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THE EFFECTS OF SUCROSE ON THE MECHANICAL PROPERTIES OF ACID MILK PROTEINS-κ-CARRAGEENAN GELS

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Abstract - Mechanical properties have been widely correlated with textural characteristics to determine the interactions during the process formation of dairy gel. These interactions are strongly affected by process conditions and system composition. In the present study, the rheological of acid-induced protein dairy gels with ($2^{(7-3)}$) and without ($2^{(6-2)}$) sucrose and subjected to small and large deformations were studied using an experimental design. The independent variables were the sodium caseinate, whey protein concentrate (WPC), carrageenan and sucrose concentrations as well as stirring speed and heat treatment time and temperature. Mechanical deformation tests were performed at 0.1, 1, 5, and 9 mm/s up to 80% of initial height. A heavy dependence of rupture stress on increasing crosshead speed and the formation of harder gels with the addition of sucrose were observed. Moreover the elastic and viscous moduli, obtained by fitting the Maxwell model to stress relaxation data, increased with increasing addition of sucrose. These results can be explained by preferential hydration of the casein with sucrose, causing an induction of casein-polysaccharide and casein-casein interactions.
Keywords: Rheology, Milk proteins; Carrageenan; Factorial design.

INTRODUCTION

Protein-polysaccharide mixtures are widely used in the food industry as they play an essential role in the structure and texture of many foodstuffs. The overall product texture and structure depend not only on properties of the individual proteins and polysaccharides, but also on the nature and strength of protein-polysaccharide interactions (Dickinson and Merino, 2002). Therefore, in order to develop desirable properties in food products, a knowledge of the mechanisms of interactions in protein-polysaccharide systems is important (Hemar *et al*, 2002). The two main classes of milk proteins are the caseins fractions ($\propto_{s1}$-, $\propto_{s2}$-, β- e κ-casein) and the whey proteins (β-lactoglobulin, α-lactoalbumin, immunoglobulins and bovine serum albumin), with casein being the major protein component. Sodium caseinate (Na-CN) and whey protein concentrate (WPC) are functional dairy ingredients widely used in many foods, particularly in dairy desserts. Na-CN is manufactured by adding acid to milk to precipitate the casein at its isoelectric point. The acid-coagulate casein is washed and then redissolved by adding an appropriate amount of sodium hydroxide to restore neutrality. Under these conditions, caseinate forms aggregates or submicelles due to the high proportion of hydrophobic aminoacid side chain that self-associate in aqueous solutions (Farrell et al.; 1990). Further association of submicelles to form casein micelles is prevented by removal of most of the calcium (Oakenfull et al., 1999). Whey protein is obtained from cheese whey by ion-exchange fractionation process or membrane filtration process

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(Mulvihill and Kinsella, 1988). Heat denaturation and the subsequent aggregation of globular whey proteins can produce desirable food texture properties. Depending on the proteins concentration, this aggregation process could result in gel formation. Three phenomena are involved in the heat-induced aggregation of globular proteins, frequently simultaneously: conformational changes, chemical reactions and physical interactions. On heating, whey proteins will partly unfold. In this denaturation step non-polar groups are exposed and in the case of β-lactoglobulin and bovine serum albumin, free sulphydryl groups become reactive. In principle, the conformational changes in this denaturation step are reversible. However, the denatured molecules may aggregate irreversibly via non-covalent and covalent bonds. The non-covalent bonds are due to van der Waals attraction forces, hydrogen bonding and electrostatic and hydrophobic interactions (Roefs et al., 1990). The structure and properties of whey protein aggregates will vary according to the extent of unfolding, the type and kinetics of process, and the nature of interactions. Changes in solution pH, co-solute concentration and heating conditions alter the aggregation and gelling properties of whey proteins (Mulvihill and Kinsella, 1988).

The milk protein gels can be formed by acidification using bacterial cultures (lactic fermentation) or by hydrolysis of the internal ester, glucono-δ-lactone (GDL). Although there are some undoubtedly differences (e.g. in the rate of lowering the pH) between the microbial and chemical acidification methods, the convenience and reproducibility of GDL method has resulted in its increased use in quantitative laboratory-base studies (Koh et al., 2002) and in the manufacture of foods such as cheeses (Serpelloni et al., 1990) and yogurts (Fly et al., 1997). Gels made by these two types of acidification also differ in their rheological properties (Lucey and Singh, 1998), which could be partly attributed to how quickly of acidification/gelation occurs. The presence of co-solutes may also modify the gelation process, contributing to the stability of the protein during milk acidification (Schkoda et al., 1999). Studies of milk protein-sugar interactions (Kulmyrzaev et al., 2000; Mora-Gutierrez *and* Farrell, 2000) have shown that sugar contributes to protein hydration and increases the degree of protein aggregation. On lowering the pH from neutral in the direction of pI, the sucrose in sodium caseinate systems strengthens protein-protein interactions with an increasing number of structural bounds (Belyakova et al., 2003). This has been observed for a wide range of sucrose concentration, from 10-78% w/v.

In addition to the milk protein ingredients, many manufactured products based on acidified milk gels also contain polysaccharides, which are added to improve the texture and shelf life. Carrageenans are anionic polysaccharides extracted from red seaweed, which are found in three main forms like kappa, iota or lambda. Kappa- and iota-carrageenans have the ability to form a gel under certain conditions. Kappa-carrageenan undergoes a coil (disordered state) to helix (ordered state), depending on the temperature and ionic environment. Gelation is achieved via junction zones formed by helices, forming a three-dimensional network (Hemar et al., 2002). Interactions between biopolymers (protein-protein, protein-polysaccharide and/or protein-solvent) are controlled as a function of system composition (Verheul and Roefs, 1998; Mleko, et al., 1997) or process condition, such as heat treatment (Capron, et al., 1999; Ju and Kilara, 1998) and shear (Walkenström, et al., 1998). These interactions can be evaluated by rheological measurements.

In this study, we used a factorial design to evaluate the effect of composition and process conditions on the mechanical properties (rheological measurements under conditions of large deformation and stress relaxation) of gels acidified with glucono-δ-lactone and formed at 10 ^{o}C. The model systems were produced using different concentrations of sodium caseinate, whey protein concentrate (WPC) and κ-carrageenan with and without the addition of sucrose. The process variables were stirring speed, heating time and temperature.

MATERIALS AND METHODS

Materials

Casein, carrageenan and glucono-δ-lactone (GDL) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The whey protein concentrate (WPC) (Alacem ^{TM} 392) was kindly supplied by New Zealand - Brazil (NZMP) and sucrose with analytical degree was acquired from Synth (São Paulo, Brazil). The composition of ingredients is summarized in Table 1.

Table 1: Mean composition of the samples biopolymers used to prepare the dairy model systems.

	Protein (%)	Na^+(%)	Ca^{2+}(%)	K^+(%)	Lactose (%)	Moisture (% wet basis)
Casein	89.66	0.80	0.40	0.40	-	7.22
Carrageenan	-	0.10	0.70	2.70	-	8.40
WPC	83.60	0.25	0.36	0.58	6.70	3.90

Preparation of the Model Systems

The aqueous solutions of sodium caseinate were prepared by dissolving the casein sample in de-ionised water by magnetic stirring. The pH of the samples was adjusted to the range between 6.8 and 7.0 using NaOH 10M. WPC was then added to the sodium caseinate solution.

The carrageenan was dissolved separately using controlled mechanical stirring, in a thermostatically controlled water bath at 40°C. Sucrose was then added to the carrageenan solution. Protein and carrageenan solutions were then mixed and subjected to different heat treatment temperature/time combinations. After the heat treatment, the final temperature was set at 35°C and the GDL added in the following ratio: GDL/caseinate = 0.135 (Braga, 2002). The process conditions and ingredient concentrations were defined according to a factorial design (section 2.4). The mixture was conditioned in plastic cylinders (30x30 mm) and stored at 10°C for 48 hours. The acidification of the systems promoted by GDL allowed that the pH was approximately 4.6 (between 4.3 and 4.8) at the end of storage time. These systems (gels) were measured by uniaxial compression.

Mechanical Properties of the Gels

The mechanical properties of gels were determined by uniaxial compression using a Universal Testing Machine (TA-XT2i, Stable Microsystems, Surrey, England) with a 25 kg load cell. The samples were subjected to large deformation and stress relaxation measurements using an acrylic cylindrical plate (35mm diameter). The surfaces in contact were lubricated with low viscosity silicon to avoid friction between the sample and the acrylic cylindrical plate. Each sample was tested in triplicate at 10 °C.

For large deformation tests, samples were submitted to 80% compression at crosshead speeds of 0.1, 1, 5 e 9 mm/s. The raw data were converted into true or Hencky strain (ε) and stress (σ) using Eqs. (1) and (2), respectively.

$$\varepsilon = \left| \ln\left(\frac{H(t)}{H_0} \right) \right| \tag{1}$$

where ε is the true strain, H(t) is the height at time (t) and H_0 is the height at the beginning of compression. The true stress (σ) was calculated assuming incompressible material.

$$\sigma = \frac{F(t)H(t)}{A_0 H_0} \tag{2}$$

where F(t) is the force at time (t) and A_0 is the initial contact area of the gel. Stress (σ_f) and strain (ε_f) at fracture were determined at the maximum point of the true stress-strain curve. The deformability modulus (E) was obtained from the slope of initial linear portion of stress-strain plot (ε< 5%) (Steffe, 1996).

The stress relaxation measurements were taken at 3% of the initial height (within linear viscoelastic domain) during 300s, using an initial crosshead speed of 5mm/s. The stress relaxation data were fitted to an exponential expression based on a Maxwell model (Steffe, 1996). Relaxation curves were dimensionless expressed using the $F(t)/F_0$ ratio vs. time, where F(t) is the instantaneous force during relaxation time, and F_0 is the initial force before the decay of the stress (Bertola et al., 1991). The used model had two Maxwellian elements and one elastic element in parallel, in accordance with equation (3):

$$F^*(t) = F(t)/F_0 = S_\infty + \\ +S_1 \exp(-t/\tau_1) + S_2 \exp(-t/\tau_2) \tag{3}$$

where S_∞, S_1, S_2 are coefficients dependent on the viscoelastic properties of the material and τ_1, τ_2 the relaxation times. In terms of the elastic moduli (E_i):

$$E_i = S_i F_0 / \varepsilon A \quad \text{with } i=1,2,\infty \tag{4}$$

where A is the compression area and $\varepsilon = 1-(L/L_0)$ is the constant compressive strain, L is the height of

cylindrical sample after the compression and L_0 is the initial height.

The values of the viscous moduli (η_i) of the liquid in the dashpot of element i were estimated as follows (Bertola et al., 1991):

$$\eta_i = E_i \tau_i \quad (5)$$

Experimental Design

For multivariate analysis of the mechanical properties of gels, two experimental designs were used: one with sucrose and other without. A factorial experimental design with $2^{(6-2)}$ trials was used for gels without sucrose and $2^{(7-3)}$ those with sucrose with four central points. Table 2 shows the range of sodium caseinate (CAS), whey protein concentrate (WPC), carrageenan (CAR) and sucrose (SUC) concentrations, as well as the stirring speed of ingredients mixture (VEL) and time (t) and temperature (T) of heat treatment used to evaluate their effect on the mechanical properties of the gels.

The responses monitored were the deformability modulus (E) and stress and strain at rupture (σ_f and ε_f) obtained at four compression rates as well as the maximum viscous modulus (η_i) and elastic modulus E_∞ obtained from Maxwell model fitted to stress relaxation data. The analysis of variance was used to detect significant effects at $p<0.05$. The Statistica software package (Statsoft, v. 5.0) was used to evaluate the significant effects of the independent variables on the responses. This software was also used to estimate the Maxwell model parameters.

Table 2: Levels of both coded and actual values of independent variables used in the factorial designs.

	CAS	WPC	CAR	SUC	T	t	VEL
Level (-1)	2.0	0.8	0.3	5	30	5	88
Level (0)	6.0	1.75	0.65	10	55	17.5	188
Level (+1)	10.0	2.7	1.0	15	80	30	288

CAS: Sodium caseinate concentration (%w/w),
WPC: Whey protein concentrate concentration (%w/w),
CAR: κ-carrageenan concentration (%w/w),
SUC: Sucrose concentration (%w/w), T: Heat treatment temperature (^oC),
t: Heating time (min), VEL: Stirring speed (rpm)

RESULTS E DISCUSSION

Large Deformation Properties of the Acidified Dairy Gels

The responses of deformability modulus and stress and strain at rupture for the systems without (factorial design $2^{(6-2)}$) and with sucrose (factorial design $2^{(7-3)}$) are shown in Table 3 and 4, respectively, as a function of ingredients concentration and process parameters. In both cases, a clear tendency for the stress at rupture and deformability modulus to an increase with in compression rate could be observed, as is typical of viscoelastic materials (Ribeiro, et al., 2004). This was also observed by Nakamura et al. (2001) and Thybo et al. (2000), who studied gellan gels and cooked potato, respectively. However, the values for strain at rupture did not show a clear trend with the increase of compression rate, as did the values for the other mechanical properties (Tables 3 and 4).

The values of the mechanical properties were normally slightly higher for gels with sucrose than for systems without, as can be observed at central points (runs 17-20). The deformability modulus and stress and strain at rupture are mechanical properties associated to firmness, hardness and elasticity, respectively. It means that the addition of sucrose can be expected to produce firmer, harder and more elastic gels than systems without.

In Figures 1 and 2 the significant effects of independent variables on the mechanical properties, obtained at different crosshead speeds (0.1, 1, 5 and 9 mm/s) for gels without and with sucrose, respectively are presented. All independent variables normally had significant effects on the mechanical properties, and the absolute values of the effects increased with compression rate.

Table 3: Coded levels for the experimental design $2^{(6-2)}$ and stress at rupture, strain at rupture and deformability modulus of model systems without sucrose evaluated at different compression rates.

							Stress at Rupture (kPa)				Strain at Rupture (-)				Deformability Modulus (kPa)			
							Crosshead speed (mm/s)				Crosshead speed (mm/s)				Crosshead speed (mm/s)			
	CAS	WPC	CAR	T	t	VEL	0.1	1.0	5.0	9.0	0.1	1.0	5.0	9.0	0.1	1.0	5.0	9.0
1	-1	-1	-1	-1	-1	-1	12.32	17.38	20.23	20.92	0.46	0.48	0.47	0.41	26.11	26.68	35.94	65.52
2	1	-1	-1	1	-1	1	20.72	33.41	33.69	38.95	0.41	0.46	0.48	0.50	57.15	73.34	88.63	89.25
3	-1	1	-1	1	1	-1	9.89	16.11	20.60	22.07	0.44	0.44	0.45	0.46	27.64	30.12	45.65	57.60
4	1	1	-1	-1	1	1	11.70	16.31	21.59	23.21	0.43	0.47	0.40	0.38	30.58	39.31	64.78	75.90
5	-1	-1	1	1	1	1	33.60	37.53	41.47	45.40	0.44	0.48	0.59	0.70	77.39	83.39	90.06	96.73
6	1	-1	1	-1	1	-1	9.38	14.26	19.52	20.79	0.37	0.38	0.41	0.43	31.53	45.03	56.16	56.63
7	-1	1	1	-1	-1	1	1.63	3.10	4.71	4.88	0.16	0.17	0.22	0.26	9.83	17.41	18.63	20.81
8	1	1	1	1	-1	-1	20.75	30.23	38.03	38.77	0.25	0.27	0.30	0.30	99.36	135.88	167.48	169.0
9	-1	-1	-1	-1	1	1	11.09	18.68	24.76	25.17	0.48	0.55	0.59	0.55	17.94	26.85	27.74	28.41
10	1	-1	-1	1	1	-1	15.12	22.95	29.22	32.21	0.38	0.38	0.38	0.40	48.68	70.31	89.83	100.6
11	-1	1	-1	1	-1	1	10.07	16.42	18.40	20.03	0.56	0.60	0.51	0.55	15.04	23.36	29.72	30.68
12	1	1	-1	-1	-1	-1	11.25	19.92	26.75	28.55	0.30	0.32	0.37	0.39	46.24	74.42	83.65	85.02
13	-1	-1	1	1	-1	-1	21.53	29.67	34.22	36.35	0.57	0.59	0.59	0.59	41.69	58.56	59.96	69.57
14	1	-1	1	-1	-1	1	18.65	25.09	34.69	36.88	0.29	0.41	0.34	0.36	88.14	70.21	122.06	123.7
15	-1	1	1	-1	1	-1	0.84	1.47	2.16	2.48	0.11	0.14	0.15	0.16	9.31	11.61	14.10	16.74
16	1	1	1	1	1	1	16.11	24.32	31.91	34.71	0.26	0.30	0.31	0.33	81.46	108.01	132.80	137.80
17	0	0	0	0	0	0	28.05	34.95	53.97	64.08	0.41	0.51	0.49	0.51	68.83	85.01	96.02	99.63
18	0	0	0	0	0	0	27.70	34.84	53.48	63.29	0.41	0.52	0.48	0.52	68.45	84.77	95.48	99.43
19	0	0	0	0	0	0	27.33	34.48	53.62	62.78	0.43	0.51	0.50	0.50	68.12	84.78	95.57	98.43
20	0	0	0	0	0	0	26.23	34.65	53.40	63.98	0.40	0.52	0.47	0.51	68.93	85.56	95.21	99.23

Table 4: Coded levels for the experimental design $2^{(7-3)}$ and stress at rupture, strain at rupture and deformability modulus of model systems with sucrose evaluated at different compression rates.

								Stress at Rupture (kPa)				Strain at Rupture (-)				Deformability Modulus (kPa)			
								Crosshead speed (mm/s)				Crosshead speed (mm/s)				Crosshead speed (mm/s)			
	CAS	WPC	CAR	SUC	T	t	VEL	0.1	1.0	5.0	9.0	0.1	1.0	5.0	9.0	0.1	1.0	5.0	9.0
1	-1	-1	-1	-1	-1	-1	-1	13.02	14.32	15.89	17.28	0.40	0.42	0.44	0.46	28.90	39.90	53.97	55.54
2	1	-1	-1	-1	1	-1	1	9.07	13.25	16.94	19.20	0.36	0.36	0.37	0.37	31.37	45.38	60.77	63.60
3	-1	1	-1	-1	1	1	-1	4.44	6.20	8.35	8.57	0.23	0.24	0.24	0.25	22.42	30.39	42.76	43.83
4	1	1	-1	-1	-1	1	1	13.70	21.27	29.15	29.44	0.33	0.38	0.39	0.43	48.69	63.13	75.52	90.91
5	-1	-1	1	-1	1	1	1	5.53	6.49	8.12	21.52	0.37	0.46	0.47	0.49	12.67	24.73	34.51	81.33
6	1	-1	1	-1	-1	1	-1	19.40	30.93	43.50	53.72	0.34	0.37	0.43	0.42	69.73	107.8	126.3	155.3
7	-1	1	1	-1	-1	-1	1	0.75	0.95	1.64	1.67	0.13	0.15	0.19	0.19	6.12	7.18	10.65	10.85
8	1	1	1	-1	1	-1	-1	16.99	26.11	33.54	34.35	0.34	0.35	0.37	0.39	70.80	104.5	126.4	127.9
9	-1	-1	-1	1	-1	1	1	9.30	14.10	14.76	18.20	0.43	0.48	0.49	0.51	26.54	37.79	46.63	52.39
10	1	-1	-1	1	1	1	-1	16.72	23.45	26.92	30.29	0.40	0.39	0.40	0.39	55.02	76.50	85.31	101.2
11	-1	1	-1	1	1	-1	1	8.41	11.91	11.28	13.54	0.61	0.54	0.58	0.46	19.41	29.36	31.44	40.80
12	1	1	-1	1	-1	-1	-1	8.23	11.65	15.69	18.47	0.35	0.35	0.35	0.38	18.33	39.88	52.96	59.37
13	-1	-1	1	1	1	-1	-1	25.86	27.21	30.66	36.17	0.52	0.52	0.53	0.53	64.04	65.62	72.46	79.47
14	1	-1	1	1	-1	-1	1	29.49	44.48	55.94	61.91	0.33	0.36	0.37	0.37	108.1	154.1	192.0	211.9
15	-1	1	1	1	-1	1	-1	1.79	2.63	3.35	3.51	0.17	0.19	0.22	0.23	11.69	15.12	16.80	19.63
16	1	1	1	1	1	1	1	18.63	28.72	41.69	52.41	0.23	0.26	0.30	0.31	103.4	142.5	192.0	253.7
17	0	0	0	0	0	0	0	32.66	54.80	71.91	79.36	0.43	0.53	0.56	0.58	96.59	135.0	153.7	173.0
18	0	0	0	0	0	0	0	33.67	55.24	72.35	80.33	0.44	0.53	0.55	0.59	96.15	133.4	163.3	175.7
19	0	0	0	0	0	0	0	32.95	54.00	72.07	79.70	0.44	0.53	0.56	0.58	95.75	134.2	148.0	174.5
20	0	0	0	0	0	0	0	32.52	54.86	72.28	80.03	0.44	0.53	0.55	0.59	96.51	135.1	171.1	175.7

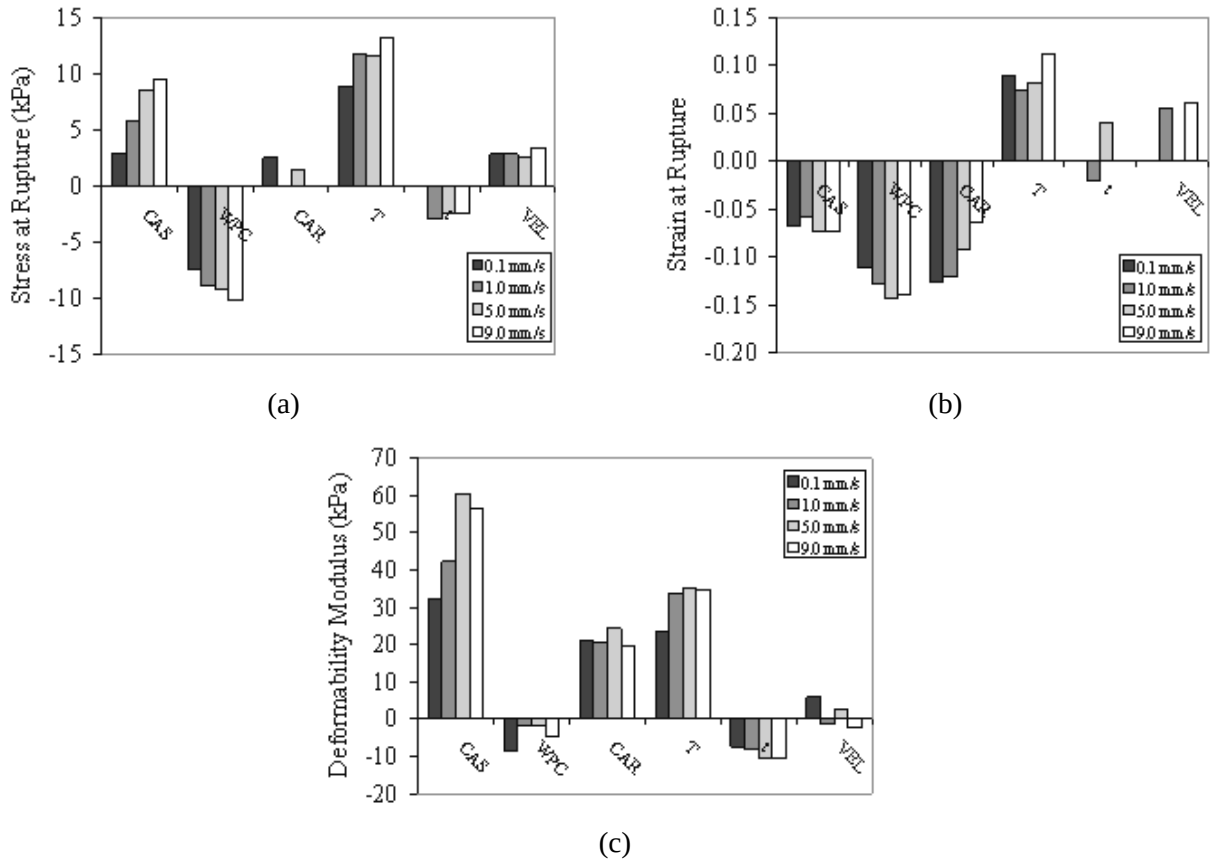

Figure 1: Significant effects of independent variables on a) stress at rupture, b) strain at rupture and c) deformability modulus in dairy model systems without sucrose. Crosshead speeds: 0.1, 1.0, 5.0 and 9 mm/s. CAS - sodium caseinate concentration, WPC - whey protein concentrate concentration, CAR - carrageenan concentration, T - heat treatment temperature, t - heating time and VEL - stirring speed.

Figure 1a shows that heating temperature was the variable that had the most pronounced positive effect on stress at rupture, followed by sodium caseinate concentration. WPC concentration had a strong negative effect on failure stress, meaning that an increase this variable yielded softer gels. The variables carrageenan concentration, heating time and stirring speed had only a minor effect on gel hardness.

Figure 1b shows that WPC concentration had the greatest negative effect on strain at rupture or gel elasticity, followed by carrageenan and sodium caseinate concentration. These results indicate that an increase in biopolymers concentrations resulted in a less elastic network. On the contrary, an increase in heating temperature contributed to the elasticity of the gels. The effects of the variables on the deformability modulus (Figure 1c) tended to be similar to the effects on stress at rupture, but the values were lower for WPC concentration and higher for sodium caseinate and carrageenan concentrations.

For gels with sucrose, sodium caseinate was the variable with the most pronounced effect on failure stress (Figure 2a), followed by carrageenan, WPC and sucrose concentrations. Stirring speed and heat treatment time and temperature showed small significant effects on stress at rupture for sweet dairy gels. The biggest differences between gels with and without sucrose were the large increase in the significant effects of carrageenan concentration and the large decrease in the effects of heat treatment temperature. The increase in hardness produced by carrageenan with sucrose could be attributed to the ability of sucrose to form hydrogen bonds, increasing the number and stabilization of junction zones (Ribeiro et al., 2004). Sucrose probably also increased the temperature for unfolding whey protein and the final rigidity of the gels (Kulmyrzaev et al., 2000), which explains the small significant effect of heating temperature on failure stress of gels with

sucrose.

All independent variables had significant effects on failure strain (Figure 2b). Heating time and sodium caseinate, carrageenan and WPC concentrations had negative effects, the latter showing the most pronounced effect on gels elasticity. On the other hand, sucrose concentration and heating temperature had significant positive effects on the elasticity. In a previous study with milk protein-carrageenan, Hemar et al. (2002) verified that strain value was independent of protein type or concentration, contrary to our results.

Apparently, the sucrose caused the biopolymers to interact, decreasing their negative effects on the gel elasticity. Mleko (1997) studied the rheological properties of desserts prepared with carrageenan, milk and whey proteins, verifying that the addition of 10-15% sucrose decreased the gelling ability of whey proteins. However, in this research, the addition of sucrose enhanced the gel elasticity. Under certain conditions, hard, elastic gels can be obtained by the addition of carrageenan to dairy systems. Nevertheless, these conditions are probably affected by shear, temperature (transition from coil to helix) and ionic strength, which could be responsible for the negative effect of polysaccharide concentration on gel elasticity.

The independent variables, sodium caseinate, carrageenan, sucrose and WPC concentrations, had the most significant effects on the deformability modulus (Figure 2c) of gels with sucrose. WPC concentration exerted a negative effect but the other variables, especially sodium caseinate contributed to an increase in gel firmness. The biggest difference between the gels with and without sucrose with respect to effect on the elasticity modulus, occurred with falls in heat treatment temperature, as in the case of failure stress.

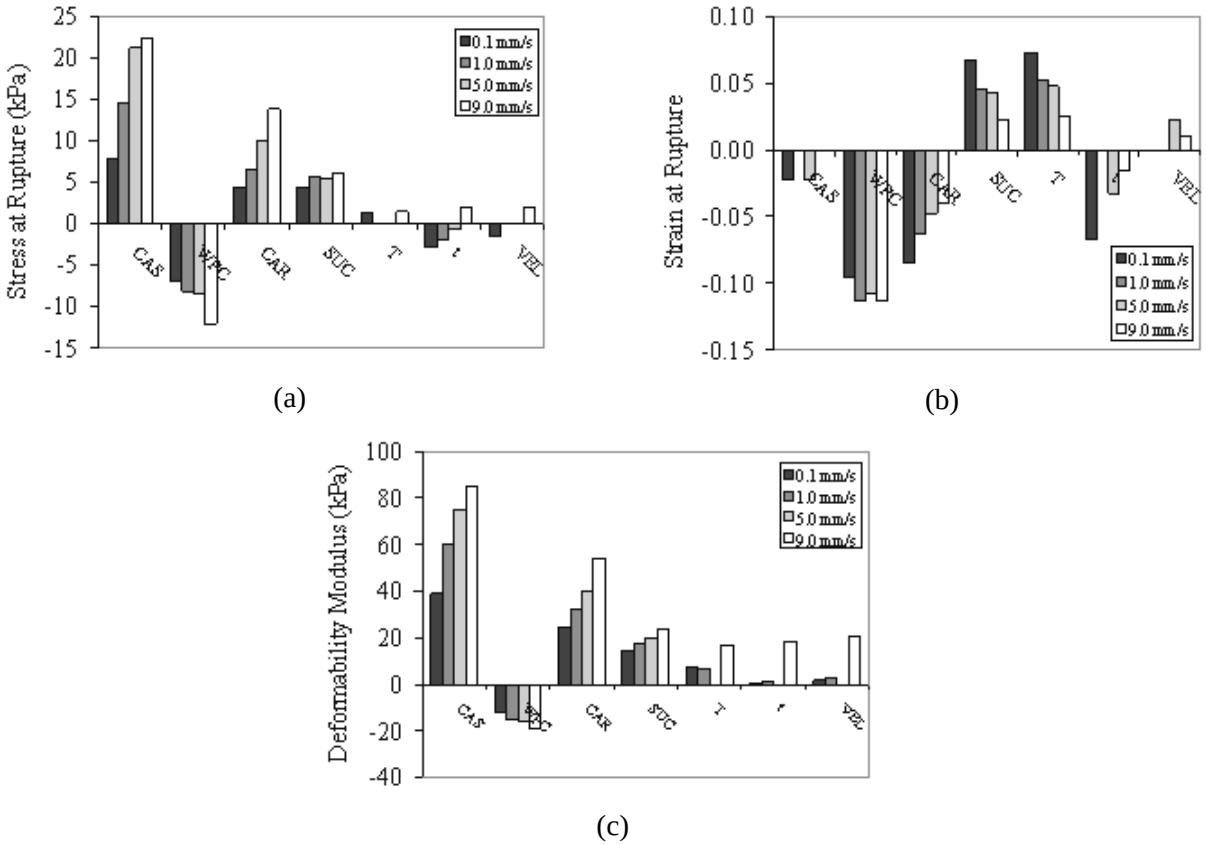

Figure 2: Significant effects of independent variables on a) stress at rupture, b) strain at rupture and c) deformability modulus in dairy model systems with sucrose. Crosshead speeds: 0.1, 1.0, 5.0 and 9 mm/s. CAS - sodium caseinate concentration, WPC - whey protein concentrate concentration, CAR - carrageenan concentration, T - heat treatment temperature, t - heating time and VEL - stirring speed.

All the results indicated that sugar can modify the interactions between protein-polysaccharide interactions or stabilize the protein in terms of conformation, aggregation state and protein-solvent interactions, as suggested by Arakawa and Timasheff (1982). Sugar changes qualitatively the structural and rheological properties, strengthening casein-casein and casein-polysaccharide interactions (Dickinson and Merino, 2002). Previous studies have shown that low-molecular-weight sugars, and particularly sucrose, can cause various marked changes in functional properties of casein: (i) an increase in the solubility of sodium caseinate in aqueous medium in the vicinity of the isoelectric point of the protein (Antipova and Semenova, 1995); (ii) an increase in the thermodynamic compatibility of casein with polysaccharides in bulk aqueous media (Antipova and Semenova, 1995; Schorsch et al., 1999); (iii) an increase in the surface activity of sodium caseinate at the planar air-water interface (Antipova et al., 1999); (iv) a substantial increase in the acid-induced gel-forming ability of sodium caseinate (Dickinson and Merino, 2002) and (v) a marked enhancement of the viscoelasticity of acid-induced casein gels due to the effects of sucrose on the self-association of sodium caseinate (Belyakova et al., 2003).

WPC concentration had negative effects on the gel hardness, firmness and elasticity in both factorial designs studied (with and without sucrose). This characteristic could be associated with lactose, since such sugar slows down the denaturation of β-lactoglobulin (Spiegel, 1999) and thus weakens the gel network. Another possible explanation for the negative effect of WPC concentration on the mechanical properties of dairy gels could be the strong interaction between caseinate and carrageenan, which could decrease the interaction of these biopolymers with WPC (Tziboula and Horne, 1999) or even exclude it from the elastic network.

Stress Relaxation of Gels

Elastic and viscous moduli were calculated using equations 4 and 5, respectively, from the analysis of stress relaxation data. These values were used as responses in factorial designs, as can be seen in the Tables 5 and 6, respectively. The significant effects and correspondent *P*-values of elastic (E_∞) and viscous η_1 (element with the highest relaxation time) moduli are presented in Tables 7 and 8 for gels without and with sucrose, respectively. Bertola et al., (1991) found values for these moduli in cheeses that had the same order of magnitude as those found in this study.

Table 5: Coded levels for the experimental design $2^{(6-2)}$ of model systems without sucrose. Elastic and viscous moduli obtained by Maxwell model.

	CAS	WPC	CAR	T	t	VEL	E_∞ (kPa)	E_1(kPa)	E_2(kPa)	η_1(kPa.s)	η_2(kPa.s)
1	-1	-1	-1	-1	-1	-1	6.74	6.34	6.49	598.89	26.51
2	1	-1	-1	1	-1	1	14.93	16.64	20.06	1399.5	81.73
3	-1	1	-1	1	1	-1	7.28	6.31	5.48	588.24	25.80
4	1	1	-1	-1	1	1	5.47	10.94	12.01	983.73	44.73
5	-1	-1	1	1	1	1	31.30	24.12	22.74	2657.4	90.95
6	1	-1	1	-1	1	-1	10.85	8.94	9.19	783.40	40.54
7	-1	1	1	-1	-1	1	8.61	6.40	5.29	661.64	25.12
8	1	1	1	1	-1	-1	43.47	40.58	54.31	3595.3	201.45
9	-1	-1	-1	-1	1	1	6.18	5.96	4.68	680.76	25.06
10	1	-1	-1	1	1	-1	17.62	21.54	26.00	1859.5	101.03
11	-1	1	-1	1	-1	1	4.71	4.64	3.42	519.69	16.39
12	1	1	-1	-1	-1	-1	14.86	18.08	24.74	1524.7	80.92
13	-1	-1	1	1	-1	-1	15.44	14.48	10.06	1430.9	42.21
14	1	-1	1	-1	-1	1	16.50	22.60	29.95	1687.2	117.18
15	-1	1	1	-1	1	-1	7.85	7.80	6.19	910.41	32.04
16	1	1	1	1	1	1	23.38	25.11	32.55	1251.6	58.29
17	0	0	0	0	0	0	12.90	14.54	11.26	1659.8	61.16
18	0	0	0	0	0	0	13.39	14.51	12.27	1510.7	60.68
19	0	0	0	0	0	0	13.22	14.65	11.78	1654.9	60.34
20	0	0	0	0	0	0	13.30	14.71	11.90	1501.0	62.64

Table 6: Coded levels for the experimental design $2^{(7-3)}$ of model systems with sucrose. Elastic and viscous moduli obtained by Maxwell model.

	CAS	WPC	CAR	SUC	T	t	VEL	E_∞(kPa)	E_1(kPa)	E_2(kPa)	η_1(kPa.s)	η_2(kPa.s)
1	-1	-1	-1	-1	-1	-1	-1	7.84	8.36	4.28	1389.4	51.31
2	1	-1	-1	-1	1	-1	1	10.95	20.87	22.41	2644.4	153.29
3	-1	1	-1	-1	1	1	-1	8.10	7.68	6.85	866.94	33.82
4	1	1	-1	-1	-1	1	1	6.09	15.47	18.94	1406.7	77.28
5	-1	-1	1	-1	1	1	1	86.17	2.98	31.01	270.21	116.06
6	1	-1	1	-1	-1	1	-1	7.43	19.69	43.08	1790.9	99.77
7	-1	1	1	-1	-1	-1	1	4.81	8.65	7.16	844.59	27.89
8	1	1	1	-1	1	-1	-1	12.42	44.96	60.55	3689.8	258.88
9	-1	-1	-1	1	-1	1	1	8.25	6.72	4.91	702.74	23.67
10	1	-1	-1	1	1	1	-1	19.14	22.38	31.90	1774.2	106.12
11	-1	1	-1	1	1	-1	1	4.95	4.61	4.34	363.18	16.28
12	1	1	-1	1	-1	-1	-1	15.60	21.55	29.44	1777.5	108.50
13	-1	-1	1	1	1	-1	-1	21.04	9.83	7.16	1274.0	36.26
14	1	-1	1	1	-1	-1	1	25.77	27.15	35.30	2399.5	124.26
15	-1	1	1	1	-1	1	-1	9.11	8.09	6.10	930.96	29.99
16	1	1	1	1	1	1	1	4.88	38.27	47.71	4372.0	309.20
17	0	0	0	0	0	0	0	14.13	11.78	12.71	1131.0	58.34
18	0	0	0	0	0	0	0	13.96	11.84	11.96	1146.0	55.94
19	0	0	0	0	0	0	0	14.92	12.65	13.04	1229.9	60.56
20	0	0	0	0	0	0	0	13.17	11.41	11.81	1097.4	49.95

Table 7: *P*-values significant effects of independent variables on the elastic and viscous moduli for gels without sucrose. CAS - sodium caseinate concentration, WPC - whey protein concentrate concentration, CAR - carrageenan concentration, T - heat treatment temperature, t - heating time and VEL - stirring speed.

		MEAN	CAS	WPC	CAR	T	t	VEL
E_∞ (kPa)	Effect	14.40	7.37	-	9.95	10.14	-	-
	P-value	$4 . 10^{-8}$	0.05	-	0.01	0.12	-	-
η_1 (kPa.s)	Effect	1373	630	-	603	684	-	-
	P-value	$3 . 10^{-7}$	0.07	-	0.09	0.06	-	-

Table 8: *P*-values significant effects of independent variables on the elastic and viscous moduli for gels with sucrose. CAS - sodium caseinate concentration, WPC - whey protein concentrate concentration, CAR - carrageenan concentration, T - heat treatment temperature, t - heating time and VEL - stirring speed.

		MEAN	CAS	WPC	CAR	SUC	T	t	VEL
E_∞ (kPa)	Effect	18.66	21.93	-	11.44	-	5.31	-	-
	P-value	$2 . 10^{-7}$	$1 . 10^{-4}$	-	0.01	-	0.02	-	-
η_1 (kPa.s)	Effect	1594	1564	-	400	-	284	-	-
	P-value	$3 . 10^{-9}$	$2 . 10^{-5}$	-	0.01	-	0.03	-	-

It can be seen that sodium caseinate concentration and carrageenan concentration and heating temperature contributed to an increase in firmness and fluidity (positive effects) of gels without (Table 7) and with sucrose (Table 8). The other independent variables were not statistically significant with either system.

The values for mean effects of elastic and viscous moduli with addition of sucrose to the dairy gels, as can be observed in Tables 7 and 8. The elastic

modulus increased almost 30% (from 14.4 kPa to 18.66 kPa) and the viscous modulus, approximately 16% (from 1373 to 1594 kPa.s). The effects of sodium caseinate and carrageenan concentrations were similar. However, the effect of heating temperature decreased significantly with addition of sucrose, showing a more pronounced effect on the elastic and viscous moduli for the gels without sucrose. These results may also be associated to stabilization of the WPC to denaturation (Kulmyrzaev et al., 2000), as occurred for mechanical properties obtained for large deformations.

Sodium caseinate had the biggest effect on gels with sucrose, with the value obtained almost twice that obtained with gels without sucrose. However, the effects of carrageenan concentration and heating temperature effects on viscous moduli decreased with the addition of sucrose. Dickinson and Merino (2002) verified that sugar addition increased substantially the rigidity of acid caseinate gels, especially at high sugar/protein ratio. These results were associated to an increase in the strength of the protein-protein interactions.

There is considerable evidence in the literature supporting the use of sugar as an enhancer of protein-protein aggregation (Dickinson and Merino, 2002), e.g. (i) an inferred increased attraction between unfolded whey protein molecules with the addition of sucrose (Kulmyrzaev et al., 2000), (ii) a stronger attractive electrostatic interaction of casein molecules caused by addition of sugar (Mora-Gutierrez et al., 1997), and (iii) a corresponding strengthening of protein-protein attractive interactions in caseinate (Antipova et al., 1999).

CONCLUSIONS

The use of factorial designs has been shown to be an important way to gain an understanding of and detect the principal effects of composition and process variables in the study of interactions in complex dairy system. Of the variables studied, sodium caseinate and carrageenan concentration exerted the most important effects on rheological properties for both large and small deformations with gels with and without sucrose. Increasing the whey protein concentration produced softer and less elastic gels, but this protein did not affect the stress relaxation data. Heat treatment temperature also exerted an important positive effect on mechanical properties of the gels, especially for systems with no addition of sucrose. Sucrose probably increased the temperature of the unfolding whey protein, making difficulty to complete protein denaturation in the temperature range studied. In a general, the addition of sucrose result in harder, more elastic, and firmer gels than those in the sugar free systems, as shown by the evaluation of the rheological properties. The addition sucrose probably produced an increase of protein-protein and polysaccharide-protein interactions, strengthening the gel network.

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