EMULSIFYING PROPERTIES OF MAILLARD CONJUGATES PRODUCED FROM SODIUM CASEINATE AND LOCUST BEAN GUM

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Abstract - Emulsifying properties of sodium caseinate - locust bean gum Maillard conjugates produced at different temperatures (54 – 96 °C), protein/polysaccharide ratios (0.3 – 1.0) and reaction times (1 – 24 hours) were evaluated. Conjugate formation was confirmed by formation of color and high molecular weight fractions and the decrease of the α_s- and β-casein bands. The emulsions stabilized by Maillard conjugates showed good stability. The mean droplet diameter (d_{32}) tended to decrease with the increase of incubation time and temperature, except at extreme conditions (24 hours and 90 °C or 96 °C) when the partial degradation of the conjugates was probably favored, resulting in phase separation of emulsions. The emulsion viscosity decreased with the increase in the protein/polysaccharide ratio and with the degradation of the conjugates. The conditions used in the experimental design made the optimization of the conjugate production viable, which showed greater emulsifier properties than the pure protein under acid conditions.
Keywords: Maillard reaction; Emulsion; Rheology; Protein; Polysaccharide.

INTRODUCTION

Emulsions consist of two immiscible liquids (usually oil and water) with one of the liquids dispersed in the other one as small (0.1–100.0 μm) spherical droplets. Emulsions are generally thermodynamically unstable systems, but they can be kinetically stabilized for a reasonable period of time by adding substances known as emulsifiers and/or thickening agents prior to homogenization (McClements, 2005). Nowadays many industries, especially the food industry, have a growing interest in the replacement of synthetic emulsifiers and thickening by natural ones, such as proteins and polysaccharides (Huck-Iriart *et al.*, 2011).

Polysaccharides are hydrophilic macromolecules used to stabilize emulsions by viscosity modification or gelation of their continuous phase (Dickinson, 2003). Locust bean gum (LBG) is a neutral polysaccharide composed of a 1-4-linked β-D-mannan backbone with 1-6-linked α-D-galactose side groups (Dea and Morrison, 1975). This macromolecule has the ability to form very viscous solutions that are almost unaffected by pH or heat processing, mainly due to the neutral character of this gum (Glicksman, 1969). On the other hand, proteins are surface active molecules used to stabilize emulsions by lowering the interfacial tension and/or forming films at the oil-water interface, providing electrostatic and steric repulsion between droplets (van Aken and van Vliet, 2002; McClements, 2005). Sodium caseinate is a protein widely used due to its emulsifying, water and fat bind-

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ing, thickening and gelation properties (Mulvihill, 1989). However, the emulsifying properties of sodium caseinate decrease at acid pH. Around its isoelectric point (pH 4.6), the net attractive forces between the casein molecules increase, resulting in a self-association of the adsorbed and non-adsorbed protein components (Allen *et al*., 2006), which could lead to emulsion destabilization.

Conjugation of the protein to a polysaccharide can improve its functional properties, such as solubility around its isoelectric point (Chevalier *et al*., 2001; Kato, 2002; Oliver *et al*., 2006a; O'Regan and Mulvihill, 2009; Yadav *et al*., 2010) and its emulsifying properties (Shepherd *et al*., 2000; Kato, 2002; Neirynck *et al*., 2004; Mu *et al*., 2006; Akhtar and Dickinson, 2007; O'Regan and Mulvihill, 2009; Xu *et al*., 2012). The formation of protein–polysaccharide conjugates by Maillard-type reactions is a chemically safe method, being suitable for food applications. These conjugates are produced by the condensation of the carbonyl group of a reducing end sugar with an unprotonated amino group of the protein (mainly the ε-amino group of a lysine residue), forming a Schiff base with the release of water. The Schiff base subsequently cyclizes to the corresponding N-glycosylamine, which then undergoes an irreversible Amadori rearrangement (Ames, 1992). The covalent bonds between the protein and polysaccharide are very stable against changes in pH, temperature or ionic strength (Dickinson, 1993; Dickinson and Euston, 1991). In the final stage of the Maillard reaction, the Amadori compound may undergo several irreversible degradation reactions, giving many colored and water-insoluble, poorly-characterized compounds called melanoidins (Friedman, 1996; Yadav *et al*., 2010). At the advanced stages of the Maillard reaction, the conjugates show reduced emulsifying activity and stability when compared with conjugates at the early stages (Corzo-Martínez *et al*., 2011).

Several proteins and carbohydrates have been combined in these conjugates by using the Maillard reaction. Specifically the casein, caseinate-dextran (Fechner *et al*., 2007), caseinate-maltodextrin (Shepherd *et al*., 2000; O'Regan and Mulvihill, 2009, 2010), casein-glucose (Gu *et al*, 2009) and casein-dextran (Aminlari *et al*., 2005; Mu *et al*., 2006) conjugates were studied. These conjugates showed better emulsifying/stabilizing properties when compared to the pure protein, mainly at pH values close to the isoelectric point. The Maillard reaction rate is affected by many factors, such as temperature, pH, protein-polysaccharide ratio, relative humidity and intrinsic properties of the reagents (Oliver *et al*., 2006a).

Thus, the aim of this work was to study the effect of temperature, protein/polysaccharide ratio and reaction time on the formation of sodium caseinate (Na-CN) - locust bean gum (LBG) conjugates. An experimental factorial design was used to formulate the Na-CN − LBG conjugates, which were characterized by electrophoresis and color analyses. The conjugates were used as emulsifiers of oil-in-water emulsions and their efficiency was evaluated by stability, rheological and droplet size measurements on the emulsions.

MATERIALS AND METHODS

Materials

The ingredients used to prepare the systems were casein (Art.-No. C7078, CAS 9000-71-9, Sigma-Aldrich Co., USA), locust bean gum (LBG) (Sigma-Aldrich Co., USA) and soybean oil (Soya, Bunge Alimentos S.A., Brazil). The moisture and ionic calcium contents of the casein powder were 6.5% and 0.14%, respectively. Sodium azide (Merck, Germany) was added to the emulsions to prevent microbial growth.

Preparation of Biopolymer Solutions

The aqueous sodium caseinate (Na-CN) solutions were prepared by dispersing casein in deionized water using a magnetic stirrer and heating at 50 °C during 3 hours. The pH was constantly adjusted to 7.0 using 10 M NaOH. Polysaccharide solutions were prepared by dispersing the LBG powder in deionized water at room temperature, followed by a heating step up to 80 °C for 30 minutes under mechanical stirring. After the heat treatment, the solutions were cooled to room temperature in an ice bath.

Preparation of Na-CN − LBG Conjugates

Protein and polysaccharide solutions were mixed in different ratios at room temperature using magnetic stirring and then these mixtures were freeze-dried. The dry powders were incubated at fixed temperatures for 1, 3 and 24 hours under a relative humidity of 79% provided by a saturated KBr solution (Akhtar and Dickinson, 2007). The protein/polysaccharide ratio and temperature of incubation were determined according to an experimental design (section 2.4). The conjugation degree of the resultant Maillard reaction was analyzed by electrophoresis and color evaluation.

Experimental Design

A rotatable central composite design was used to define the conditions of Na-CN − LBG conjugate preparation, considering two factors as independent variables (temperature of reaction and Na-CN / LBG ratio). Five levels of each variable were chosen for

the trials, including two central points and two axial points, giving a total of 10 combinations (Table 1). Analysis of variance was used to detect effects significant at $p < 0.1$. Statistica 5.5 software (Statsoft, USA) was used to analyze the results.

Table 1: Coded and real values of the independent variables used for experimental design.

Trial	Coded values		Real values	
	Temperature	Na-N/LBG ratio	Temperature (°C)	Na-CN/LBG ratio
1	-1	-0.6	60	0.50
2	+1	-0.6	90	0.50
3	-1	+1	60	0.88
4	+1	+1	90	0.88
5	-1.41	0	54	0.65
6	+1.41	0	96	0.65
7	0	-1.41	75	0.30
8	0	+1.41	75	1.00
9	0	0	75	0.65
10	0	0	75	0.65

Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis (PAGE) was carried out to identify conjugates, following the method of Laemmli (1970). The conjugates were diluted with an equal volume of pH 6.8 buffer (62.5 mM Tris-HCl, 20% glycerol, 2% SDS, 0.05% bromophenol blue) and applied to the polyacrylamide gels. Commercial molecular weight markers (molecular weight range between 6.0 and 181.5 kDa) (Sigma-Aldrich Co., USA) and a solution of 6% (w/v) sodium caseinate were also applied to the gels. These were run at 120 V through the stacking (4% polyacrylamide) and separating (12% polyacrylamide) gels using a Mini Cell Protean electrophoresis unit (Biorad Laboratories, USA). The gels were stained with 0.25% (v/v) Coomassie Brilliant Blue, in ethanol:acetic acid: water (45:10:45, v/v), followed by destaining with acetic acid:ethanol:water (5:10:85, v/v).

Color Measurements

The color of the conjugate powders was measured using an Ultra Scan Vis 1043 colorimeter (Hunter Lab, USA), with the CIELab scale (L*, a*, b*). The color measurements were expressed in terms of the b* value (+b*=yellow and -b*=blue).

Emulsion Preparation

Aqueous solutions were prepared by dissolution of the conjugate powders in deionized water using magnetic stirring at room temperature for approxi-

mately 3 hours. After the dissolution, sodium azide (0.02% w/v) was added and the pH adjusted to 3.5 with 1.5 M HCl. Oil-in-water (O/W) emulsions were prepared by homogenizing the soybean oil in the aqueous solutions with an Ultra Turrax model T18 (IKA, Germany) at 14000 rpm for 4 minutes. The conjugate and oil concentrations were fixed at 1% (w/v) and 30% (v/v), respectively, for all systems. The emulsions were evaluated in relation to stability, microstructure and rheology.

Evaluation of Emulsion Stability

Immediately after preparation, 10 mL of each emulsion was poured into a cylindrical glass tube (internal diameter = 15.5 mm, height = 65 mm), sealed with a plastic cap and stored at 25 °C for a period of 4 days. After storage, some emulsions separated into a top cream phase and a bottom serum phase. The creaming stability was measured by the height of the serum layer (H) with the storage time. The creaming index (CI) was reported as CI (%) = (H/Ho) × 100, where Ho represents the initial height of the emulsion (Keowmaneechai and McClements, 2002).

Optical Microscopy and Image Analysis

The microstructure of emulsions was evaluated one day after their preparation. For this, the samples were placed on slides, covered with cover slips and observed using a conventional optical microscope: Carl Zeiss Model mf-AKS 24 x 36 EXPOMET (Zeiss, Germany) with 40x objective lens.

The microscopy images were analyzed with the public domain software Image J v1.36b (http://rsb. info.nih.gov/ij/). Micrographs were transformed into 8-bit grey scale binary images of 640x480 pixels and were then segmented by 'thresholding'. The threshold was set from the median of the grey level histogram of each image (Pugnaloni *et al.*, 2005). During this process, pixels were deemed to be detected only if their grey value was lower than the threshold setting. The pixel-scale values were converted into microns by a scaling factor. The droplet areas were then measured and the volume-surface mean diameter (d_{32}) of the emulsion droplets was calculated considering spherical shape, according to Equation (1).

$$d_{32} = \frac{\Sigma n_i d_i^3}{\Sigma n_i d_i^2} \qquad (1)$$

where n_i is the number of droplets with diameter d_i.

Rheological Measurements

Rheological measurements on the emulsions were made using a modular compact rheometer Physica MCR301 (Anton Paar, Germany). All measurements were made 1 day after emulsion preparation at 25 °C using a 5 cm parallel plate geometry with a gap of 200 μm. Flow curves were obtained between 0 and 300 s^{-1}.

Statistical Analysis

Significant differences between color, droplet size and rheological parameters were determined by the Tukey test. Statistical analyses were performed using the software Statistica 5.5 (Statsoft Inc., Tulsa, USA) and the level of confidence was 90%.

RESULTS AND DISCUSSION

Characterization of the Conjugates

SDS-PAGE Analysis

SDS-PAGE analysis was used to evaluate the covalent coupling between Na-CN and LBG for different conditions of temperature, protein/polysaccharide ratio and incubation time. Figure 1 shows molecular weight distributions of the conjugates formed at different temperatures and incubation times. The behavior was the same independent of the protein/polysaccharide ratio. The formation of Na-CN/LBG conjugates was suggested by the presence of bands with molecular weights higher than 182 kDa and some protein fractions visualized at the interface of the stacking and separating gels, indicating the presence of materials with very high molecular weight developed after covalent attachment between the protein and polysaccharide (Al-Hakkak and Al-Hakkak, 2010; O'Regan and Mulvihill, 2010; Miralles *et al.*, 2007). Moreover, the bands ascribed to α_s- and β-casein (between 25.9 and 37.1 kDa) gradually disappeared with reaction time, especially for conjugates produced at 24 hours of incubation and at higher temperatures of reaction (lanes 9 and 12 of Figure 1). Despite the increase of conjugate formation with the increase of incubation time and temperature, the residual presence of α_s and β-caseins bands in the samples prepared at 54, 60 and 75 °C (Figure 1) indicated that not all the protein had reacted with the polysaccharide and that the formation of conjugates was probably not totally complete under these heating conditions (Fechner *et al.*, 2007; Akhtar and Dickinson, 2007). The bands of κ-casein were not detectable due to the lower molecular weight of this fraction (~19kDa).
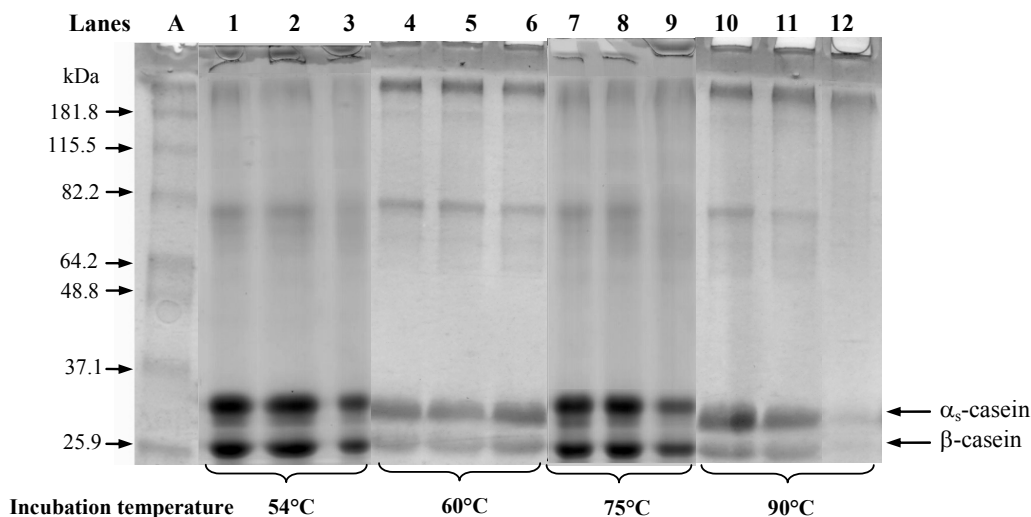
Figure 1: SDS-PAGE electrophoretogram of Na-CN and LBG conjugates produced at different temperatures (54, 60, 75 and 90 °C) and incubation times: 1 hour (lanes 1, 4, 7 and 10), 3 hours (lanes 2, 5, 8 and 11) and 24 hours (lanes 3, 6, 9 and 12). Lane A = commercial molecular mass markers.
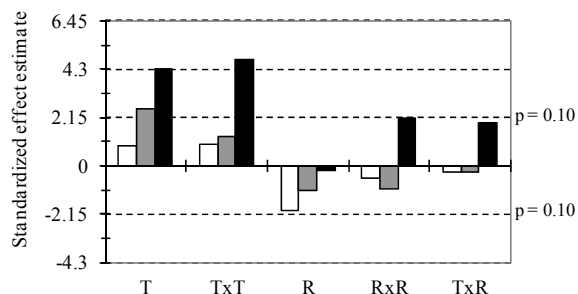
Color

The formation of conjugates was confirmed by the increase of the b*-value (Table 2), as observed by others authors (O'Regan and Mulvihill, 2009; Einhorn-Stoll *et al*., 2005). The b*-values of these materials are an indicator of the yellow color resulting from the Maillard-reaction (Einhorn-Stoll *et al*., 2005). The increase of the b*-values was verified mainly after 24 hours of incubation (Table 2). The standardized effects of temperature and Na-CN/LBG ratio on the conjugate color can be visualized in Figure 2. At 1 hour of incubation time, none of the variables showed a significant effect (p < 0.1) on the b*-value. At 3 hours of incubation time, temperature showed a positive linear effect, while at 24 hours, the temperature showed a higher positive (linear and quadratic) effect on the b*-value, in accordance with the SDS-PAGE results (Figure 2). Thus, the increase of temperature favored the occurrence of the Maillard reaction. The Na-CN/LBG ratio did not show a significant effect on the conjugate color at any temperature or incubation time.

Table 2: Influence of processing conditions on the b*-value of powder conjugates.

Trial	Temperature (°C)	Na-CN/LBG ratio	Incubation Time		
			1 h	3 h	24 h
1	60	0.50	3.83[a]	3.85[a]	4.51[b]
2	90	0.50	4.06[a]	4.81[a]	6.84[b]
3	60	0.88	3.93[a]	4.39[a]	5.23[b]
4	90	0.88	3.91[a]	5.27[b]	7.88[c]
5	54	0.65	4.44[a]	3.99[a]	5.25[b]
6	96	0.65	5.66[a]	7.36[b]	8.80[c]
7	75	0.30	4.90[a]	4.37[a]	6.08[b]
8	75	1.00	2.56[a]	2.50[a]	3.70[b]
9	75	0.65	4.55[a]	4.68[a]	3.23[b]
10	75	0.65	4.11[a]	4.60[a]	4.06[a]

Different superscript letters indicate significant difference (p > 0.1) between systems at the same line.

Figure 2: Standardized effects of temperature (T) and Na-CN/LBG ratio (R) on color (b*-value) of conjugates. Incubation time: (□) 1 h, (■) 3 h and (■) 24 h.

Evaluation of Emulsifying Properties of the Conjugates

Stability of Emulsions Stabilized by Na-CN – LBG Conjugates

Emulsions were prepared using Na-CN – LBG conjugates as emulsifiers. The evaluation of stability showed that the emulsions were stable up to 4 days of storage, with the exception of emulsions stabilized by conjugates prepared at higher temperatures (90 and 96 °C) and incubation time (24 hours), which showed phase separation. Trials 2, 4 and 6 (Table 1) at 24 hours of incubation time led to emulsions with values of the creaming index (CI) of 22%, 28% and 30%, respectively, after 4 days of storage. This occurred because the Maillard reaction probably proceeded faster at higher temperatures, leading to an irreversible degradation of conjugates, giving many colored and water-insoluble compounds called malanoidins (Yadav *et al*., 2010). However, the conjugates prepared at 1 and 3 hours using the same conditions of temperature and Na-CN/LBG ratio produced stable emulsions. These results indicated that emulsions stabilized by Na-CN – LBG conjugates were highly stable, even when produced using a rotor-stator homogenizer (Ultra Turrax), which did not occur for the emulsions stabilized by non-conjugated Na-CN and LBG containing low concentrations of polysaccharide (< 0.8% w/v) (Perrechil and Cunha, 2010).

Droplet Size of Emulsions Stabilized by Na-CN – LBG Conjugates

The evaluation of the mean droplet diameter (Table 3) showed that all emulsions were comprised of droplets with d_{32} between 7.35 μm and 12.84 μm, which were smaller than those of emulsions stabilized only by sodium caseinate at pH values of 3.7 and 2.7 (~14 μm) (Perrechil and Cunha, 2010). The smallest oil droplet (7.79 μm) was produced for the highest temperature (96 °C) and 1 hour of incubation time. Comparing the droplet diameters for different incubation times, it was found that d_{32} tended to decrease with the incubation time for the most of samples (Table 3), which could be associated with the good emulsifying properties of Na-CN – LBG conjugates. The conjugates can combine the hydrophobic property of proteins to adsorb strongly at the oil-water interface, with the hydrophilic property of polysaccharides to solubilize in an aqueous medium (Dickinson and Galazka, 1991), improving the stability and reducing the droplet size of emulsions.
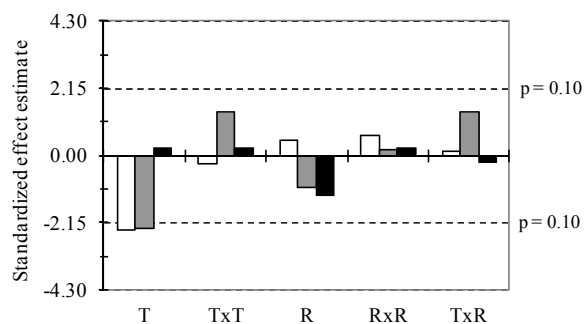
Table 3: Mean droplet diameter (d_{32}) of emulsions stabilized by Na-CN – LBG conjugates.

Trial	Temperature (°C)	Na-CN/LBG ratio	d_{32} (µm)		
			1 h	3 h	24 h
1	60	0.50	9.57[a]	11.04[a]	10.35[a]
2	90	0.50	7.35[a]	7.87[a]	12.84[b]
3	60	0.88	11.91[b]	10.39[ab]	8.56[a]
4	90	0.88	9.95[a]	10.08[a]	9.61[a]
5	54	0.65	11.55[b]	11.67[b]	9.50[a]
6	96	0.65	7.79[a]	8.59[a]	7.94[a]
7	75	0.30	11.46[b]	10.33[a]	10.25[a]
8	75	1.00	9.51[b]	7.21[a]	8.08[a]
9	75	0.65	9.88[b]	8.93[a]	8.31[a]
10	75	0.65	10.49[b]	9.30[a]	9.26[a]

Different superscript letters indicate significant difference (p > 0.1) for the mean droplet diameter (d_{32}) between systems in the same row.

However, for the conjugates produced at higher temperatures (90 and 96 °C), the diameters were similar or tended to increase with time (Table 3). This occurred due to the degradation of the protein-polysaccharide conjugates under these extreme conditions of heat treatment, confirming the results of emulsion stability. The same effect was verified for conjugates of egg white and pectin produced by Maillard reaction at 60 °C and a relative humidity of 79 °C, which showed improved emulsification properties up to1 12 hours of incubation time, after which emulsions with larger oil droplets were produced (Al-Hakkak and Al-Hakkak, 2010).

Evaluating the effects of variables (temperature and protein/polysaccharide ratio) on the mean droplet diameter (d_{32}) of emulsions (Figure 3), it was verified that only the temperature showed a significant effect (linear) for 1 hour and 3 hours of incubation.

Figure 3: Effects of temperature (T) and protein/polysaccharide ratio (R) on droplet diameter (d_{32}) for emulsions stabilized by conjugates. Incubation time: (□) 1 h, (■) 3 h and (■) 24 h.

This negative effect means that an increase in temperature led to smaller droplets, which is in accord with the results in Table 3. However, no variable showed a significant effect for the reaction time of 24 hours. This probably occurred because, at this incubation time, some conjugates were still being produced, while those prepared at higher temperatures were degraded in the final stage of the Maillard reaction.

Rheology

Rheological measurements indicated that all emulsions showed shear-thinning behavior (i.e., the apparent viscosity decreased with increasing shear rate). This behavior is the most common for emulsions and can occur for a variety of reasons, such as the changes of spatial distribution, the alignment of non-spherical droplets or deformation and disruption of flocs with the shear application (McClements, 2005).

The data of shear stress (σ) versus shear rate ($\dot{\gamma}$) were fitted to the power law model ($\sigma = k \cdot \dot{\gamma}^n$). Table 4 shows the values of the consistency index (k) and the flow behavior index (n) obtained from the fitting of the flow curves. In addition, the apparent viscosity at 10 s^{-1} (η_{10s}^{-1}) (Table 4) was evaluated. This shear rate was chosen because it is typical for food processes such as flow under gravity. The results showed that n tended to increase, while k and η_{10s}^{-1} had a tendency to decrease upon increasing the incubation time. The Maillard conjugate formation can be associated with an increase of the solution viscosity when compared to untreated proteins (Manzocco and Maltini, 1999; Oliver et al., 2006b; Hiller and Lorenzen, 2010) due to the increase of the molar mass of the ingredients (Hiller and Lorenzen, 2010). Nevertheless, the emulsion viscosity decreased with incubation time, probably due to the reduction of free polysaccharide in solution and to the partial Maillard conjugate degradation, as suggested in the previous discussion. We can assume that the improvement in the emulsifying properties is not due to the increased continuous phase viscosity, but to the molecular properties of the covalently-linked conjugate (Kim et al., 2003).

The effects of protein/polysaccharide ratio and temperature of incubation on the flow behavior index (n) and apparent viscosity at 10 s^{-1} of emulsions stabilized by Na-CN – LBG conjugates are shown in Figure 4. The results for the consistency index are not shown because they were very similar to those for apparent viscosity. At 1 hour of incubation time, the flow behavior index was mainly affected by the

Na-CN/LBG ratio (positive effect) (Figure 4(a)), indicating that the conjugates containing a higher amount of protein led to greater n. However, at 24 hours of incubation time, only the temperature showed a negative effect, i.e., the shear-thinning behavior became less pronounced with the increase of temperature (Figure 4(a)).
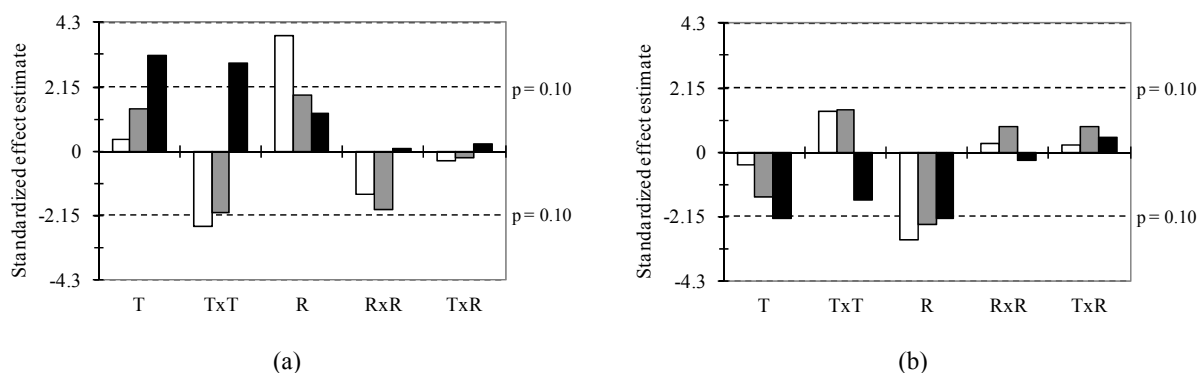
The protein/polysaccharide ratio showed a linear and negative effect on the apparent viscosity of the emulsions stabilized by the conjugates produced at 1, 3 and 24 h incubation time (Figure 4(b)). In this case, lower values of the protein/polysaccharide ratio, i.e., higher amounts of polysaccharide, were responsible for the increase of viscosity. On the other hand, the temperature showed a significant negative effect on the viscosity of emulsions stabilized by conjugates at 24 hours of incubation time (Figure 4(b)). The increase of temperature decreased the viscosity (and increased n), probably due to conjugate degradation after 24 hours of incubation time.

Table 4: Values of consistency index (k), flow behavior index (n) and apparent viscosity at 10 s^{-1} (η_{10s}^{-1}) of emulsions stabilized by Na-CN – LBG conjugates at pH 3.5

	k (Pa.s^n)			n (–)			η_{10s}^{-1} (Pa.s)		
Trial	1 h	3 h	24 h	1 h	3 h	24 h	1 h	3 h	24 h
1	8.68 [b]	8.50 [b]	4.52 [a]	0.43 [a]	0.44 [a]	0.52 [b]	2.34 [b]	2.34 [b]	1.51 [a]
2	7.48 [b]	3.68 [b]	1.58 [a]	0.46 [a]	0.55 [b]	0.64 [c]	2.15 [c]	1.30 [b]	0.69 [a]
3	1.92 [a]	2.18 [b]	1.36 [c]	0.60 [b]	0.58 [a]	0.65 [c]	0.77 [b]	0.83 [b]	0.61 [a]
4	1.92 [b]	1.24 [b]	0.24 [a]	0.60 [a]	0.65 [a]	0.81 [b]	0.77 [b]	0.55 [b]	0.16 [a]
5	4.58 [b]	4.68 [b]	1.08 [a]	0.52 [a]	0.51 [a]	0.67 [b]	1.50 [b]	1.51 [b]	0.51 [a]
6	3.45 [b]	3.29 [b]	0.05 [a]	0.54 [a]	0.53 [a]	0.91 [b]	1.19 [b]	1.12 [b]	0.04 [a]
7	5.63 [b]	5.40 [b]	2.63 [a]	0.46 [a]	0.47 [a]	0.58 [b]	1.64 [b]	1.58 [b]	0.99 [a]
8	0.89 [a]	2.31 [c]	1.36 [b]	0.66 [c]	0.56 [a]	0.58 [b]	0.40 [a]	0.84 [c]	0.52 [b]
9	1.91 [a]	2.06 [b]	2.22 [c]	0.62 [b]	0.62 [b]	0.58 [a]	0.80 [a]	0.87 [b]	0.84 [b]
10	1.94 [b]	1.45 [a]	1.86 [b]	0.63 [b]	0.64 [c]	0.61 [a]	0.83 [c]	0.64 [a]	0.75 [b]

Different letters indicate significant difference (p > 0.05) for the consistency index (k), flow behavior index (n) and apparent viscosity between systems in the same row.

(a)   (b)

Figure 4: Effects of protein/polysaccharide ratio (R) and temperature (T) on (a) flow behavior index (n) and (b) apparent viscosity at 10 s^{-1} (η_{10s}^{-1}) for conjugates prepared at different incubation times: (□) 1 h, (■) 3 h and (■) 24 h.

CONCLUSIONS

The study of sodium caseinate – LBG conjugates from the Maillard reaction showed that sodium caseinate was successfully conjugated to LBG and the covalent linkage was confirmed by SDS-PAGE and color evaluation. The properties of these conjugates were influenced by the temperature of the Maillard reaction, the protein/polysaccharide ratio and the incubation time. In general, the incubation time (1 and 3 hours) and temperature of reaction favored the conjugate formation, which produced stable emulsions with smaller droplet size. However, 24 hours of incubation time at high temperatures produced unstable emulsions with lower viscosity, less pronounced shear-thinning behavior and bigger droplet sizes, suggesting partial conjugate degradation. From these results, extreme conditions of temperature and incubation time can be identified as the process limitations. The protein/polysaccharide ratio only significantly affected the rheological properties of the emulsions. The decrease of the Na-CN/LBG ratio (increase of polysaccharide content) led to more viscous emulsions with more pronounced shear-thinning behavior (lower n values). From these results, it is possible to choose the adequate conjugates to obtain stable emulsions with the desired rheological behavior. In addition, these conjugates are an excellent alternative to substitute pure proteins and synthetic emulsifiers in emulsions produced under acid conditions (pH 3.5).

ACKNOWLEDGEMENTS

NOMENCLATURE

CI	creaming index	%
d_{32}	mean droplet diameter	µm
d_i	droplet diameter	µm
H	height of serum layer	mm
H_o	initial height of emulsions	mm
k	consistency index	$Pa.s^n$
LBG	locust bean gum	
n	flow behavior index	
Na-CN	sodium caseinate	
n_i	number of droplet	
R	protein/polysaccharide ratio	
T	temperature	°C

Greek Symbols

$\dot{\gamma}$	shear rate	s^{-1}
$\eta_{10s^{-1}}$	apparent viscosity at $10s^{-1}$	Pa.s
σ	shear stress	Pa

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