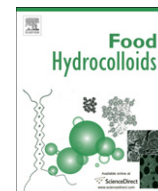




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Short Communication

Relative viscoelasticity of soy protein hydrolysate and polysaccharides mixtures at cooling conditions analyzed by response surface methodology

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ABSTRACT

The objective of this work was to study the relative viscoelasticity of soy protein hydrolysate and polysaccharides mixtures at cooling conditions analyzed by response surface methodology.

Systems of soy protein hydrolysate (HSP) of 4% degree of hydrolysis, a hydroxypropylmethylcellulose (E4M) and kappa-carrageenan (κ C) were made with concentrations conformed by Doehlert matrix as experimental design used.

The samples were subjected to dynamic rheological studies with a control stress rheometer, Paar Physica MCR 300, with a program with a heating and a cooling period. At the end of the cooling at 10 °C the relative viscoelasticity ($\tan \delta$) was evaluated from these measurements.

To relate the relative viscoelasticity with the components of systems and their concentrations at cooling conditions the response surface methodology was used to obtain this information.

The results obtained indicate that E4M promoted in general a decrease of relative viscoelasticity only in the combined systems. When E4M was in combination with HSP, two regions in the plot with the lowest $\tan \delta$ can be possible to obtain. One of them was at lower HSP and E4M concentrations and the other at the HSP and E4M highest concentrations. In similar way, when E4M was in combination with κ C an increase of relative viscoelasticity was observed at the lowest E4M and κ C concentrations and other region was found at the highest E4M and κ C concentrations.

In other hand, κ C would enhance a higher relative viscoelasticity, however, when this polysaccharide was used in combination with hydrolyzed soy protein and/or E4M, a decrease of relative viscoelasticity was observed in the mixed systems.

It can be concluded that E4M is the principal component which determines high viscoelastic characteristics in combination with hydrolyzed soy proteins and κ C at 10 °C.

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1. Introduction

The use of soy proteins as functional ingredients in food manufacturing is increasing because of their role in human nutrition and health. Native soy protein, because of its quaternary and compact tertiary structure has limited foaming (Kinsella, 1979; Utsumi, Matsumura, & Mori, 1997, chap. 9; Yu & Damodaran, 1991) and emulsifying (Kinsella, 1979; Liu, Lee, & Damodaran, 1999) properties. However, structural modifications by chemical methods such as hydrolysis, allowing greater conformational flexibility of protein, may improve its surface behavior and functionality (Carp, Wagner, Bartholomai, & Pilosof, 1997; Kim & Kinsella,

1987a, 1987b; Wagner & Guéguen, 1999). Nevertheless, the structural modification decreases their foam stability, and the addition of polysaccharides as stabilizers would be required.

Hydroxypropylmethylcellulose (HPMC) is used in the food industry, printing technology, and has pharmaceutical applications because is nontoxic and possesses good mechanical properties. The usefulness of HPMC is essentially based upon four attributes: efficient thickening, surface activity, film forming ability, and the capacity to form thermal gels that melt upon cooling. These interesting properties are given by methyl substitutes along the cellulose backbone that constitute strong hydrophobic zones and hydroxypropyl groups that are more hydrophilic. Although several works have been focused on certain properties of HPMC such a water affinity (Ford & Mitchell, 1999; Fyfe & Blazek-Welsh, 2000; Tritt-Goc & Pislewski, 2002) and gelation (Sarkar & Walker, 1995) there is scarce literature concerning their behavior in mixed systems formed with proteins.

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κ -Carrageenan (κ C) is an anionic sulphated polysaccharide extracted from red algae which is widely used in the food industry as a thickening, gelling and stabilizing agent.

It forms thermo reversible gels in the presence of potassium ions and its gelling properties may be affected by protein addition (Tziboula & Horne, 1998a, 1998b, Ould Eleya & Turgeon, 2000). κ C is used for the stabilization and formation of structure in refrigerated foamed products (mousses, meringues, etc.).

Many food products are multi-component mixtures, in which one or more gelling biopolymers are incorporated to obtain essential texture and sensory characteristics. It would be very valuable to obtain a new ingredient and use it as foaming agent at cooling after a heating process. However, the rheological properties of continuous phase of foams or emulsions and the relative concentrations of components could determine their stability. A high viscous phase would retard all destabilizations process as liquid drainage and disproportion of droplets (Kinsella, 1981). When the continuous phase presents yield stress or is gelled, these processes would be decelerated (Carp, Baeza, Bartholomai, & Pilosof, 2004).

Despite the many studies on mixed protein-polysaccharide systems, literature on mixtures involving a hydrolyzed protein is scarce. Regarding rheological of thermal studies of hydrolysates-polysaccharides mixtures, no references have been found so far.

Hence, the system studied in the present work involves an aqueous solution of soy protein hydrolysate (HSP) and two polysaccharides; an HPMC (E4M) and κ C, with changes of concentrations according to Doehlert matrix (Doehlert, 1970) used as experimental design. Thus, relative viscoelasticity at 10 °C (end of cooling period of rheological studies) of mixed system was studied.

Accordingly, the response surface methodology was used to analyze the behavior and concentration effect on relative viscoelasticity of each component in a 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; Ylimaki, Hawrish, Hardin, & Thomson, 1988), dough baking process (Leon, Duran, & Benedito de Barber, 1997), egg white proteins (Kobylasni, Perez, & Pilosof, 2004), κ C-whey proteins mixed systems (Tziboula & Horne, 1998a), caseins (Lundin & Hermansson, 1998) and β -lactoglobulin-PGAs systems (Baeza, Carp, Pérez, & Pilosof, 2002) between others.

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

Therefore, the aim of this work was to study the relative viscoelasticity at 10 °C of hydrolyzed protein-polysaccharides mixtures obtained by dynamic rheological studies relating the behavior and concentrations effect of biopolymers gaining knowledge on dispersed systems applications at refrigerated food products.

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 polysaccharide (PS) were used without further

purification: κ -carrageenan (κ C), from Sanofi Bioindustries, Argentina, and an hydroxypropylmethylcellulose (HPMC) E4M from Dow Chemical Co.

2.2. Enzymatic hydrolysis

SP isolate (72 g in 1200 ml of 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 of 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 then kept at 70 °C for 15 min, then cooled to 10 °C at 10 °C/min. The relative viscoelasticity ($\tan \delta$) reached at the end of the cooling period were evaluated from the dynamic measurements.

Relative viscoelasticity values reported are means of at least two replicates and the error was less of 10%.

2.4. Experimental design

The combined effect of HSP, E4M and κ C concentrations were evaluated by response surface methodology. A Doehlert design was selected to elaborate the experiment, which is associated with second order models detecting optimum values and interactions (Doehlert, 1970). This experiment involved 3 factors (HSP, E4M and κ C) 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 κ C.

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

The response or dependent variable evaluated was the relative viscoelasticity of systems ($\tan \delta$) at the end of cooling phase of the experiment. A second-degree polynomial model was fitted for the dependent variable, as follows:

$$\tan \delta = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2^2 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3$$

where b_0 , b_{ii} and b_{ij} are regression coefficients and x_i the coded independent variables, linearly related to HSP, E4M and κ C 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 surfaces were developed using significant parameters ($P < 0.05$) of the polynomial equations, obtained by holding one of

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)

the independent variables at a constant value (usually at the central point) and changing the levels of the other two variables using Statgraphics Plus 3.0. software.

3. Results

3.1. Relative viscoelasticity of mixed systems

Table 2 shows the relative viscoelasticity ($\tan \delta$) at the end of cooling phase (10 °C).

Multiple regression analysis has been done for $\tan \delta$ response. The regression coefficients obtained are showed in Table 3 and the best explanatory equation is also shown.

The R^2 coefficient was high, thus, the model was able to explain more than 80% of the observed response (Cornell, 1990).

The “lack of fit” resulted significant, 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 coefficient R^2 as evidence of the applicability of the regression model between the range of variables included.

Table 2

Experimental points (EP) and the corresponding results obtained for relative viscoelasticity ($\tan \delta$) at ending of cooling phase (10 °C).

EP	$\tan \delta^a$
1	0.2276
2	0.2127
3	0.1908
4	0.1319
5	0.1343
6	0.2414
7	0.2230
8	0.1464
9	0.0773
10	0.4897
11	0.2152
12	0.1403
13	0.1313
14	0.1448
15	0.1434

^a Mean \pm SD % less of 10% for $\tan \delta$ of at least two replicates.

Table 3

Model coefficients estimated by multiple linear regression for relative viscoelasticity ($\tan \delta$) at ending of cooling phase.

Factor	$\tan \delta$
Constants	0.1680
Linear	
HSP	−0.0497*
E4M	0.0810*
κC	−0.0714*
Quadratic	
HSP ²	0.0616*
E4M ²	0.0351*
κC ²	0.0287*
Interactions	
(HSP) × (E4M)	−0.2151*
(HSP) × (κC)	0.0511*
(E4M) × (κC)	−0.1656*
R^2	0.896*
Lack of fit	*

*Significant value at $P < 0.05$. Reduced equations for relative viscoelasticity: $\tan \delta = 0.1680 - 0.0497\text{HSP} + 0.0810\text{E4M} - 0.0714\kappa\text{C} + 0.0616\text{HSP}^2 + 0.0351\text{E4M}^2 + 0.0287\kappa\text{C}^2 - 0.2151\text{HSP} \times \text{E4M} + 0.0511\text{HSP} \times \kappa\text{C} - 0.1656\text{E4M} \times \kappa\text{C}$.

All correlations coefficients resulted significant for the three components showing linear, quadratic and interactions effects ($P < 0.05$). Significant linear terms corresponding to HSP and κC were negative, thus indicating a decrease of $\tan \delta$ (or relative viscoelasticity increase) during cooling when these two components are present. The quadratic terms indicate the existence of a minimum.

In other hand, it was observed that E4M promoted an increase of $\tan \delta$ (or decrease of relative viscoelasticity), with also a minimum presence (positive quadratic term). However, when this polysaccharide was in the presence of HSP or κC the studied response decreased, it means, a higher relative viscoelasticity could be possible to obtain in the mixed system analyzed. The dissociation of the HPMC gel during cooling, giving a fibrillar structure in the presence of denatured proteins, could favor the formation of a stronger structure (Haque, Morris, & Richardson, 1994). Therefore, E4M would play a very important role on the interactions at these mixed systems in their molten state at 10 °C.

At same time, it can be seen that even though HSP and κC would favor the relative viscoelasticity of the system by increasing it, their interaction would be unfavorable when both are present in the mixed sample (positive coefficient of $\text{HSP} \times \kappa\text{C}$ interaction). Thus, the presence of E4M would be fundamental in a mixed system conformed by a hydrolyzed soy protein and polysaccharides as showed the present work.

Nevertheless, dissimilar results were obtained by Rosell and Foegeding (2007). They investigated the possible interaction between HPMC and gluten proteins to understand the effect of the HPMC in bakery products. Dynamic oscillatory studies at small deformations indicated that the presence of varying concentrations of HPMC induces a softening effect in the gluten dough. During heating of HPMC–gluten mixtures, HPMC gelation only became evident when a minimum amount of HPMC was present (HPMC/gluten ratio was 0.075), however, the presence of HPMC did not modify the viscoelastic behavior of gluten dough during cooling.

In Fig. 1a–c are shown the response surfaces obtained with the regression model corresponded to the relative viscoelasticity as a function of significant factors, thus, three surfaces were constructed, fixing the third component at the central point value in each case.

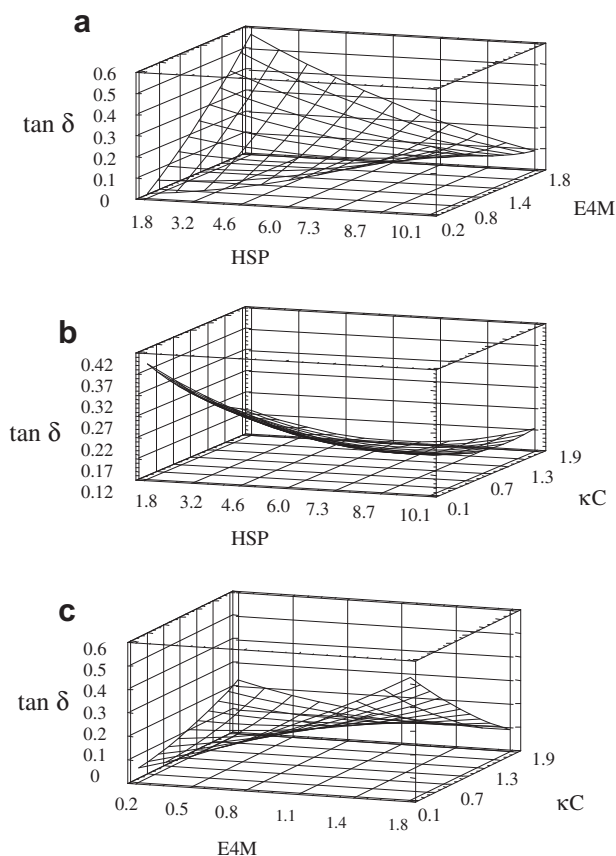


Fig. 1. $\tan \delta$ corresponding to: (a) response surface plot as a function of concentration of HSP and E4M, in a concentration of κC at the central point replication (1%wt/wt); (b) response surface plot as a function of concentration of HSP and κC , in a concentration of E4M at central point replication (1%wt/wt) and (c) response surface plot as a function of concentration of E4M and κC , in a concentration of HSP at central point replication (6%wt/wt).

When relative viscoelasticity response was studied as a function of HSP and E4M concentrations, (Fig. 1a) two regions in the plot with the lowest $\tan \delta$ can be possible to obtain. One of them was at lower HSP and E4M concentrations (i.e. EP number 5, with a $\tan \delta$ of 0.1343) and the other, at higher HSP and E4M concentrations (i.e. EP number 4 or 8, with $\tan \delta$ at about of 0.13–0.14). It seems that the HSP–E4M biopolymers should be in similar range of concentrations to obtain a system with a high relative viscoelasticity at cooling conditions.

While, relative viscoelasticity response was analyzed as a function of HSP and κC concentrations, (Fig. 1b) a central range with the lowest $\tan \delta$ response can be possible to obtain. This range corresponds to HSP intermediate concentrations and all concentrations range for κC . The EP numbers 1, 2, 3, 8 and 12 could be seen as examples giving $\tan \delta$ values between 0.14 and 0.22. It can be said that despite the fact that HSP– κC interaction promoted a viscoelasticity decrease, some concentrations levels of this components can generate an acceptable high relative viscoelasticity of mixed systems at cooling as plot showed.

When relative viscoelasticity response was studied as a function of E4M and κC concentrations, (Fig. 1c) a similar tendency as HSP–E4M concentrations response plot was found. However, in this case, a more wide favorable range was observed with lower $\tan \delta$ (higher relative viscoelasticity). Some examples of EP numbers 8 and 9 can be given, resulting $\tan \delta$ between 0.07 and 0.14 for this case.

As a result, E4M in a molten state at 10 °C, showed a determinant role on the relative viscoelasticity in mixed systems formed by hydrolyzed soy proteins and κC .

4. Conclusions

The rheological properties of continuous phase of refrigerated foams and emulsions and the relative concentrations of components may determine their stability in this condition.

For this purpose, a mixed systems were made with different polysaccharides and concentrations according to Doehlert design. Response surface methodology was applied to analyze the components behavior and concentrations effect on relative viscoelasticity.

When regression coefficients were obtained, a great number of significations could be determined, indicating all kind of interactions between the components.

Among all significant variables, through their corresponding response surfaces plots, it can be observed that E4M promoted a decrease of relative viscoelasticity, however, when E4M was in combination with HSP or/and κC , an increase of relative viscoelasticity of system was observed. When E4M was in combination with HSP, two regions in the plot with the lowest $\tan \delta$ can be possible to obtain. One of them was at lower HSP and E4M concentrations and the other at the HSP and E4M highest concentrations. In similar way, when E4M was in combination with κC an increase of relative viscoelasticity was observed at the lowest E4M and κC concentrations and other region was found at the highest E4M and κC concentrations.

In other hand, κC would enhance a higher relative viscoelasticity, however, when this polysaccharide was used in combination with HSP and/or E4M, a decrease of relative viscoelasticity was observed in the mixed systems.

It can be concluded that E4M–HSP and E4M– κC interactions mainly controlled a high relative viscoelasticity of mixed systems at 10 °C. Therefore, the use of hydroxypropylmethylcelluloses in combination with hydrolyzed soy proteins and κC would domain the relative viscoelasticity of continuous phase of dispersed systems in refrigerated food products.

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