viscoelastic characteristics of complex mixture at 70 °C, due to the gelled structure produced during the heating process.

As κC is not gelled at this temperature in a “random coil” state, it is supposed that it would only contribute with the viscosity effect on elastic and viscoelastic properties of the system at 70 °C.

It could be observed that the elastic, viscous components and tan δ at the end of heating (70 °C) in some experimental points (5, 9, 11 and 12) presented low elastic module values. Their corresponding diagrams obtained were different from the rest of the experimental points, suggesting that a defined gelled structure did not take place. The statistical analysis showed that the presence of high HSP and/or κC concentration would inhibit the E4M gel formation which resulted in a low G' and high tan δ values. This explains why in those experimental points a defined gelled structure formation was not detected.

In the case of T_mE4M, a direct relation between E4M melting temperature and E4M concentration of mixed system was not observed. However, when regression coefficients were obtained, a great number of significations could be determined, indicating all kinds of interactions between the components.

Nevertheless, when $T_g\kappa$C was analyzed, a similar tendency between this response and the κC concentration in the experimental design was observed. Moreover, the statistical analysis showed that E4M showed the best performance for both T_mE4M and $T_g\kappa$C responses at the same time.

On the other hand, it was observed that the T_mE4M was higher in the presence of κC; however, the same relation of polysaccharides used or hydrolyzed soy protein added can revert this situation by decreasing the response. It can be concluded that the use of these specific polysaccharides for the continuous phase stability purpose that gel at heating or cooling conditions can be highly improved by adding hydrolyzed soy protein.

In another way, when κC concentration was lower, the E4M presence produced an increase of $T_g\kappa$C. This increase can be attributed to a thermodynamic incompatibility phenomenon at neutral pH between biopolymers in the solution.

5. Conclusions

The G' and tan δ of mixed systems on heating (70 °C) were mainly determined by E4M because this polysaccharide is gelled on heating. However, with high HSP or κC concentration, the E4M gelation was prevented.

The statistical analysis showed that E4M exhibited the best performance for both T_mE4M and $T_g\kappa$C responses.

Thus, from these obtained results a potential enzymatically modified ingredient could be possibly used and combined with cellulose polysaccharide derivative for industrial application on new functional foods.

Impact statement

Limited enzymatic treatment of soy proteins can substantially improve foamability but it has been reported that it can decrease foam stability. Therefore, because of the decreased foam stability, their use would require the addition of polysaccharides as stabilizers.

The complex mixture we studied could be used as a foaming agent under refrigeration or heating conditions because of the presence of one polysaccharide that gels on heating (hydroxypropylmethylcellulose, HPMC called E4M) and the other that gels on cooling (kappa-carrageenan, κC), together with the hydrolyzed soy protein (HSP) at 4% degree of hydrolysis.

The concentration effect of each biopolymer on rheological behavior at 70 °C (elastic component module, G', and relative viscoelasticity, tan δ) and thermal behavior (melting temperature of E4M, T_mE4M; and gelling temperature of κC, $T_g\kappa$C) of the mixture was studied. For this purpose, a Doehlert design and a response surface methodology were used to design the experiment and analyze it respectively.

The G' and tan δ of mixed systems on heating (70 °C) were mainly determined by E4M because this polysaccharide gels on heating. However, a high HSP or κC concentration E4M gelation was prevented.

The statistical analysis showed that E4M showed the best performance for both T_mE4M and $T_g\kappa$C responses.

Acknowledgments

This research was supported by Universidad de Buenos Aires, Agencia Nacional de Promoción Científica (PICT 2008-1901) and Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

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