Dessert models: Effect of calcium chloride on the rheological and sensory properties of pea protein/κ-carrageenan systems.

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Abstract

Food industry is looking for alternative protein sources, plant proteins are an option and they have functional and nutritional properties. The effects of calcium chloride on the rheological and sensory properties of pea protein/κ-carrageenan systems were studied. Those pea protein systems without and with gum addition exhibited different rheological and sensory properties affected by the concentration of calcium chloride The systems prepared only with pea protein (6.0-10.0 g/100 g) behaved as liquid like items (beverage model systems), while those systems with pea protein and κ-carrageenan (0.05 and 0.1 g/100 g) behaved as weak gels (semisolid model systems). Addition of calcium chloride (0.05-0.15 g/100 g) contributed to form a more complex structure; samples with calcium chloride showed higher consistency, thixotropy and viscoelasticity. Additionally, sensory assessment was completed to monitor the effect of salt level on pea protein/κ-carrageenan systems, in both analyses the particular behavior was function of the three studied components.

Keywords: Flow properties, viscoelasticity, sensory analyses, dessert model systems, pea protein, carrageenan,

1. Introduction

Nowadays, protein concentrates and isolates used by the food industry are mostly derived from animal, dairy, soy or wheat origin. Due to the proper restrictions of this stuff, food manufacturers as well as consumers are looking for alternative protein sources (Elzerman, Hoek, van Boekel & Luning, 2011; Boye et al., 2010). Particularly, animal sources of dietary protein, despite providing a complete protein and numerous vitamins and minerals, have some health concerns; similarly soy and wheat proteins have been related to some allergenic problems in humans (Hoffman & Falvo, 2004).

Therefore, an important number of researches have analyzed proteins from animal origin such as whey protein, caseinate concentrates and isolates, while plant proteins have received lower attention. Plant proteins perform also an important role in the world food supply due to their nutritional value and functional properties (Batista, Portugal, Sousa, Crespo, & Raymundo, 2005; Eltayeb, Ali, & Haron, 2010). As a result, challenges and efforts to replace animal proteins by plant proteins in novel food products are increasing and being a common practice. Additionally, there are economic and ecological reasons, by a considerable less energy usage for the production of plant proteins that favours and increases their use worldwide (Dijkink & Langelaan, 2002; Elzerman et al., 2011).

Vegetable seed proteins and their preparations are being gradually used as ingredients for the food industry. The main plant source is soybean, now used in industrial production of isolates and concentrates, for its high protein content and a well-developed technology of manufacturing. Other seeds such as legume, lentil, lupine pea, peanut, and sunflower, have been also introduced as potential raw materials for production of protein isolates (Tömösközi, Lµsztity, Haraszi, & Baticz, 2001; Makri, Papalamprou, & Doxastakis, 2005). The utilization of legume proteins for curd preparation is one of the most promising methods for increasing their functional and marketing values (Cai, Klamczynska, & Baik, 2001).

Pea, similar to other pulse and grain commodities, is relatively inexpensive and highly nutritious. It is high in soluble and insoluble fiber, high in protein, low in sodium and fat, and an excellent source of complex carbohydrates, B vitamins and minerals such as calcium, iron, and potassium (Roy, Boye, & Simpson, 2010). Furthermore, peas exhibit high nutritional value which is attributed to a well balanced amino acid composition of the protein, especially rich in two essential amino acids: tryptophan and lysine, and relatively low content of anti-nutrients (Borowska, Zadernowski, & Konopka, 1996; Nunes, Batista, Raymundo, Alves, & Sousa, 2003). Pea proteins are storage nutrients mainly comprised of albumins and two globulins (legumin and vicilin), characterized by high lysine content, which is a disadvantage in other proteins from plant origin including cereals. They play an important role in protein supply of numerous countries (Tömösközi et al., 2001), and are an important food source for people of low incomes (Makri et al., 2005).

Typical functional properties for proteins include emulsification, gelation, hydration, water binding, and viscosity, texture, whipping and foaming modification. These functional properties are influenced by several environmental conditions, such as temperature, pressure, pH, ionic strength and salt, affecting solubility and other related properties. Due to the variety of protein sources, notable differences in isoelectric point and functionality are observed at different pH values or salt concentrations (Kinsella & Melachouris, 1976). Since gelation is an important functional property of the globular proteins used to modify consistency, it is essential to understand which factors determine the gel network and how they are affected by ingredients. The presence of proteins, salts and ionic species are formulation ingredients that together with processing parameters affect the gel network formation, and they can be manipulated to influence this physicochemical phenomenon (Sun & Arntfield, 2010). On the other hand, proteins in combination with polysaccharides are

of wide utilization to control structure, texture and stability of food network systems (Andrade, Azevedo, Musampa, & Maia, 2010).

Gums and polysaccharides have been incorporated in dairy desserts and food systems in order to improve the rheological properties and quality attributes of dairy products and others foods. Carrageenans are linear polysaccharides with alternating α-1, 3- and β-1, 4- linked galactose residues, they have the ability to form a wide range of gels at room temperature and they are also able to thicken, suspend and stabilize food products. Particularly, the κ-carrageenan is soluble in water and forms viscous solutions, it contributes to the constitution of double helical structures (Chapman & Baek, 2002; Andrade et al., 2010), favouring gel formation.

Although some studies have been conducted on mixtures of pea protein and κ-carrageenan at different ratios (Ipsen, 1995), analyzing their rheological, microstructural and functional characteristics affected by different factors, such as temperature in cooling (Nunes, Raymundo, & Sousa, 2006a) or heating (Andrade et al., 2010); carbohydrates addition (Nunes, Raymundo, & Sousa, 2006b); ionic strength (Musampa, Alves, & Maia, 2007); and extraction methods (Sun & Arntfield, 2010; Liu, Elmer, Low, & Nickerson, 2010); the effect of calcium chloride on functionality of this mixture has been scarcely reported. Further, the information on pea protein gel formation is limited, although such information is very important for food products development. Therefore, the objective of this work was to study the influence of calcium chloride addition on rheological and sensory properties of pea protein and pea protein/κ-carragenan mixtures, as potential dessert products.

2. Material and methods

2.1 Materials

Pea protein Pisane C9 (Cosucra, Belgium), κ-carrageenan Satiagel ADF 23 (Cargill, Spain), calcium chloride ($CaCl_2$, Merk, Spain), granular sucralose (Splenda, USA) and cocoa powder Valencia S-5310.02 (Natra cacao, Spain) were used in this study. Rheology and sensory analyses required samples with different preparation. For rheological measurements, samples of 200 g were prepared varying the levels of pea protein (6.0, 8.0 and 10.0 g/100 g), κ-carrageenan (0, 0.05 and 0.10 g/100 g) and calcium chloride (0, 0.05, 0.10 and 0.15 g/100 g) in deionised water.

For each sample, ingredients were weighted in a flask and mixed under mechanical stirring during 10 min with a propelled stirrer. The flask was placed in a water bath at 90±1ºC and constantly stirred during 10 min (taking 5 min to the product to reach a temperature of 85±1ºC, temperature at which carrageenan begins to be dissolved). After the heating process the evaporated water was replaced gravimetrically in order to maintain the concentration levels. The sample was cooled down in a water bath at 25±1ºC and then transferred to a closed flask to be stored in refrigeration at 4±1ºC for 24 h.

For sensory evaluation, chocolate flavoured model systems containing 8.0 g of pea protein/100 g were prepared in batches of 800 g. Two series of samples, one without κ-carrageenan (beverage model systems) and other one, with 0.1 g of κ-carrageenan/100 g (semisolid model systems) were prepared. In each series, samples varied in the $CaCl_2$ concentration (0, 0.05, 0.10 and 0.15 g/100 g). Sucralose and cocoa powder were also incorporated to both systems, in which the levels were selected through preliminary tests (data not shown). The concentrations were different for beverage systems, with 0.010 g of sucralose/100 g and 1.5 g of cocoa/100 g, whereas for semisolid systems, 0.015 g of sucralose and 2 g of cocoa/100 g were used. For each sample, ingredients were weighted in a flask and mixed under mechanical stirring during 10 min with the same propelled stirrer. Each flask was placed in a water bath at 90±1ºC and constantly agitated for 15

min. The sample was cooled down in a water bath at 25±1ºC, transferred to a closed flask and stored in refrigeration (4±1ºC) during 24 h before sensory evaluation.

2.2. Rheological measurements

All rheological measurements were carried out in a controlled stress rheometer (RheoStress RS1, Thermo Haake, Karslruhe, Germany), monitored by a Rheowin Projob Manager, using a plate/plate sensor system (6 cm diameter and 0.5 mm gap). During measurements, temperature was kept constant at 10±1ºC using a Phoenix P1 Circulator device (Thermo Haake, Karslruhe, Germany). Before being tested, samples were carefully placed in the measuring system and allowed to rest for 10 min, in order to structure recovery and temperature equilibration. Two batches of each formulation were prepared and at least one measurement was performed on each batch. A fresh sample was loaded for each measurement.

2.2.1 Flow properties

Flow behavior was determined by recording shear stress values (σ) when shearing the samples at linearly increasing shear rates ($\dot{\gamma}$), up from 1 to 200 s^{-1} through 60 s and down in reverse sequence for the same time. Data from the ascending flow curve were fitted to the Ostwald-deWaele model ($\sigma = K\gamma^n$), where K (Pa s^n) is the consistency coefficient and n (dimensionless) is the flow behavior index, with very good fittings. To quantify time dependence of the flow response, the thixotropic area (Pa s^{-1}) was calculated. These calculations were performed using Rheowin Pro software (version 2.93, Haake).

2.2.2 Viscoelastic properties

Dynamic parameters were measured using small amplitude oscillatory shear tests. To determine the linear viscoelastic region, stress sweeps were run at 1 Hz. Then the frequency sweeps were performed over the range of 0.01–10 Hz and the values of the storage modulus (G', Pa), loss modulus (G'', Pa), loss tangent angle (tan δ, dimensionless) and complex viscosity (η^*, Pa s), as a function of frequency, were calculated using the Rheowin Pro software (version 2.93, Haake) (Tárrega, Durán, & Costell, 2005).

2.3 pH measurement

A Crison GLP21 digital potentiometer (Crison Instruments, Barcelona, Spain), previously calibrated, was used to measure pH. Measurements were done at room temperature and twice in each sample.

2.4 SDS–PAGE analysis

Systems with 0, 0.05, 0.10 and 0.15 g/100 g of calcium chloride without κ-carrageenan where taken for SDS-PAGE analysis. An aliquot of 20 μL was taken and an equal volume of 2×SDS buffer (30 mM Tris-HCl, pH 6.8, 4 g/100 g of SDS, 10 g/100 g of glycerol, 1 g /100 g of mercaptoethanol, 0.01 g/100 mL of bromophenol blue) was added, before heat denaturation, in Eppendorf centrifuge test tubes, for 1min in a boiling water bath. Fractions were analyzed by SDS-PAGE and performed on 12 g/100 g of polyacrylamide slab gels with 0.04 g/100 g of ammonium persulfate, and 0.028 g/100 g of TEMED. The gel was Coomassie stained (0.2 g/100 mL of Coomassie Blue, 0.2 g/100 mL of amido black, 10 g/100 mL of isopropanol, 10

g/100 mL of acetic acid) over the night and then destained with 10 g/100 mL of acetic acid until bands could be visualized.

2.5 Sensory analysis

The effect of calcium chloride concentration on sensory properties of chocolate flavored model systems containing 8.0 g of pea protein/100 g was studied. Two series of samples, one without κ-carrageenan (beverage model systems) and other one with 0.1 g of κ-carrageenan/100 g (semisolid model systems) were studied. The sensory evaluation was carried out at 10±1ºC by a group of 50 assessors using ranking tests (ISO, 1993). Each group of samples was evaluated in a separate session. Assessors were asked to rank the four samples according to the intensity of each attribute (chocolate flavour, sweetness and consistency), as well as the preference. The samples (30 mL) were presented in white plastic cups coded with three digits random numbers. Mineral water was provided for mouth-rinsing. All sessions were carried out in the morning (11:00–13:00 h) in separate booths, in a standardised test room (ISO, 2007). Data acquisition and analysis were performed using the software Compusense five, release 5.0 (Compusense Inc., Guelph, ON, Canada)

2.6 Statistical analysis

The effect of pea protein (three levels), calcium chloride (four levels) and κ-carrageenan (three levels) concentrations on rheological parameters and pH values were analyzed by a three factors ANOVA with interactions. The Fisher test ($\alpha = 0.05$) was used to calculate the minimum significant difference.

Friedman analysis of variance was applied to the sensory data obtained in the rank tests, significance of differences between samples was determined by the Fisher test ($\alpha = 0.05$), modified for non-parametric data.

3. Results and discussion

3.1 Flow behavior of protein-carrageenan systems

The flow curves of systems were obtained to observe the changes caused by the addition of $CaCl_2$ in different protein–carrageenan systems. In Figure 1 the flow curves for 6.0 and 10.0 g/100 g of pea protein samples without and with κ-carrageenan are shown as examples. All systems showed shear thinning or pseudoplastic behavior and for some of them, the up curve and down curve formed a hysteresis loop indicating time dependent flow behavior. As expected, curves clearly change depending on the protein and carrageenan content in the system. As can be observed in Figure 1, when protein and carrageenan content were increased, the flow curve of the system appeared at higher shear stress values and the hysteresis loop was more evident. The addition of $CaCl_2$ also caused changes in the flow behavior although the effect was different depending of the samples composition.

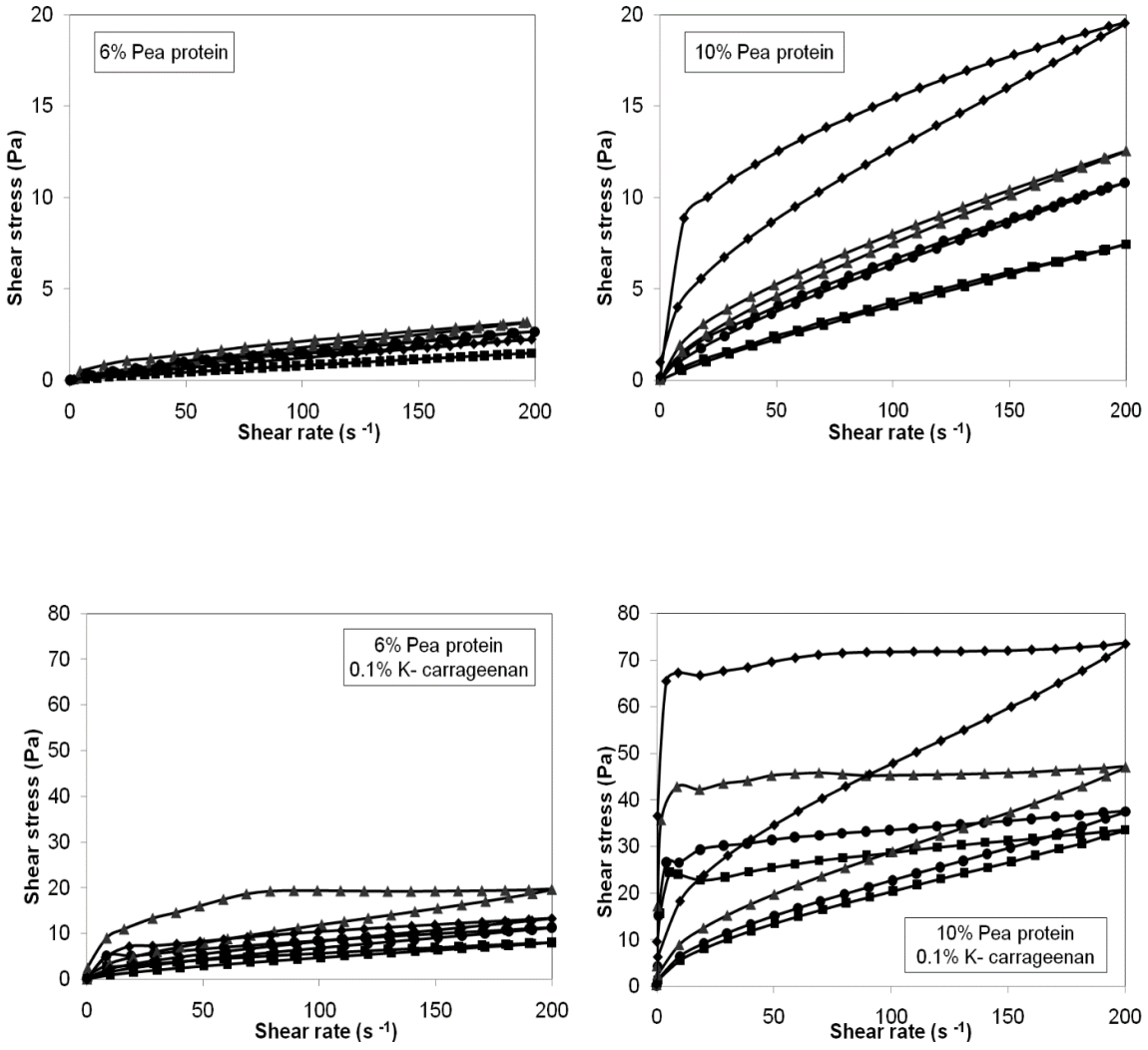


Fig. 1. Flow curves for 6.0 and 10.0 g/100 g protein systems: **a**) without or **b**) with κ-carrageenan (0.1 g/100 g) and varying the calcium chloride concentration: 0 ( ), 0.05 ( ), 0.10 ( ) and 0.15 g/100 g ( ).

To analyze these differences, the flow behavior was characterized by fitting the upward flow curve to the Ostwald-deWaele model ($0.976 \leq R^2 \leq 0.998$) with their calculated parameters, the consistency coefficient (K) and flow behavior index (n), while the time dependence was expressed by mean of the thixotropic area; Table 1 includes values for all the studied systems. For systems without κ-carrageenan, only the addition of 0.15 g/100 g of $CaCl_2$ in the 8.0 g/100 g and 10.0 g/100 g protein samples caused a significant increase in consistency, pseudoplasticity (decreasing of n) and thixotropy. The addition of lower calcium chloride concentrations only caused significant increase in those samples with κ-carrageenan.

Table 1.Mean values (n=2) for the flow parameters of pea protein-carrageenan systems at selected concentrations of calcium chloride.

Protein (g/100g)	κ-carr (g/100g)	$CaCl_2$ (g/100g)	K (Pa s^n)	n (dimensionless)	Thixotropic area (Pa/s)
6.0	0	0	0.039 ^{h}	0.751 ^{abc}	7.9 ^{o}
		0.05	0.058 ^{h}	0.719 ^{bcd}	10.6 ^{o}
		0.1	0.124 ^{h}	0.625 ^{def}	38.5 ^{o}
		0.15	0.098 ^{h}	0.672 ^{bcd}	33.5 ^{o}
	0.05	0	0.138 ^{h}	0.655 ^{cde}	39.7 ^{o}
		0.05	0.074 ^{h}	0.779 ^{ab}	22.9 ^{o}
		0.1	1.046 ^{h}	0.408 ^{ijklmn}	229.0 ^{lmno}
		0.15	0.469 ^{h}	0.537 ^{efgh}	134.1 ^{no}
	0.1	0	0.584 ^{h}	0.513 ^{fghi}	135.4 ^{no}
		0.05	1.524 ^{gh}	0.360 ^{klmno}	235.0 ^{lmno}
		0.1	3.922 ^{fg}	0.370 ^{jklmno}	611.5 ^{ij}
		0.15	2.130 ^{fgh}	0.333 ^{lmno}	323.0 ^{klmn}
8.0	0	0	0.039 ^{h}	0.861 ^{a}	3.1 ^{o}
		0.05	0.065 ^{h}	0.790 ^{ab}	13.8 ^{o}
		0.1	0.665 ^{h}	0.436 ^{ghijkl}	166.4 ^{mno}
		0.15	2.299 ^{fgh}	0.321 ^{lmno}	408.0 ^{jklm}
	0.05	0	0.905 ^{h}	0.482 ^{ghij}	181.5 ^{mno}
		0.05	4.239 ^{f}	0.543 ^{efg}	753.0 ^{hi}
		0.1	3.794 ^{fg}	0.294 ^{nop}	540.5 ^{ijk}
		0.15	10.539 ^{de}	0.157 ^{rs}	1272.0 ^{f}
	0.1	0	4.606 ^{f}	0.260 ^{opqr}	888.0 ^{gh}
		0.05	9.152 ^{e}	0.163 ^{rs}	1087.0 ^{fg}
		0.1	12.313 ^{d}	0.200 ^{pqrs}	1691.5 ^{e}
		0.15	21.560 ^{c}	0.144 ^{rst}	2274.5 ^{d}
10.0	0	0	0.150 ^{h}	0.773 ^{abc}	44.8 ^{o}
		0.05	0.237 ^{h}	0.718 ^{bcd}	48.0 ^{o}
		0.1	0.964 ^{h}	0.464 ^{ghijk}	193.5 ^{lmno}
		0.15	4.148 ^{fg}	0.286 ^{opq}	615.0 ^{ij}
	0.05	0	2.597 ^{fgh}	0.423 ^{hijklm}	448.5 ^{jkl}
		0.05	1.552 ^{gh}	0.491 ^{ghi}	313.0 ^{klmn}
		0.1	4.780 ^{f}	0.314 ^{mnop}	1059.5 ^{fg}
		0.15	22.115 ^{c}	0.122 st	2284.0 ^{d}
	0.1	0	12.965 ^{d}	0.172 ^{qrs}	1757.5 ^{e}
		0.05	22.145 ^{c}	0.100 st	2926.5 ^{c}
		0.1	27.950 ^{b}	0.113 st	3399.0 ^{b}
		0.15	60.855 ^{a}	0.028 ^{t}	4601.5 ^{a}

Columns with different superscripts are significantly different at $p \leq 0.05$ according to Fisher test.

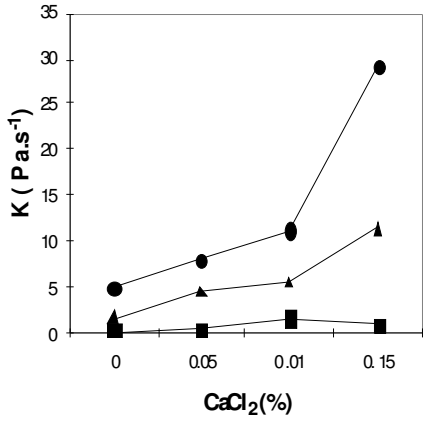
Analysis of variance indicated that consistency, pseudoplasticity, and thixotropy of samples varied significantly depending on the composition (Table 2), either as main factors or their interactions. The influence of each factor of study may be appreciated in Figures 2 and 3, in which the binary effect of calcium chloride-protein is more uniform and notable than the binary effect of salt-carrageenan on the two flow parameters, consistency coefficient and thixotropic area. Particularly, the system with 6.0 g/100 g of protein dropped in consistency when calcium chloride increased from 0.10 (K = 0.124 Pa s^n) to 0.15 g/100

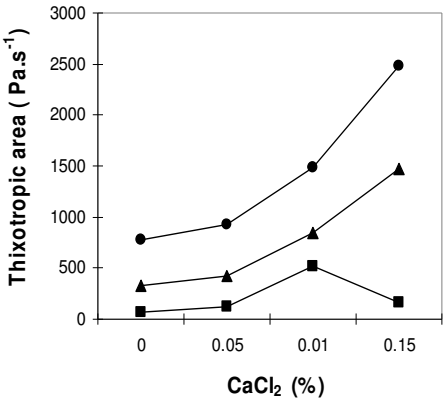
g (K = 0.098 Pa s^n), attributed to the change in pH (7.62 and 7.23, respectively), which did not allow the formation of a homogeneous mixture. However in systems with 0.15 g/100 g of calcium chloride, the consistency coefficient increased notably when protein increased from 6.0 to 10.0 g/100 g (0.098 to 4.8 Pa s^n) and κ-carrageenan, from 0 to 0.10 g/100 g (0.098 to 60.9 Pa s^n). This change may be considered as a synergistic effect of both components, in agreement with the synergy observed by Ipsen (1995) for pea protein concentrate and κ-carrageenan. Similarly, the pseudoplasticity increased (lower flow index) as the percentages of protein, carrageenan and calcium chloride increased.

Table 2. Analysis of variance of pH and rheological parameter values, F and p values.

	pH		K		n		Thixotropic area	
	F	p	F	p	F	p	F	p
Main Effects								
A: Protein	9.3	0.001	545.7	< 0.001	121.6	< 0.001	522.5	< 0.001
B: $CaCl_2$	225.1	< 0.001	255.4	< 0.001	112.2	< 0.001	221.5	< 0.001
C: Carrageenan	0.2	0.797	752.0	< 0.001	338.5	< 0.001	964.4	< 0.001
Binary Interactions								
AxB	2.0	0.088	98.9	< 0.001	5.2	0.001	52.5	< 0.001
AxC	0.3	0.879	254.8	< 0.001	9.0	< 0.001	166.5	< 0.001
BxC	1.1	0.378	64.0	< 0.001	17.5	< 0.001	45.2	< 0.001
Ternary Interactions								
AxBxC	0.2	0.999	27.2	< 0.001	3.6	0.001	10.7	< 0.001

2a)





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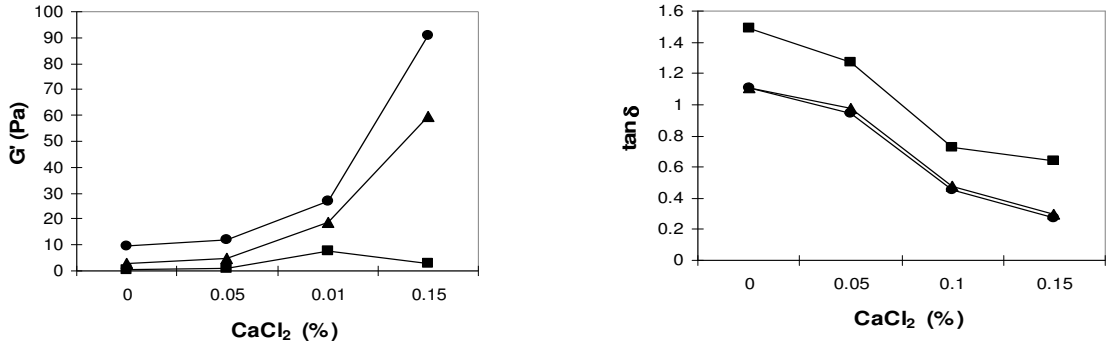
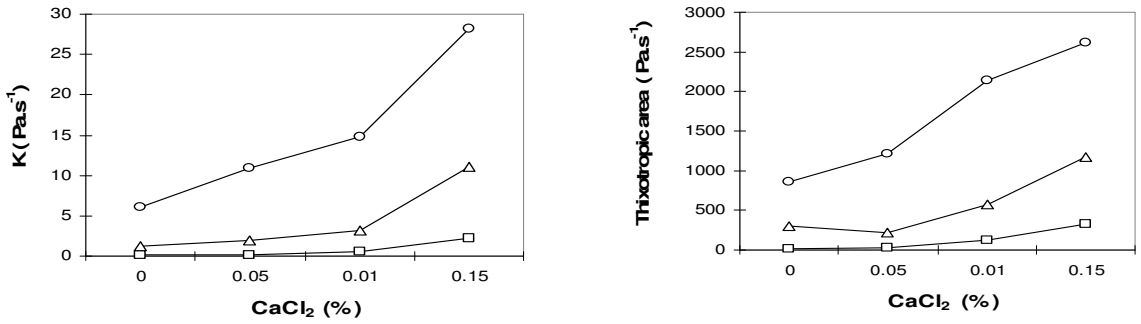


Fig. 2. Mechanical spectra for systems without carrageenan: **a**) 6.0 g/100 g and **b**) 10.0 g/100 g of pea protein; and systems with carrageenan (0.1 g/100 g), **c**) 6.0 g/100 g and **d**) 10.0 g/100 g of pea protein, with calcium chloride at 0 (■), 0.10 (●) and 0.15 g/100 g (▲). Filling symbols denote the storage moduli (G´) and open symbols the loss moduli (G") of the systems.

3a)



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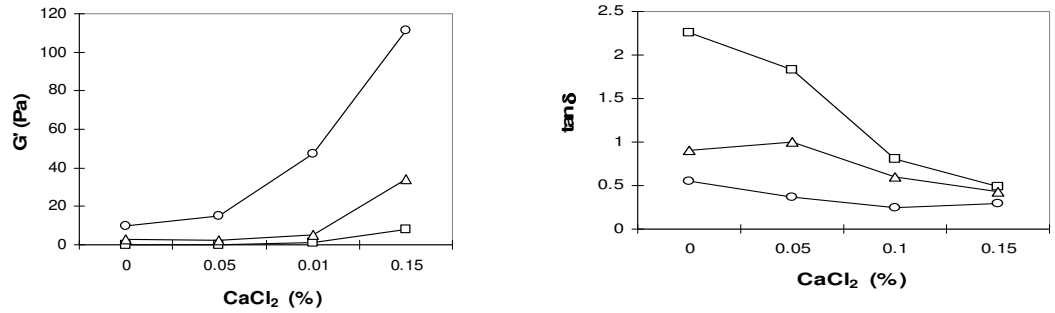


Fig. 3. Effect of interactions between $CaCl_2$ concentration and protein concentration on rheological parameter values. Protein concentration, 6.0 g/100 g (square), 8.0 g/100 g (triangle) and 10.0 g/100 g (circle): **a**) flow parameters and **b**) dynamic parameters.

The depressing effect of the calcium chloride seems to be higher at the concentration of 0.15 g/100 g. At this salt level and with the minimum pea protein concentration raises a depletion in all parameters, the consistency coefficient, thixotropic area and flow index, was observed. This response was due to the decreasing pH by the salt adding that at high concentrations promote a sharply drop in the solubility of proteins. On the other hand and in general, increasing calcium chloride concentration had no negative effect

in the κ-carrageenan systems. The consistency coefficient and thixotropic area increased with salt concentration. In most of the cases, the pH did not affect the behavior of the systems, which was attributed to the κ-carrageenan capacity of trapping water and improving gel network.

3.2 Effect on viscoelasticity

Mechanical spectra for each one of the systems were obtained in order to study their viscoelastic properties (Fig. 4). Some of them showed the typical response of liquid dispersions, pea protein at 6.0 and 10.0 g/100 g without κ-carrageenan and without salt for instance (Figs. 4a and 4b), while other systems behaved as weak gels, with G' values above of G'' as may be observed for systems without and with carrageenan but with calcium chloride (Figs. 4c and 4d). In all the cases, both dynamic parameters showed similar frequency dependency; and as it is observed, the increasing in pea protein, carrageenan and salt contributed to the system viscoelasticity, leading to the formation of weak gels.

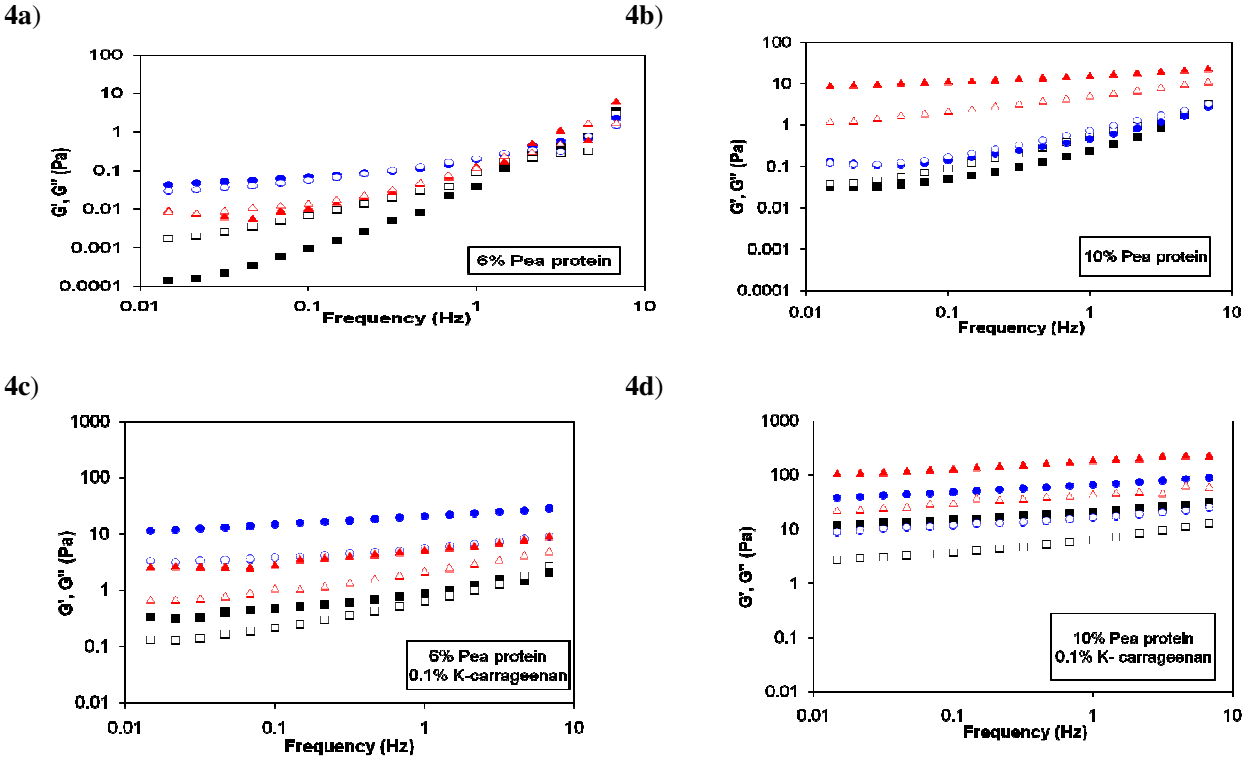


Fig. 4. Effects of interaction between $CaCl_2$ concentration and κ-carragenan concentration on rheological parameter values. κ-carragenan, 0 g/100 g (square), 0.05 g/100 g (triangle) and 0.10 g/100 g (circle): **a**) flow parameters and **b**) dynamic parameters.

Samples with 6.0 g/100 g pea protein, without carrageenan and with added calcium chloride, under oscillatory shear behaved as liquid like. Whereas, the mechanical spectra obtained for the rest of samples, with salt and mainly with hydrocolloid, was a typical response of weak gelled systems. Similar to the increment in flow parameters when the concentration of salt increased, also the storage moduli increased becoming higher in magnitude than the loss moduli, which lead to a more structured network.

For comparison purposes, G', G'', η* and tan δ values at a frequency of 1 Hz were considered (Table 3). Storage modulus and complex viscosity increased while loss angle tangent decreased with pea protein, carrageenan and salt concentrations, indicating an increase in the relative elastic contribution of these

ingredients to the viscoelasticity of the systems. The magnitude of the dynamic parameters for the studied systems was similar to values reported for gels of chickpea protein added with sodium and calcium chloride salts (Zhang, Juang, & Wang, 2007) and for that of lupin and soy proteins (Batista et al., 2005).

Table 3. Mean values (n=2) for the viscoelastic parameters of pea protein-carrageenan systems at selected concentrations of calcium chloride at 1 Hz.

Protein (g/100g)	κ-carrag (g/100g)	$CaCl_2$ (g/100g)	G' (Pa)	G" (Pa)	η* (Pa s)	tan δ (dimensionless)
6.0	0	0	0.06 ^{a}	0.12 ^{a}	0.02 ^{a}	1.96 ^{bcd}
		0.05	0.1 ^{a}	0.17 ^{ab}	0.03 ^{a}	1.71 ^{cde}
		0.1	0.17 ^{a}	0.18 ^{ab}	0.04 ^{a}	1.09 ^{f}
		0.15	0.17 ^{a}	0.12 ^{a}	0.03 ^{a}	0.76 ^{fghij}
	0.05	0	0.17 ^{a}	0.26 ^{abc}	0.05 ^{a}	1.53 ^{e}
		0.05	0.18 ^{a}	0.28 ^{abc}	0.05 ^{a}	1.58 ^{de}
		0.1	1.09 ^{abc}	0.83 ^{abcd}	0.22 ^{ab}	0.82 ^{fghi}
		0.15	1.02 ^{abc}	0.77 ^{abcd}	0.2 ^{ab}	0.76 ^{fghij}
	0.1	0	0.68 ^{ab}	0.61 ^{abcd}	0.15 ^{a}	0.98 ^{fg}
		0.05	2.01 ^{abc}	1.07 ^{bcd}	0.36 ^{abc}	0.53 ^{hijklm}
		0.1	22.28 ^{h}	5.64 ^{j}	3.66 ^{h}	0.25 ^{lm}
		0.15	7.49 ^{de}	2.9 ^{efg}	1.28 ^{de}	0.39 ^{jklm}
8.0	0	0	0.07 ^{a}	0.17 ^{ab}	0.03 ^{a}	2.24 ^{ab}
		0.05	0.14 ^{a}	0.23 ^{abc}	0.04 ^{a}	1.75 ^{cde}
		0.1	1.47 ^{abc}	0.94 ^{abcd}	0.28 ^{abc}	0.65 ^{ghijkl}
		0.15	7.59 ^{de}	2.78 ^{ef}	1.29 ^{de}	0.37 ^{jklm}
	0.05	0	1.57 ^{abc}	1.14 ^{cd}	0.31 ^{abc}	0.73 ^{fghij}
		0.05	1 ^{ab}	0.89 ^{abcd}	0.21 ^{ab}	0.89 ^{fgh}
		0.1	4.39 ^{bcd}	2.31 ^{e}	0.79 ^{bcd}	0.52 ^{hijklm}
		0.15	36.97 ^{j}	10.17 ^{l}	6.1 ^{j}	0.28 ^{lm}
	0.1	0	7.66 ^{de}	2.76 ^{ef}	1.3 ^{de}	0.36 ^{jklm}
		0.05	12.63 ^{fg}	3.79 ^{gh}	2.1 ^{fg}	0.30 ^{klm}
		0.1	51.05 ^{k}	12.51 ^{m}	8.37 ^{k}	0.25 ^{lm}
		0.15	134.15 ^{n}	34.44 ^{p}	22.05 ^{n}	0.26 ^{lm}
10.0	0	0	0.18 ^{a}	0.43 ^{abc}	0.07 ^{a}	2.57 ^{a}
		0.05	0.26 ^{a}	0.51 ^{abcd}	0.09 ^{a}	2.02 ^{bc}
		0.1	2.01 ^{abc}	1.38 ^{d}	0.39 ^{abc}	0.69 ^{ghijk}
		0.15	16.14 ^{g}	5.21 ^{ij}	2.7 ^{g}	0.32 ^{klm}
	0.05	0	7.69 ^{de}	3.52 ^{fgh}	1.35 ^{de}	0.46 ^{ijklm}
		0.05	4.95 ^{cd}	2.61 ^{ef}	0.89 ^{cd}	0.53 ^{hijklm}
		0.1	9.65 ^{ef}	4.33 ^{hi}	1.68 ^{ef}	0.45 ^{ijklm}
		0.15	64.22 ^{l}	17.14 ^{o}	10.58 ^{l}	0.27 ^{lm}
	0.1	0	20.47 ^{h}	6.1 ^{j}	3.4 ^{h}	0.30 ^{klm}
		0.05	30.23 ^{i}	8.34 ^{k}	4.99 ^{i}	0.28 ^{lm}
		0.1	68.88 ^{m}	15.34 ^{n}	11.24 ^{m}	0.22 ^{m}
		0.15	192.2 ^{o}	44.05 ^{q}	31.38 ^{o}	0.23 ^{m}

Columns with different superscripts are significantly different at $p \leq 0.05$ according to Fisher test.

For all dynamic parameters, ANOVA showed a significant influence of main studied factors and also of almost all of the interactions (Table 4, Figs. 2b and 3b); the effect of system composition on viscoelasticity was different as a function of the three studied factors. All samples showed a decreasing tan δ with increasing carrageenan and salt concentrations, in which a lower tan δ value is representative of firmer three-dimensional structure. However, the tan δ showed similar behavior in systems containing 8.0 to 10 g/100 g of pea protein, with similar loss modulus to storage modulus ratios.

Table 4. Analysis of variance of viscoelastic parameter values. F and p values.

	G'		G''		η*		tan δ	
S	F	p	F	p	F	p	F	p
Main effects								
A:Protein	1627.5	< 0.001	1860.6	< 0.001	1737.1	< 0.001	15.3	< 0.001
B:$CaCl_2$	2269.4	< 0.001	2414.7	<0.001	2402.4	< 0.001	108.2	< 0.001
C:carrageenan	3371.1	< 0.001	3361.8	< 0.001	3553.3	< 0.001	174.6	< 0.001
Binary interactions								
AxB	591.4	< 0.001	574.5	< 0.001	622.8	< 0.001	0.5	0.817
AxC	630.2	< 0.001	532.5	< 0.001	657.3	< 0.001	5.4	0.002
BxC	807.1	< 0.001	753.9	< 0.001	851.2	< 0.001	17.4	< 0.001
Ternary interactions								
AxBxC	188.0	< 0.001	162.7	< 0.001	197.4	< 0.001	4.1	0.001

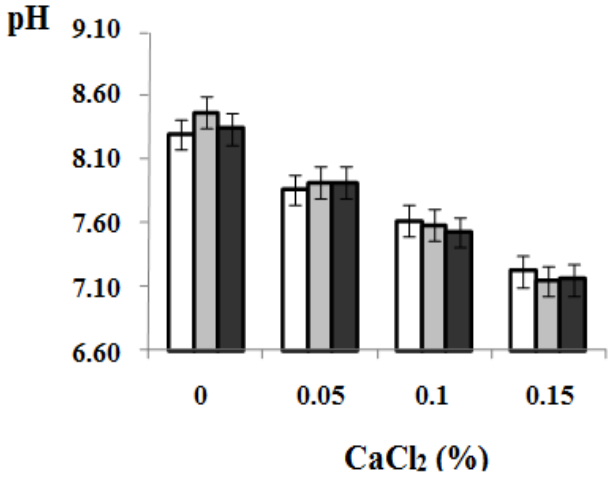
For systems with 6.0 g/100 g of pea protein that moduli ratio was higher than for systems 8.0 and 10.0 g/100 g, due to the loss modulus values were greater than those of the storage modulus (G'' > G'), being less structured systems. For the three protein content, tan δ decreases with increasing content of calcium chloride, indicating a more structured system due to the addition of this salt (Fig. 3a). On the other hand, the relationship of storage to loss moduli was affected significantly by the addition of κ-carrageenan (Fig. 3b).

3.3 Effect on pH and protein gelation

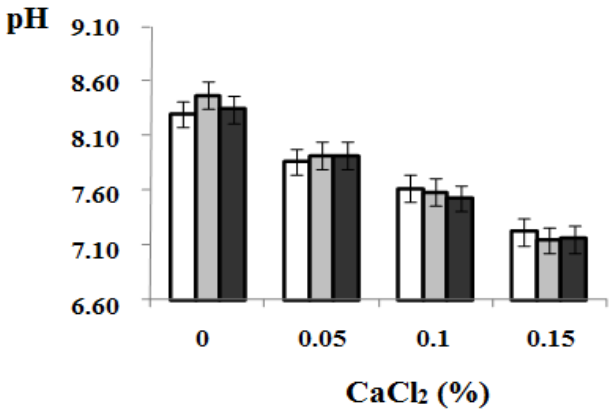
In addition to rheological properties, the presence of $CaCl_2$ influenced the pH and gel structure. When working with proteins, the pH is a very important factor, due to promotion of the denaturation and gelation phenomena, pH influences the number of charged reactive groups on the protein surface (Liu, Low, & Nickerson, 2009).

A pH of 8.3-8.5 was measured for those systems prepared only with pea protein, and it was reduced as a function of the concentration of $CaCl_2$, regardless the concentration of carrageenan (Fig. 5). Statistical analysis showed significant differences of pH in terms of both factors the protein concentration and salt percentage.

5a)



5b)



5c)

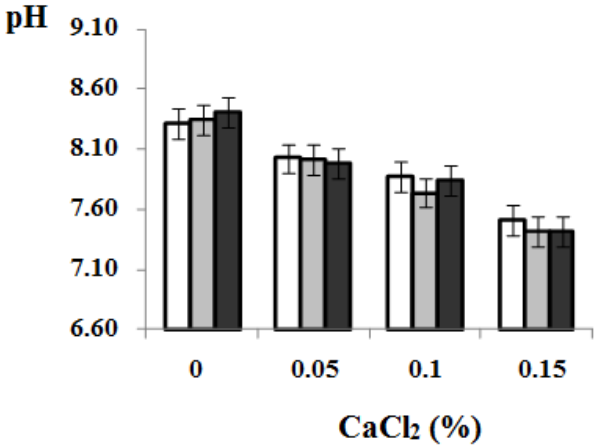


Fig. 5. pH of systems with selected percentages of κ-carrageenan, 0 (□), 0.1 (▒), and 0.15 g/100 g (■) for: **a**) 6.0 g/100 g, **b**) 8.0 g/100 g and **c**) 10.0 g/100 g of pea protein.

Calcium chloride improved the consistency of all systems, in which salt-protein interaction had a greater effect on systems without carrageenan. The lowest concentration of pea protein (6.0 g/100 g) with the highest concentration of salt (0.15 g/100 g) reached a minimum pH of 7.23 for systems without the addition of carrageenan resulting in a gel with different characteristics, i.e. this concentration of pea protein did not allowed the gel formation, due to the abundance of salt ions decreases the solvating power of the systems and as a result the solubility of the proteins decreases, provoking gel syneresis and system precipitation. The $CaCl_2$ competes for the protein bond water molecules depriving the proteins of water, thus increasing the protein-to-protein interaction and decreasing the dispersed water molecules (Graham, 1977).

Carrageenan addition contributed to form a weak gel with the ability to hold water in the matrix system, although the pH of the systems remained at lower levels when the salt was added. Furthermore, the calcium chloride did improve the consistency of the systems in combination with carrageenan, giving place to a more structured and firm gel in which the formed network was capable of entrapping the aqueous phase (Graham, 1977; Vieira, Biasutti, Capobiango, Afonso, & Silvestre, 2006).

Complementarily, the pea protein composition without and with calcium chloride as was separated by the SDS-PAGE test, allowed to know the protein integrity and the effect of added salt. Analysis of pea protein subunits (Fig. 6) showed exactly the same pattern in absence of and at the three different concentrations of calcium chloride that may be interpreted as the pea protein without notable modifications. Since in the electrophoresis the migration distance in the gel is directly linked only to the size of the unfolded protein, the selected concentration of the salt did not exhibit molecular change in the protein structure, similar bands were obtained in the distinct columns. Therefore, the observed gel behavior was a consequence of protein-salt interaction and not a denaturation phenomenon.

Figure 6

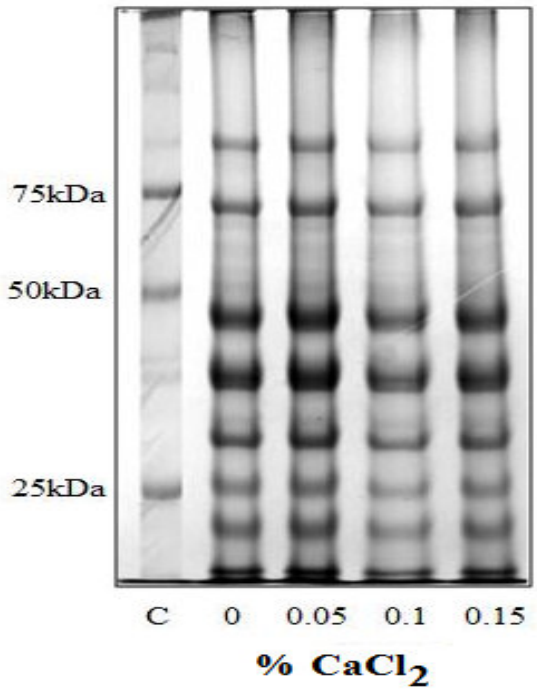
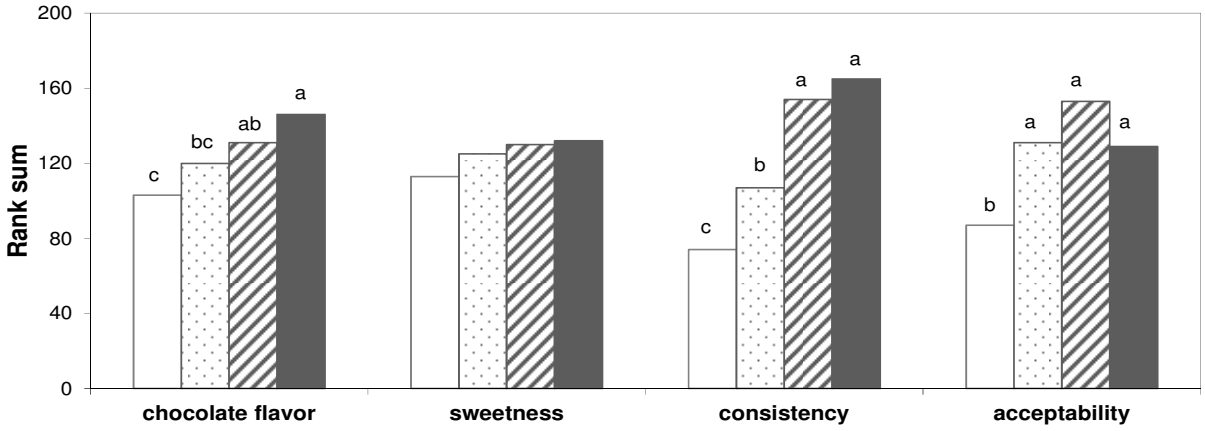


Fig. 6. SDS-PAGE electrophoretic patterns of heat-treated pea protein systems with 0, 0.05, 0.10 and 0.15 g/100 g $CaCl_2$. In control band C the marker was run.

3.4 Effect on sensory assessment

The effect of calcium chloride on sensory properties of pea protein (8.0 g/100 g) enriched products was evaluated in chocolate flavoured model systems of beverage and semisolid products (without and with κ-carrageenan, respectively). Two aspects were evaluated, first if rheological changes were sensory perceived as differences in consistency; and secondly, if the presence of this salt could influence other sensory properties and affect the acceptability of model systems. Analysis of data obtained from ranking tests are shown in Figure 7, for both types of systems (beverage and semisolid models), salt concentration significantly affected chocolate flavour intensity, consistency and overall acceptability of samples. F values, from 11.8 to 76.9 were in all cases higher than the theoretical F value of 9.49. However, calcium chloride concentration did not cause a significant variation of the product sweetness in any case.

7a)



7b)

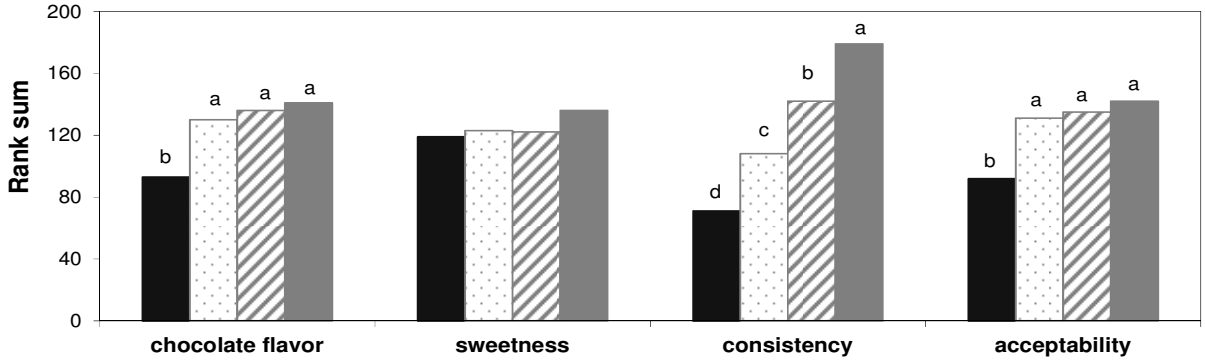


Fig. 7. Sensory evaluation of chocolate flavour, sweetness, consistency and acceptability for systems, **a**) without κ-carrageenan with different calcium chloride concentration: 0 ( ), 0.05 ( ), 0.10 ( ) and 0.15 g/100 g ( ), and **b**) with 0.10 g/100 g of κ-carrageenan with different calcium chloride concentration: 0 ( ), 0.05 ( ), 0.10 ( ) and 0.15 g/100 g ( ). Different letters on top of bars mean significant differences ($\alpha = 0.05$). Different letters on top of bars mean significant differences ($\alpha = 0.05$).

As can be observed in Figure 7, samples varying in the salt concentration differed mainly in the perceived consistency that increased with the salt concentration. Regarding chocolate flavor, all samples with calcium chloride were perceived with a more intense chocolate flavour than systems without it, being this effect more evident in the beverage model systems. Those samples containing the calcium chloride were more liked by the assessor panel.

According to these results, the addition of the calcium chloride at concentrations between 0.05 and 0.15 g/100 g can be used to effectively increase the thickness of pea protein based systems. Furthermore the presence of calcium salt did not affected sweetness of samples, slightly increased the chocolate flavour intensity and did not affect negatively the acceptability.

4. Conclusions

Systems of pea protein without and with κ-carrageenan exhibited different rheological properties and sensory characteristics at different calcium chloride concentrations. From the rheological analysis, it was seen that all systems presented shear thinning response, time dependent behavior and weak gel structure, allowing the conclusion that formulations with added salt in carrageenan-protein mixtures present a practical advantage over formulations including the gum-protein blends or only with protein systems. The developed protein gel was modified by the presence of the other two components that provided structure, water holding capacity and flavor to the systems. The addition of calcium chloride to pea protein systems not only improved the consistency, but it also contributed to the perception of chocolate flavour and acceptability. This combination of ingredients may be useful in food applications and new products development.

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