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ORIGINAL PAPER

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Gelled vegetable desserts containing pea protein, κ-carrageenan and starch

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Abstract Due to recent animal diseases, cholesterol intake worries and strong demand for healthy food, there is a greater pressure for the direct consumption of vegetable proteins in food products. In this work, the objective is to develop alternative of strictly vegetable origin desserts based on gelled systems with required physical structure and perceived texture. For this reason, it is important to control the properties of the biopolymer mixtures and understand the phase separation behaviour under different physicochemical conditions. The firmness and storage modulus of different formulations of pea protein/κ-carrageenan/starch systems processed and cooled at different conditions are compared with those parameters obtained for commercial products. Formulation and thermal conditions were determined to influence the texture and storage modulus of the mixed systems. Confocal microscopic images showed that phase separation between pea protein and κ-carrageenan takes place, leading to the formation of two network systems. The binding of water effect, of the starch swollen granules, promotes the concentration of pea protein and κ-carrageenan, reinforcing the gel structure.

Keywords Pea protein · κ-carrageenan · Starch · Mixed gel · Rheology · Texture · Confocal laser scanning microscopy

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Introduction

The challenge in this work is to develop novel protein foods using plant proteins for imparting texture and appearance to the traditional animal protein based products, with subsequent multiple advantages from the economic and health-hazard points of view [1, 2].

Dairy desserts are an interesting source of protein in the diet of infants and elderly people. Although these products are not a novelty in the market since there are already commercial alternatives produced with soybean protein, their flavour and poor texture are not always appealing to the consumer.

The present work is part of a research project that aims to develop "dairy desserts"-like products in which egg and milk proteins are fully replaced by a pea protein isolate. Besides nutritional characteristics, the good gelling properties of pea protein [3–9], have led to a greater interest in this protein source as a promising food ingredient. Moreover, in the last decades gel research focuses more strongly on protein/polysaccharide mixtures as a result of industrial demands for new materials with specific textures [10–12].

In the most of the binary systems studied, some extent of phase separation is observed prior to, or during the gelation process of one, or both, of the components. This is due either to thermodynamic incompatibility or to a depletion flocculation mechanism [10–14]. However, real food products have generally a more complex composition, involving more than two types of biopolymers. In gelled milk desserts, κ-carrageenan, a sulphated anionic polysaccharide, is frequently used in combination with starch. In a previous work [14], it was demonstrated that the gelation ability of a mixture of pea protein, κ-carrageenan and native maize starch could be used as an interesting alternative to commercial desserts.

To design a "vegetable gel" similar to a dairy dessert, it is important to understand how the complex mixture of pea protein and polysaccharides behave under food processing conditions. The phase separation of the mixed biopolymer systems, their final structure and mechanical properties are

strongly dependent on processing temperature/time, cooling rate, protein and polysaccharide contents, pH and salt conditions [10, 11, 13]. The effect of temperature and time of thermal treatment on the microstructure and mechanical properties of the pea protein/κ-carrageenan/starch mixed system was already investigated [15]. It was concluded that temperature favours protein unfolding and biopolymers interactions, resulting in a significant increase on gel texture and viscoelastic parameters. In this work it was also determined that the mechanical properties are significantly affected by gel composition, increasing with pea protein, κ-carrageenan and starch content. In another work [16], the influence of cooling conditions on the gel-forming kinetics, final mechanical properties and microstructure of this complex mixture was evaluated. It was shown that slower cooling rates allowed for more complete phase separation with protein molecules structurally organised in larger aggregates, resulting in a decrease of the storage modulus and texture parameters.

To optimise the processing variables and formulation in order to develop a vegetable gelled dessert with good physical properties, it is necessary to use a commercial standard. In a previous work [17], 12 commercial desserts were characterised in order to define a target and the range of variation of the most representative physico-chemical properties. Additionally, in this work the mechanical spectra of five representative commercial desserts were obtained under similar conditions used for the pea protein/κ-carrageenan/starch mixed gels.

The main objective of the present work is to compare the values of texture and rheological parameters for vegetable gels at different formulation and thermal processing conditions with the range of variation of each variable obtained for commercial desserts. The comparison of two of the gel properties (firmness and storage modulus at 1 Hz) will help to set the adequate formulation and processing conditions to produce a vegetable dessert with physical properties close to the commercial dairy puddings.

Materials and methods

Materials

Pea protein isolate (Pisane®, Cosucra, Belgium), κ-carrageenan (Satiagel™ AMP45, Degussa, France) and native maize starch (Vitena A, Copan, Portugal) were kindly provided by the respective manufacturers. Sucrose was of commercial grade.

Methods

Preparation of the mixtures

Biopolymer mixtures (500 g) were made by dispersing the dry ingredients in demineralised water, under mechanical stirring (300 rpm, 1 h) at room temperature. The suspensions were treated in a thermally controlled water bath and, immediately after, poured into 6 cm diameter cylindrical containers filled up to 3.5 cm height, and allowed to set at a temperature of (5–7)°C in a domestic refrigerator, for texture analysis. No adjustment was made to the natural pH of the systems which varied between 7.1 and 8.2. In this type of food products with milk, eggs and starch, pH values are typically around neutral values. The range found for commercial desserts was 6.3 up to 7.06 [17]. The higher pH values of the pea gels must be due to the pH of pea protein, of 7.8±0.5 accordingly to Pisane® technical sheet. No extra salt was added.

Table 1 Independent variables (time and temperature) tested at five levels

	$-\alpha$ (-1.414)	-1	0	$+1$	$+\alpha$ (1.414)
Time (min)	1	5.2	15.5	25.8	30
Temperature (°C)	60	66	80	94	100

Effect of thermal treatment conditions

To study the effect of thermal treatment on the mechanical properties of the mixed gel, a central composite rotatable experimental design based on the response surface methodology (RSM) was used [18, 19]. The independent variables considered were time (from 1 to 30 min) and temperature (from 60 to 100°C), tested at five levels (Table 1), resulting in nine different experiments, with four replicates of the central point [15]. Statistical analysis was performed using the software *Statistica* (version 6.0, StatSoft Inc., USA), with texture parameters as the dependent variables. A formulation studied in a previous work [14], with 2.34% (w/w) of pea isolate, 0.15% (w/w) of κ-carrageenan, 2.5% (w/w) of starch and 15% (w/w) of sucrose, was adopted.

Effect of cooling conditions

To determine the effect of cooling conditions the suspensions were heated up to 95°C and hold for 5 min. After thermal processing, samples were immediately loaded onto the rheometer-measuring device. For texture, the samples poured into cylindrical containers, were then placed in the rheometer water bath. Four cooling conditions were studied: (i) 75°C down to 5°C at 0.1°C/min (in 11.7 h); (ii) 75°C down to 5°C at 0.5°C/min (in 2.3 h); (iii) 40°C down to 5°C at 0.5°C/min (in 1.2 h); (iv) quenching rapidly to 5°C [16]. This temperature of 5°C was used since it is the storage temperature of these products in a domestic refrigerator.

Effect of composition

The influence of different proportions of the ingredients on the gel mechanical properties was studied according to the RSM methodology. The independent variables chosen were pea isolate (P), κ-carrageenan (C) and starch (S) content, tested at five levels (Table 2), resulting in 15 formulations, with seven replicates of central point. The

Table 2 Independent variables (pea protein isolate, κ-carrageenan and starch content) tested at five levels

	$-\alpha$ (-1.682)	-1	0	$+1$	$+\alpha$ (1.682)
Pea protein isolate (%, w/w)	0.0	0.81	2.0	3.19	4.0
κ-Carrageenan (%, w/w)	0.0	0.06	0.15	0.24	0.30
Starch (%, w/w)	0.0	1.01	2.50	3.99	5.0

concentration range studied was 0–4.0% (w/w) for the pea isolate, 0–0.30% (w/w) for κ–carrageenan and 0–5.0% (w/w) for starch. Sucrose content was fixed at 15% (w/w). In this study, the time/temperature of thermal treatment was 95°C/5 min [15]. The dependent variables considered were texture parameters.

Texture characterisation

Macrostructure of the gels was evaluated from the texture profile analysis (TPA) performed in a TA-XT2i (Stable Micro Systems, UK) texturometer (cylindrical probe of 25 mm diameter, 10 mm of penetration, 5 s of waiting time and 2 mm s^{-1} of crosshead speed). The experiments were carried out 8 days after preparation, in order to allow full maturation of the mixed gels. Gels were allowed to equilibrate at 20°C for approximately 3 h in a temperature-controlled room [15, 16]. Results for each gel were determined at least three times.

To compare the firmness of the vegetable gels and commercial desserts, for which probes with different diameters were used, firmness was calculated as the height of the force peak during the first compression cycle, divided by the probe contact area, and thus expressed in N m^{-2}. These gels break under puncture so a true peak is obtained before 10 mm distance, and they showed no fracturability. Adhesiveness, representing the work necessary to pull the probe away from the sample, was recorded as the negative force area of the first compression (−N.s).

Rheological measurements

According to the methodology described in previous works [15, 16], dynamic oscillatory measurements were conducted in a controlled-stress rheometer (RS-300, Haake, Germany), using cone-plate geometry (35 mm, 2°). After thermal treatment, mixed systems were immediately transferred to the rheometer-measuring device, which was at 40°C, covered with a layer of paraffin oil to prevent moisture loss, and cooled down to 5°C at 0.5°C/min, except for the systems with different cooling conditions, for which the cooling profiles described above were used. Time sweep tests were conducted at 5°C, during a reasonable period of time (24 or 35 h), at a constant frequency of 1 Hz (6.28 rad/s), in order to study the development of the gel structure. After temperature and time sweep tests, frequency sweeps were conducted at 5°C, with oscillation frequencies ranging from 0.01 to 115.6 rad/s. A constant shear stress within the linear viscoelastic region of the material was used in all measurements. Each test was repeated at least three times.

Five representative commercial desserts were analysed. The samples were stored at (5±2)°C prior to testing and allowed to equilibrate for 1 h at 5°C on the rheometer sensor device. Frequency sweeps (from 0.01 to 115.6 rad/s) were conducted at 5°C. The values of storage modulus G′ at 1 Hz (6.28 rad/s) were calculated using RheoWin Pro Data Manager version 2.97 software (Germany, 1997).

Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was performed with a Leica Microsystems (SP2, AOBS), using 40x/UV/1.25 NA/oil/HCXPL-APO CS objective lens. The light source was an argon laser. According to the procedure described by Nunes et al. [16], fluorescent probe rhodamine B (Sigma, USA), added during the mechanical stirring of the mixtures (0.01 g rhodamine/500 g), was used for the labelling of protein and starch.

Syneresis

Gel syneresis, a measure of water-holding ability, was measured by centrifugation as the percentage of supernatant liquid after centrifugation of the gel [20, 21]. After thermal treatment, samples (around 10 g) were placed into centrifuge tubes, stored in a vertical position at (5–7)°C for 1, 2, 3 and 4 weeks and centrifuged at 1359×g (5°C) for 20 min using a 12111-H rotor in a Sigma (3K3OH, Germany) ultracentrifuge.

Results and discussion

The texture properties of twelve commercial desserts (eight dairy desserts and four soya desserts) previously characterised by Batista et al. [17] and the storage modulus at 1 Hz ($G'_{1\,Hz}$) of five representative commercial desserts, shown in Table 3, will be used as a target to optimise the processing conditions.

Thermal treatment conditions

Experimental design and results for the texture parameters of the gels subjected to different thermal conditions are presented in Table 4. The statistical equations obtained from the multiple regressions of the results (Eqs. (1) and (2))

Table 3 Range of variation of firmness, adhesiveness and storage modulus at 1 Hz ($G'_{1\,Hz}$) for commercial desserts

Mechanical property	Dairy desserts	Soya desserts
Firmness (N m^{-2})	1815–4222	720–1371
Adhesiveness (−N.s)	1.28–2.68	0.40–0.57
$G'_{1\,Hz}$ (Pa)	564–1435	194

Table 4 Response surface methodology matrix and texture responses for pea protein/κ-carrageenan/starch systems subjected to different heat treatments

Temperature (°C)	Time (min)	Firmness (N)	Adhesiveness (−N.s)
66	5.2	0.347	0.000
94	5.2	0.724	0.208
66	25.8	0.397	0.007
94	25.8	0.724	0.128
80	1.0	0.445	0.094
80	30.0	0.447	0.156
60	15.5	0.367	0.000
100	15.5	0.763	0.181
80	15.5	0.452–0.479[a]	0.136–0.150[a]

[a](min–max) values

showed that firmness (F) and adhesiveness (A) quadratically increase with temperature (T) and are not dependent on time (t):

$$F(N) = 1.46 - 0.036T + 0.0003T^2 \quad (R^2_{aj} = 0.958,\ MS_{residual} = 0.00089) \qquad (1)$$

$$A(-N.s) = -1.18 + 0.028T - 0.00014T^2 \quad (R^2_{aj} = 0.823,\ MS_{residual} = 0.00090) \qquad (2)$$

As it can be seen, gels prepared under milder heat exposure (60–80°C) showed much lower firmness values (from 0.347 to 0.479 N) compared with gels processed at higher temperatures (94° and 100°C), for which firmness varied between 0.724 and 0.763 N. Temperature influences all the components of the system as well as their interactions. Thermal gelation of globular proteins is a process that requires denaturation and subsequent interaction of the exposed residues to form aggregates [4, 22]. High temperature induces protein unfolding with exposure of additional hydrophobic residues to the aqueous solvent. κ-Carrageenan requires high temperatures to achieve maximal hydration [23] and the extension of starch gelatinization is also dependent on temperature [24]. Consequently, high temperatures favours biopolymer interactions resulting in lower phase separation between pea protein and κ-carrageenan, with evenly distribution of protein smaller aggregates, as demonstrated by Nunes et al. [15]. At neutral pH values, both biopolymers are negatively charged with predominant repulsive forces. Therefore the building up of the structure results from a dynamic process of competition between phase separation and gel formation. κ-Carragenan induces the aggregation of denatured protein molecules through depletion flocculation mechanism, and hence promoting the gelation process. However, if this depletion is too extensive, phase separation will become dominant and visible even at macroscopic level and gelation will be overcomed.

In Fig. 1, firmness of gels processed at different thermal treatment conditions are compared with firmness values obtained for the dairy and soya commercial desserts.

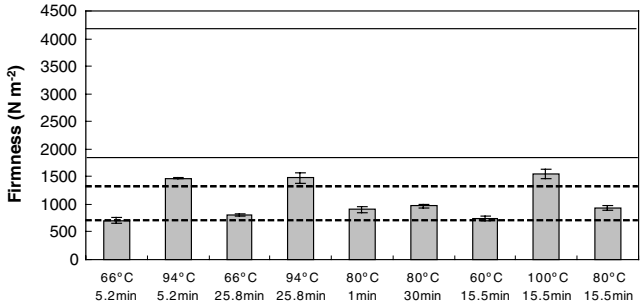

Fig. 1 Firmness values for commercial desserts and pea protein/κ-carrageenan/starch gels processed at different time and temperature of thermal treatment. The *solid lines* represent the range of variation for commercial dairy desserts. The *dotted lines* represent the range of variation for commercial soya desserts

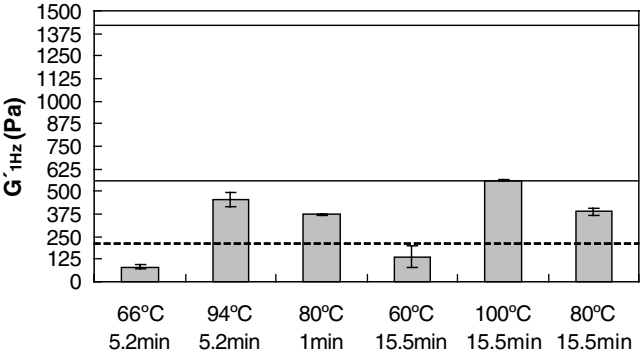

Fig. 2 Storage modulus values at 1 Hz ($G'_{1\,Hz}$) for commercial desserts and pea protein/κ-carrageenan/starch gels processed at different time and temperature of thermal treatment. The *solid lines* represent the range of variation for commercial dairy desserts. The *dotted line* represents the value for a commercial soya dessert

Since time had no effect on gel texture, rheology measurements were only performed for gels prepared at time-temperature conditions from 6 points of the design matrix: 60°C–15.5 min, 66°C–5.2 min, 80°C–1 min, 80°C–15.5 min, 94°C–5.2 min and 100°C–15.5 min. The storage modulus at 1 Hz ($G'_{1\,Hz}$) of these gels is shown in Fig. 2. Higher temperatures of heat exposure resulted in gels with a higher degree of internal structure as reflected by the increase of $G'_{1\,Hz}$, in agreement with texture results.

Firmness (Fig. 1) and $G'_{1\,Hz}$ (Fig. 2) values of the vegetable gels were compared with the range of variation of the dairy and soya commercial desserts. The goal was to obtain pea protein/κ-carrageenan/starch gels with firmness and $G'_{1\,Hz}$ values similar to those of the dairy desserts, since low firmness and $G'_{1\,Hz}$ indicate poor gelation while high firmness and $G'_{1\,Hz}$ indicate enhanced gelation properties. All the mixed gels have mechanical properties within the range of variation of soya desserts, but lower than dairy puddings. Nevertheless, physical properties of gels processed at 94° or 100 °C were closer to dairy desserts. Adhesiveness values for all the mixed gels were lower compared to the commercial desserts (0.40 to 2.68 −N.s), varying from 0 to 0.21 −N.s. In the subsequent experiments the biopolymer mixtures were heated up to 95°C and hold at this temperature for 5 min.

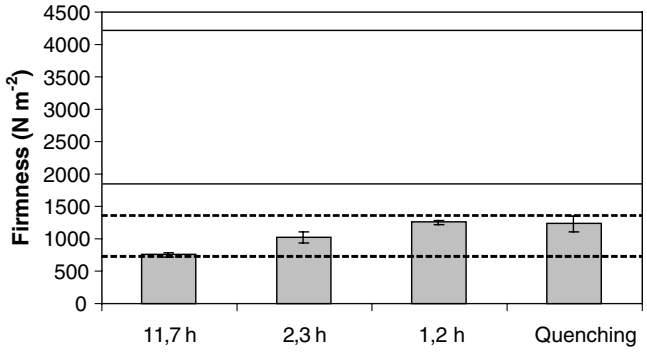

Fig. 3 Firmness values for commercial desserts and pea protein/κ-carrageenan/starch gels processed at different cooling conditions. The *solid lines* represent the range of variation for commercial dairy desserts. The *dotted lines* represent the range of variation for commercial soya desserts

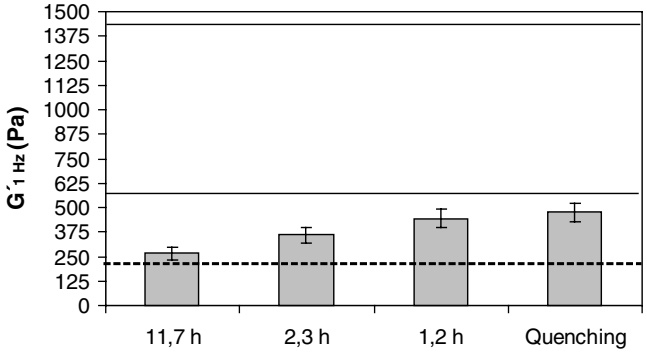

Fig. 4 Storage modulus values at 1 Hz ($G'_{1\,\mathrm{Hz}}$) for commercial desserts and pea protein/κ-carrageenan/starch gels processed at different cooling conditions. The *solid lines* represent the range of variation for commercial dairy desserts. The *dotted line* represents the value for a commercial soya dessert

Cooling conditions

Firmness and $G'_{1\,\mathrm{Hz}}$ values of gels cooled at different conditions are shown in Figs. 3 and 4, respectively. Pea protein gels had firmness values within the range of variation of soya desserts and $G'_{1\,\mathrm{Hz}}$ values higher than the soya dessert analysed. Faster cooling rate allowed the formation of gels with higher firmness and $G'_{1\,\mathrm{Hz}}$ values, closer to dairy puddings. Firmness and $G'_{1\,\mathrm{Hz}}$ values of the gel cooled down to 5°C in 1.2 h were not significantly different from the values of the gel quenched to 5°C.

Composition

To optimise formulation, the incorporation levels of the three biopolymers were studied according to the RSM design and texture responses are presented in Table 5. Statistical equations obtained for firmness (F) and adhesiveness (A) as function of biopolymers concentration were:

$$\mathrm{F(N)} = 0.09 - 0.09\mathrm{P} - 1.39\mathrm{C} - 0.13\mathrm{S} + 2.08\mathrm{PC} + 0.07\mathrm{S}^2 \left(R^2_{aj} = 0.847,\ MS_{\mathrm{residual}} = 0.0337\right) \quad (3)$$

Table 5 Response surface methodology matrix and texture resp-onses for pea protein/κ-carrageenan/starch systems with different composition

Pea isolate (%, w/w)	κ-Carrageenan (%, w/w)	Starch (%, w/w)	Firmness (N)	Adhesiveness (−N.s)
0.81	0.06	1.01	0.102	0.000
0.81	0.06	3.99	0.218	0.003
0.81	0.24	1.01	0.104	0.000
0.81	0.24	3.99	0.433	0.225
3.19	0.06	1.01	0.228	0.000
3.19	0.06	3.99	0.526	0.237
3.19	0.24	1.01	0.945	0.156
3.19	0.24	3.99	1.811	0.283
0.00	0.15	2.50	0.117	0.000
4.00	0.15	2.50	0.734	0.206
2.00	0.00	2.50	0.118	0.000
2.00	0.30	2.50	0.818	0.237
2.00	0.15	0.00	0.129	0.000
2.00	0.15	5.00	1.698	0.283
2.00	0.15	2.50	0.421–0.487 [a]	0.080–0.136 [a]

[a](min-max) values

$$\mathrm{A(-N.s)} = -0.22 + 0.05\mathrm{P} + 0.67\mathrm{C} + 0.05\mathrm{S} \left(R^2_{aj} = 0.859,\quad MS_{\mathrm{residual}} = 0.0014\right) \quad (4)$$

From the above equations, it can be seen that firmness shows a negative linear dependence in all the biopolymers which is overcomed by a stronger positive interaction between pea protein and κ-carrageenan and a quadratic effect on starch. Adhesiveness is linearly dependent on all the three macromolecules being this dependence stronger on κ-carrageenan.

Firmness values of gels with different composition are presented in Fig. 5 in comparison with values obtained for the commercial desserts. It is possible to observe that firmness for three formulations (3.19%P–0.24%C–1.01%S; 3.19%P–0.24%C–3.99%S; 2.0%P–0.15%C–5.0%S) was within the range of variation of those values for dairy desserts. This means that different formulations could be used to produce pea protein gels similar to dairy desserts. The selection will depend on nutritional value and industrial cost, since commercial desserts have a protein content varying from 2.3 to 4.0% (w/w) [17]. Adhesiveness values for all the mixed gels were lower compared to the commercial desserts (0.40 to 2.68 −N.s), varying from 0 to 0.28 −N.s. Sensory analysis of these formulations in terms of consumer acceptance will be considered in future.

Rheology of the central point of the experimental design, and of the star points which formed a gel, was explored. $G'_{1\,\mathrm{Hz}}$ values obtained for these formulations (Fig. 6) are consistent with firmness results, since it is possible to obtain $G'_{1\,\mathrm{Hz}}$ values closer to those of dairy desserts, increasing pea protein (formulation A to B), κ-carrageenan (A to C) or starch (A to D) content.

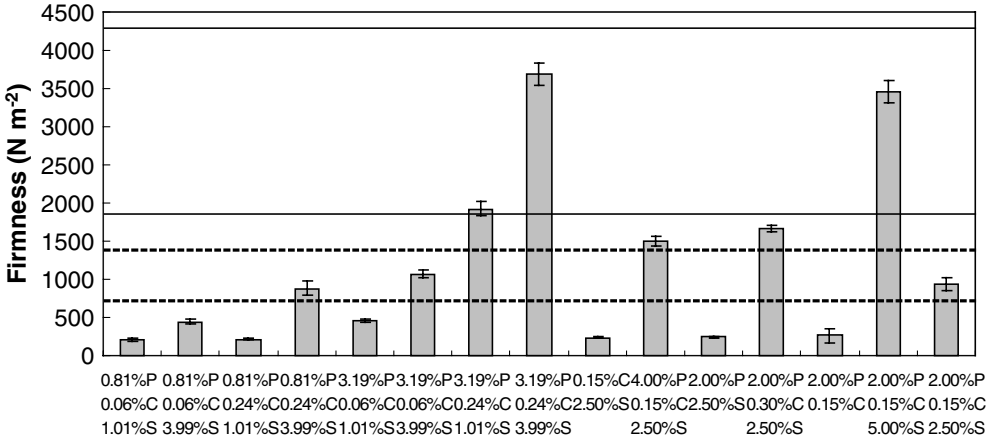

Fig. 5 Firmness values for commercial desserts and pea protein/κ-carrageenan/starch systems with different composition. The *solid lines* represent the range of variation for commercial dairy desserts. The *dotted lines* represent the range of variation for commercial soya desserts

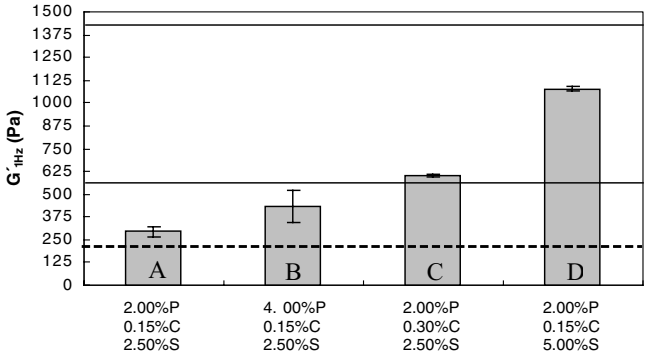

Fig. 6 Storage modulus values at 1 Hz ($G'_{1\,\mathrm{Hz}}$) for commercial desserts and pea protein/κ-carrageenan/starch gels with different composition. The *solid lines* represent the range of variation for commercial dairy desserts. The *dotted line* represents the value for a commercial soya dessert

An important result is the need for the presence of all the three macromolecules, since formulations with only two of the components (0.15%C–2.5%S; 2.0%P–2.5%S; 2.0%P–0.15%C) did not produce a gel but a viscous suspension. This is valid in the experimental domain studied. Gelling is accomplished only above a certain level of each of the three biopolymers. Total concentration required to form a gel is much lower than the critical gelation concentration for each of the biopolymers that reflects a synergistic effect between the three components of the mixed system [12]. Synergistic gel formation occurs as consequence of the mutual exclusion of pea protein and κ-carrageenan from the polymer domain of the other, with increase in effective concentration of both, when gelation takes place.

CSLM images (Fig. 7) showed that phase separation between protein (white areas) and κ-carrageenan (grey areas) takes place. Dark areas represent swollen starch granules. From 2.0%P–0.15%C–2.5%S to 2.0%P–0.15%C–5.0%S an increase on dark spots is noticeable.

Interaction between pea protein and κ-carrageenan results in phase separation, probably by the depletion-flocculation mechanism, promoting the formation of a κ-carrageenan network and a network of protein aggregates. Depletion-flocculation must be due to electrostatic repulsion since both macromolecules are negatively charged at pH 7. Starch granules bind the water, promoting the concentration of pea protein and κ-carrageenan in the solvent. This result is supported by the statistical equation obtained for firmness (Eq. (3)).

Syneresis is an undesirable parameter and of great importance from a practical point of view because it is not well accepted by the consumer. Syneresis is due to contraction of the gel and depends on factors that affect the polymer–polymer and water–polymer interactions, such as the degree of thermal treatment, type and concentration of solids, pH and salt additions. From Fig. 8 it can be seen that

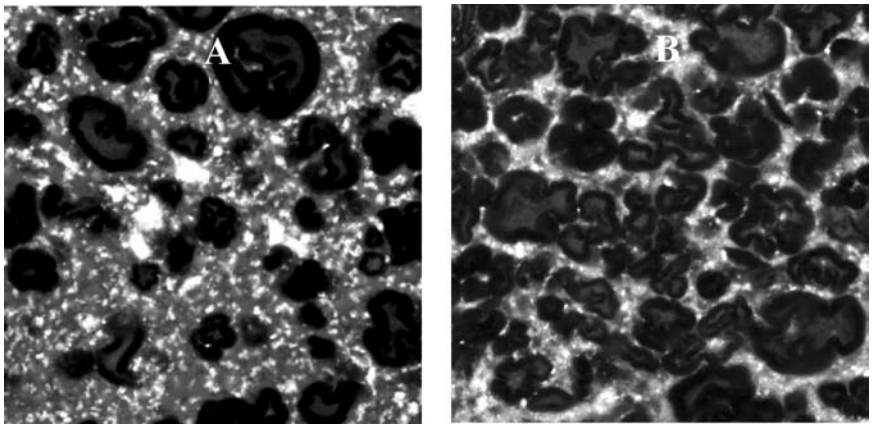

Fig. 7 CSLM images ($188\times188\ \mu m^2$) of pea protein/κ-carrageenan/starch gels with different composition. A) 2.0%P–0.15%C–2.5%S. B) 2.0%P–0.15%C–5.0%S

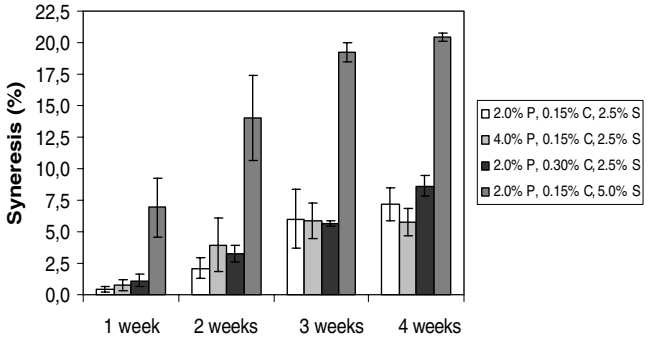

Fig. 8 Syneresis of pea protein/κ-carrageenan/starch gels with different composition

syneresis of the mixed gel increases with storage time and higher starch concentration. The three gels with the lowest level of starch (2.5%) had better water-binding characteristics. The starch granules bind water when swallowed, but this is reversible with time since they tend to shrink with subsequent water release, being the gels more susceptible to syneresis.

Conclusions

Texture parameters and storage modulus of pea protein/κ-carrageenan/starch systems could be greatly influenced by formulation and thermal conditions on heating and on cooling to gel setting.

Microscopic observations showed that interaction between pea protein and κ-carrageenan results in phase separation, probably by depletion-flocculation mechanism, which increases local polymer concentrations. The binding of water effect of the swollen starch granules, promoting the concentration of pea protein and κ-carrageenan in their own phases reinforces the gel structure.

To produce well-structured gels with this selection of polysaccharides high temperatures, above 95°C, are needed. As an indication for the industrial process one can say that the cooling operation after thermal treatment should be done quickly, in approximately 1 h. The selection of the formulation to produce a vegetable dessert with physical properties close to the commercial products will depend on nutritional value and industrial cost. Gels with higher starch content showed higher syneresis. Further work will be necessary to evaluate these gels on the basis of sensory characterisation.

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