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Novel foods with microalgal ingredients – Effect of gel setting conditions on the linear viscoelasticity of *Spirulina* and *Haematococcus* gels

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ABSTRACT

Microalgae represent an alternative and innovative source of natural ingredients that can be used in the development of novel food products. Biologically active compounds (e.g. carotenoids) are naturally encapsulated within microalgal cells, being able to resist harsh technological conditions involved in food technology processes. The aim of this work was to study the effect of adding *Haematococcus pluvialis* and *Spirulina maxima* microalgal biomass on the linear viscoelastic behaviour of vegetarian food gels prepared from pea protein, κ-carrageenan and starch. The gelation process was monitored *in situ* through dynamic oscillatory measurements, under different thermal profile conditions. Increasing temperature (70–90 °C, 5 min) resulted in more structured gels, while the effect of time (5–30 min, at 90 °C) was less pronounced. The effect of heating and cooling rates on gel setting was also studied. *Haematococcus* gels were highly structured and less dependent on gel setting conditions. *Spirulina* gels presented lower values of viscoelastic functions than the control (gel matrix without microalgae), but this was overcome when using lower heating/cooling rates.

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1. Introduction

Modern food industry leads to cheaper, healthier and more convenient products, in response to increasingly demanding consumers. The use of natural ingredients, like polyunsaturated fatty acids (PUFA) and antioxidant pigments, can have a strong impact on health, contributing to control chronic diseases incidence, which is strongly considered of capital importance in Europe, where aging population and welfare costs are fatal for public resources management. Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications (Pulz and Gross, 2004). These microscopic organisms can be grown under certain controlled environmental conditions (e.g. temperature, salinity, light, nutrients) that can stimulate or inhibit the biosynthesis and accumulation of bioactive compounds (e.g. astaxanthin from *Haematococcus pluvialis*) in large amounts. The possibility of not only harvesting microalgae but also growing them at different conditions enables its use as natural reactors at a large scale (Plaza et al., 2009). Therefore, microalgae can be used to enhance the nutritional value of food products, due to their well-balanced chemical composition as well as a source of highly valuable molecules, such as polyunsaturated fatty acids, pigments (e.g. carotenoids, phycobilins), sterols, vitamins, hydrocolloids and other biologically active compounds.

Some microalgae species, such as *Chlorella* and *Spirulina*, have been used for many centuries as a nutrient-dense food in Asia, Africa and Mexico. However, commercial large-scale production of microalgae only started in the early 1960s (Japan), and nowadays microalgae are mainly marketed as food supplements, commonly sold in the form of tablets, capsules or liquids. Additionally, there is an increasingly growing market for food products with microalgae addition such as pastas, biscuits, bread, snack foods, candy bars or gums, yoghurts, drink mixes, soft drinks, etc., either as nutritious supplement, or as a source of natural food colourant (Becker, 2004). In some countries such as Germany, France, Japan, USA, China or Thailand, food production and distribution companies have already started serious activities to market functional foods with microalgae and cyanobacteria (Pulz and Gross, 2004). The biotechnological exploitation of microalgae resources for human nutrition purposes is restricted to very few species, due to the strict food safety regulations, commercial factors, market demand and specific preparation (Pulz and Gross, 2004). However, foods supplemented with microalgae biomass might be sensorially more

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convenient and varied, thus combining health benefits with attractiveness to consumers, namely in terms of colour. For example, several microalgae when correctly processed have an agreeable or piquant taste and could be thus well incorporated into many types of foods, adding not only nutritional value, but also new, unique and attractive tastes (Richmond, 2004).

In the last years, some research has been carried out regarding the development of a range of novel attractive healthy foods, prepared from microalgae biomass, rich in carotenoids and polyunsaturated fatty acids with antioxidant effect and other beneficial properties. Traditional food products, like biscuits (Gouveia et al., 2007, 2008a), pasta (Fradique et al., 2010), mayonnaises/salad dressings (Raymundo et al., 2005; Gouveia et al., 2006) and puddings/gelled desserts (Batista et al., 2008; Gouveia et al., 2008b) have been previously studied as vehicles to microalgae addition. In general, the developed products presented appealing and stable colours, with added value in terms of health benefits, considering the antioxidant properties and PUFA-ω3 content of the microalgae, resulting in stable, attractive and healthier foods with enormous potential in the functional food market.

Natural biopolymers, such as globular proteins and polysaccharides, have been widely used in the formulation of structured gelled food products (Doublier et al., 2000). Recently, pea protein isolate has been successfully used in combination with kappa-carrageenan and starch polysaccharides, to develop strictly vegetable gelled desserts, as a vegan alternative to dairy-desserts (Nunes et al., 2003, 2006a,b). Freeze-dried biomass from various microalgae – *Chlorella vulgaris*, *H. pluvialis*, *Spirulina maxima* and *Diacronema vlkianum* – has been used as colouring agent in these mixed gel systems (Batista et al., 2008; Gouveia et al., 2008b). Besides colouring purposes, from a novel product development point of view, it is essential to define the texture/sensorial and rheological characteristics of these systems, since they reflect the microstructural modifications that might arise as a consequence of microalgal biomass addition to gel matrixes, which may distress the product stability.

The gelation process of these mixed systems is thermally induced, involving extensive denaturation and/or conformation changes of the biopolymers and subsequently the development of a gel network upon cooling. The thermal profile used on gel-setting is determinant for the development of the gel structure which is reflected by its rheological properties (Nunes et al., 2006a). The aim of the present work was to study the effect of different gel setting conditions (time/temperature treatment, heating/cooling rates) on the linear viscoelastic properties of protein–polysaccharide mixed gels with microalgal biomass addition. *S.* (*Arthrospira*) *maxima* and *H. pluvialis* were selected according to previous studies (Batista et al., 2008). The interaction of these microalgae with pea protein, kappa-carrageenan and starch biopolymers, in model binary and ternary gel systems, has been recently studied in terms of linear viscoelastic behaviour and fluorescence microscopy (Batista et al., 2011).

2. Materials and methods

2.1. Microalgae production

S. maxima (LB 2342) and *H. pluvialis* (INETI 33) microalgae were produced at the LNEG – Bioenergy Unit (Lisbon). The microalgae were cultivated in airlift bioreactors with bubbling air, using appropriate growth media (Vonshak, 1986), under low light conditions (150 $\mu Em^{-2} s^{-1}$) and optimal growth temperatures (*Sp*: 34 °C; *Hp*: 25 °C). *Spirulina* was harvested during the stationary growth phase, while *Haematococcus* was first submitted to a carotenogenesis process by nitrogen starvation, NaCl addition and high luminosity, enhanced by culture dilution (Gouveia and Empis, 2003) before harvesting. Microalgal biomass was harvested by simply stopping agitation and concentrated by centrifugation and subsequently freeze dried.

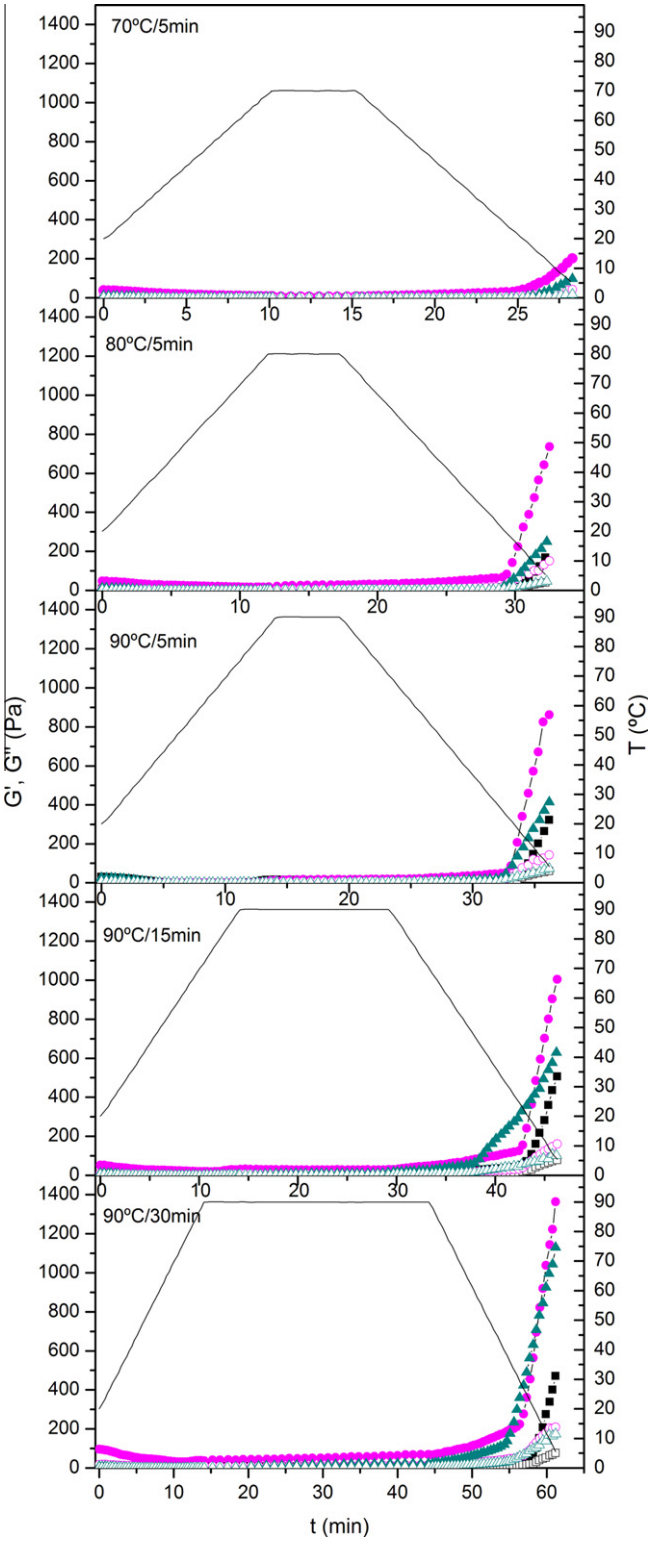

Fig. 1. Evolution of G' and G'', of pea/κ-carrageenan/starch suspensions (■) with *Haematococcus* (●) and *Spirulina* (▲), during thermal treatment, performed at different temperature/time conditions (70 °C/5 min, 80 °C/5 min, 90 °C/min, 90 °C/15 min and 90 °C/30 min). (Heating/cooling rates: ±5.0 °C/min). G' (closed symbol), G'' (open symbol), T (line).

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Table 1
Gelation temperature (T_{gel}), parameters of exponential decay, and calculated G'_{eq} (Eq. (3)), experimental G' values after 24 h maturation (G'_{24h}), and maturation index (G'_{24h}/G'_{eq})×100 of pea/κ-carrageenan/starch gels with *Haematococcus* and *Spirulina*, after thermal treatment performed at different gel setting conditions.

T/t (°C/min)	Heating/cooling rate (°C/min)	T_{gel} (°C)	y_0 (Pa)	A_1 (Pa)	b_1 (h^{-1})	A_2 (Pa)	b_2 (h^{-1})	χ^2	R^2	G'_{24h} (Pa)	G'_{eq} (Pa)	$(G'_{24h}/G'_{eq}) \times 100$ (%)
Haematococcus												
70/5	+5.0/−5.0	18.6	674	585	0.045	750	0.546	151	0.997	1642	2010	81.7
80/5	+5.0/−5.0	18.8	1504	389	0.044	827	0.605	284	0.994	2428	2720	89.3
90/5	+5.0/−5.0	20.7	1551	1694	0.019	871	0.423	448	0.992	2510	4116	61.0
90/15	+5.0/−5.0	22.9	1767	786	0.053	787	0.744	402	0.994	2890	3340	86.5
90/30	+5.0/−5.0	27.2	2084	1531	0.033	920	0.622	707	0.993	3430	4535	75.6
90/5	+0.5/−0.5	25.6	1894	1387	0.037	708	0.367	452	0.995	2971	3989	74.5
90/5	+1.0/−1.0	24.7	1648	1149	0.037	774	0.401	421	0.995	2733	3571	76.5
90/5	+5.0/−0.5	22.5	1444	1901	0.029	397	0.566	258	0.995	2266	3742	60.6
90/5	+5.0/−1.0	21.1	1260	543	0.441	2166	0.224	269	0.995	2141	3969	53.9
90/5	+5.0/−10.0	18.7	1582	553	0.690	2540	0.017	350	0.987	2328	4675	49.8
Spirulina												
70/5	+5.0/−5.0	21.6	–	–	–	–	–	–	–	414	–	–
80/5	+5.0/−5.0	21.8	495	186	1.089	299	0.028	9	0.996	720	980	73.5
90/5	+5.0/−5.0	22.9	702	339	0	194	1	4	0.997	917	1235	74.3
90/15	+5.0/−5.0	37.4	726	214	0.114	264	1.022	18	0.998	1128	1204	93.7
90/30	+5.0/−5.0	41.6	1597	457	0.070	213	0.252	100	0.994	2033	2268	89.7
90/5	+0.5/−0.5	34.8	2257	663	0.041	408	0.366	462	0.985	2871	3329	86.2
90/5	+1.0/−1.0	35.6	1544	328	0.036	471	0.523	103	0.994	2079	2343	88.7
90/5	+5.0/−0.5	24.7	820	175	0.552	216	0.062	9	0.998	1095	1211	90.4
90/5	+5.0/−1.0	24.7	644	181	0.732	152	0.069	7	0.998	900	977	92.1
90/5	+5.0/−10.0	21.0	–	–	–	–	–	–	–	519	–	–
Control												
70/5	+5.0/−5.0	10.3	231	309	0.071	497	0.791	9	0.999	878	1037	84.7
80/5	+5.0/−5.0	14.9	651	317	0.081	515	1.153	10	0.999	1338	1482	90.3
90/5	+5.0/−5.0	16.4	881	463	0.048	669	0.731	35	0.999	1706	2013	84.8
90/15	+5.0/−5.0	20.6	1127	657	0.039	809	0.732	96	0.998	2122	2593	81.8
90/30	+5.0/−5.0	25.0	1118	660	0.049	721	0.725	100	0.998	2094	2498	83.8
90/5	+0.5/−0.5	23.6	1464	664	0.051	636	0.533	158	0.997	2346	2763	84.9
90/5	+1.0/−1.0	23.6	1393	615	0.047	719	0.521	928	0.983	2312	2726	84.8
90/5	+5.0/−0.5	18.1	1066	457	0.062	517	0.632	169	0.995	1780	2040	87.2
90/5	+5.0/−1.0	18.2	887	589	0.684	549	0.049	58	0.998	1683	2025	83.1
90/5	+5.0/−10.0	16.6	–	–	–	–	–	–	–	1081	–	–

The gross chemical composition (% dw) of the microalgal biomass is (Batista et al., 2008): *Spirulina*: 44.9% protein; 3.6% fat; 16.6% carbohydrates; 30.9% ashes (2.1% K; 0.8% Ca; 0.3% Mg; 7.0% Na); 0.9% carotenoids; 7.1% phycocyanin; *Haematococcus*: 10.2% protein; 40.7% fat; 33.6% carbohydrates; 8.9% ashes (0.9% K; 0.2% Ca; 0.2% Mg; 5.5% Na); 3.0% carotenoids.

2.2. Gel preparation

Gels were prepared according to the formulation previously optimised by Nunes et al. (2006b) – 4% (w/w) pea protein isolate (Pisane F9®, Cosucra, Belgium), 0.15% (w/w) κ-carrageenan (Satiagel AMP45®, Degussa, France), 2.5% (w/w) native maize starch (Vitena A®, Copam, Portugal) and 15% sucrose (commercial grade). All these ingredients were kindly provided by the respective manufacturers. Microalgal biomass was added at 0.75% (w/w) concentration. The ingredients were dispersed in demineralised water, by mechanical agitation (300 rpm, 1 h) at room temperature. No adjustments were made to the natural pH of the systems and no salts were added to keep in solution solely the salts carried along with the biopolymers and microalgae.

2.3. Rheological measurements

The gelation process was monitored *in situ* in a controlled stress rheometer (RS-300, Haake, Germany) coupled to an UTC–Peltier system, using cone-plate geometry (35 mm Ø, 2° angle), through dynamic small amplitude oscillatory shear measurements (SAOS) under different thermal profile conditions. The samples were heated from 20 to 70–90 °C, maintained at this temperature for 5–30 min, and then cooled down to 5 °C, at varying heating/cooling rates (0.5–10.0 °C/min). These temperature sweep tests were carried out at $\omega = 6.28$ rad/s and $\tau = 0.2$ Pa (in the linear viscoelastic regime). Subsequently, time sweep tests were conducted at 5 °C during 24 h ($\omega = 6.28$ rad/s, $\tau = 0.5$ Pa). The samples were covered with paraffin oil to prevent moisture loss and each test was performed in duplicate.

3. Results and discussion

3.1. Effect of processing temperature and time

The effect of thermal treatment on the gels' rheological behaviour was studied by setting different maximum temperature and time combinations – 70, 80, 90 °C/5 min, 90 °C/15 min and 90 °C/30 min. Fig. 1 presents G' and G'' evolution upon thermal treatment, as well as through heating (up from 20 °C) and cooling (down to 5 °C) ramps (±5 °C/min).

Pea/κ-carrageenan/starch suspensions at 20 °C presented very low G' and G'' values, which were of the same order of magnitude (around 1 Pa). Heating the samples induced a decrease on the viscoelastic functions, until a slight increase occurs around 84 °C corresponding to starch gelatinization. This phenomenon is related to the swelling of the starch granules, and subsequent amylose solubilisation, which causes an increase on the systems viscosity (Morris, 1990). Therefore, in the samples heated only at 70 °C and 80 °C starch gelatinization did not occur, at least at its full length, causing a negative impact on the gels mechanical properties. In all the samples with *Spirulina* addition, this transition was not detected indicating that this microalga interferes with the starch gelatinization process, perhaps by competing for water binding sites during the granules hydration process, as proposed in previous studies

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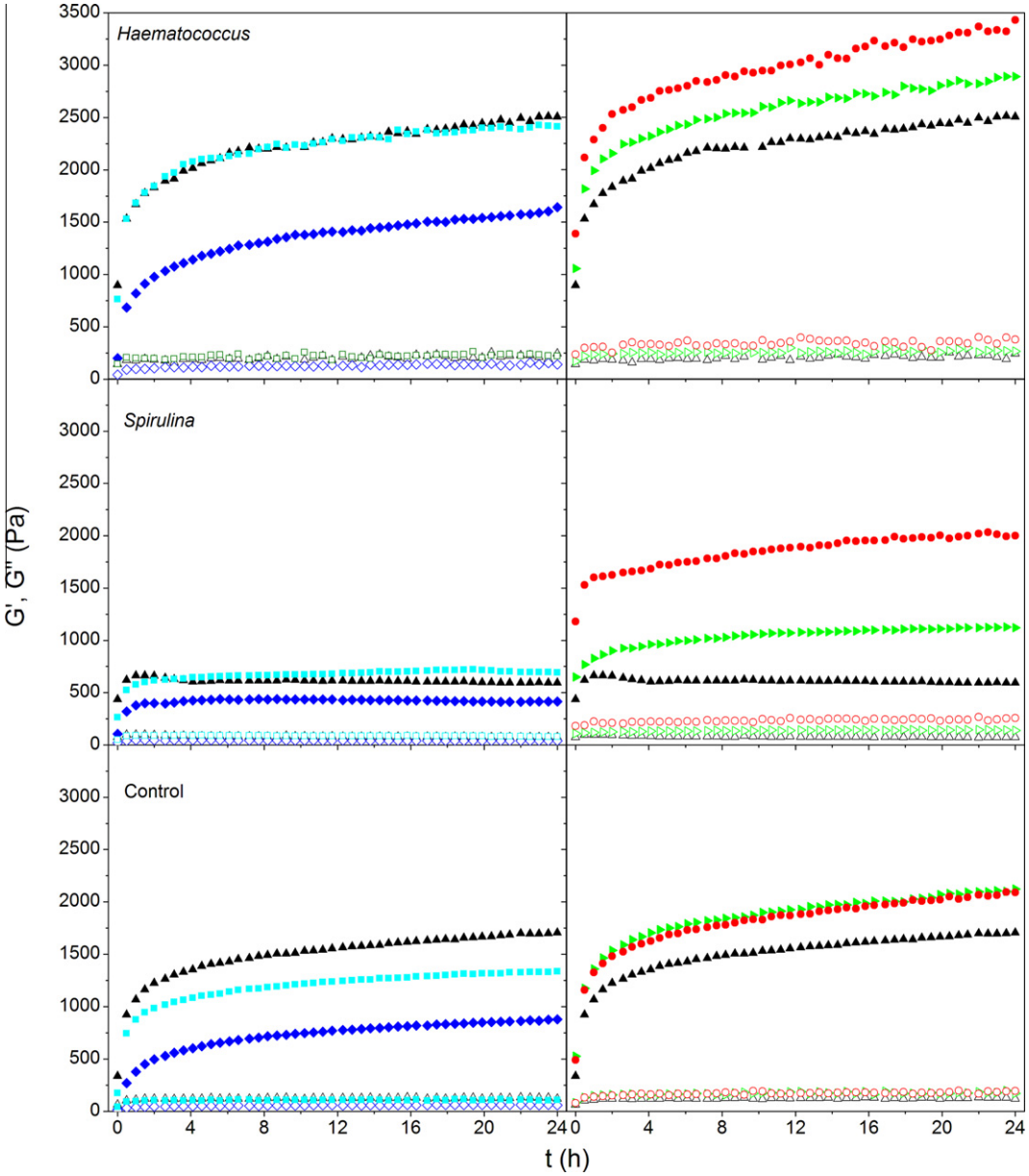

Fig. 2. Maturation kinetic curves at 5 °C, of pea/κ-carrageenan/starch gels with *Haematococcus* and *Spirulina*, after thermal treatment at 70 °C/5 min (♦), 80 °C/5 min (■), 90 °C/5 min (▲), 90 °C/15 min (▶) and 90 °C/30 min (●) (heating/cooling rates: ±5.0 °C/min). G' (closed symbol), G'' (open symbol).

(Batista et al., 2011). *Haematococcus* suspensions always presented $G' > G''$ during gel setting, with initial G′ values around 60 Pa, suggesting the existence of an incipient internal microstructure in this state.

After thermal treatment, and upon cooling to 5 °C, it is observed a small increase in the viscoelastic functions, until a sharp rise in G' arises (Fig. 1), which is coincident with a dramatic decrease in phase angle (δ), revealing an important structural reinforcement. The temperature at which this occurs may be regarded as a "*gelation temperature*" which reflects the changes in the rheological properties of the gel network formation (Chronakis, 2001; Verbeken et al., 2004). These "*gelation temperatures*" (T_{gel}) should not be regarded as true "*gel points*", meaning the appearance/disappearance of an infinite network, as defined by other authors (Winter and Chambon, 1986). These gelation temperatures are presented in Table 1. T_{gel} values of control gel increased from 10.3 to 16.4 °C with increasing temperatures (70–90 °C/5 min); and up to 25.0 °C, by extending thermal treatment duration to 90 °C/30 min. Samples with microalgal biomass addition presented the same behaviour with a higher T_{gel} with increasing temperature and time, that for *Haematococcus* ranged from 18.6 to 27.2 °C and for *Spirulina* ranged from 21.6 to 41.6 °C. The achievement of a higher T_{gel} means that gel formation occurs earlier during the cooling process, being possible to obtain gels at room temperature by increasing the intensity of the thermal treatment. From the results obtained, it can be assumed that by using microalgae biomass it is possible to attain higher T_{gel} while using milder thermal processing conditions, which enable the preservation of natural compounds such as natural pigments (e.g. astaxanthin and phycocyanin).

The maturation behaviour of the gels is typical of biopolymer gelation (Clark et al., 2001), with G' increasing rapidly at first and then more slowly, as a result of continuous reorganization of the polymeric molecules in the gel network (Fig. 2). The evolution found for gel maturation kinetic curves (Fig. 2) allows a simple def-

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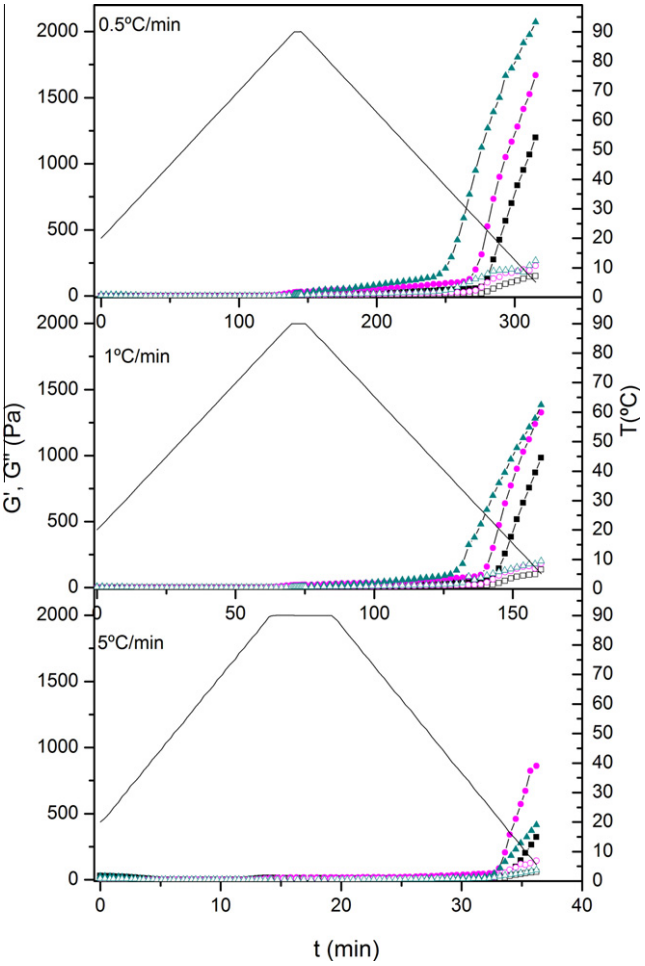

Fig. 3. Evolution of G' and G'', of pea/κ-carrageenan/starch suspensions (■) with *Haematococcus* (●) and *Spirulina* (▲), during thermal treatment (90 °C/5 min), performed at the same heating and cooling rates (±0.5, 1.0 and 5.0 °C/min). G' (closed symbol), G'' (open symbol), T (line).

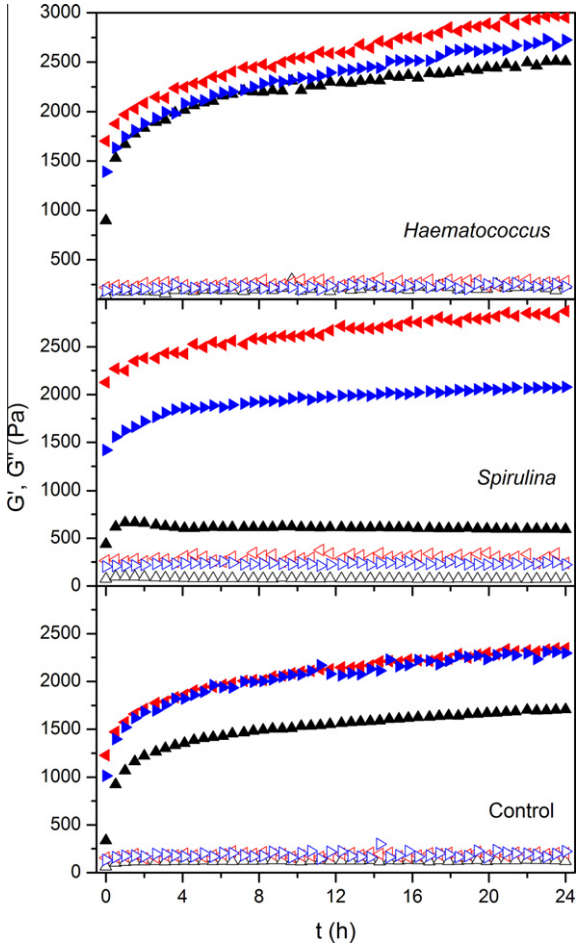

Fig. 4. Maturation kinetic curves at 5 °C, of pea/κ-carrageenan/starch gels with *Haematococcus* and *Spirulina*, after thermal treatment (90 °C/5 min) performed at the same heating and cooling rates, ±0.5 °C/min (◀), ±1.0 °C/min (▶) and ± 5.0 °C/min (▲). G' (closed symbol), G'' (open symbol).

inition of a G' value at the pseudo-equilibrium-state (G'_{eq}), i.e. the value of G' at infinite time where the gel reaches a stable and fully developed structure (Nunes et al., 2003), considering:

$$G'_{eq} = \lim_{t\to\infty} G'(t) \tag{1a}$$

or alternatively

$$G'_{eq} = \lim_{\frac{1}{t}\to 0} G'(t) \tag{1b}$$

These experimental data can be fitted to a second order exponential decay of the form:

$$G'(k) = y_0 + A_1 e^{\frac{-k}{b_1}} + A_2 e^{\frac{-k}{b_2}} \tag{2}$$

Where y_0, A_1, A_2, b_1 and b_2 are the equation parameters and k is the reciprocal time, i.e. $1/t$. Calculated G'_{eq} values are listed in Table 1 along with the experimental G' attained after 24 h maturation (G'_{24h}). These parameters are compared by means of a maturation index, given by the ratio $(G'_{24h}/G'_{eq}) \times 100$.

Pea/κ-carrageenan/starch control gels presented increasing G'_{24h} and G'_{eq} when increasing processing temperature from 70 to 90 °C. The effect of processing time was less important (small variations between 15 and 30 min), which is in agreement with previous studies (Nunes et al., 2004). High temperature induces globular protein unfolding and exposure of higher number of linking sites with subsequent reinforcement of gel structure (van Vliet et al., 2002). κ-Carrageenan also requires high temperatures to achieve maximal hydration (Schmidt and Smith, 1992) which will support subsequent gelation involving conformational transition (single chain to double helix) and aggregation when the system is cooled (Morris et al., 1980). Starch gelatinization process is also temperature dependent, as discussed previously.

Haematococcus gels presented much higher G'_{24h} and G'_{eq} values than the control gel (Table 1). This structural reinforcement action may be related to its high fat content (41%) (Batista et al., 2008), since fat droplets can act as active filler particles embedded in the protein matrix (Houzé et al., 2005). In fact, even when submitted to a milder thermal treatment (70 °C/5 min) *Haematococcus* gels presented a G'_{eq} of 2010 Pa, similar to the control gel heated at 90 °C/5 min (2013 Pa). This enables the use of lower temperatures to reach a similar product, avoiding thermal degradation of natural biomolecules (e.g. astaxanthin). All gels presented higher viscoelastic properties with increasing temperature, as a result of improved protein unfolding and biopolymer interaction. The effect of heating time was less pronounced on the gel properties.

In the case of *Spirulina* gels, they always presented lower G'_{24h} and G'_{eq} values than the control gel (Table 1), which is in agreement with previous studies (Batista et al., 2008). In fact, for the different thermal treatments applied *Spirulina* gels provide G'_{24h} values about half of those found for the control, except in the case of 90 °C/30 min, where the values are of the same order of magnitude (2033 Pa and 2094, respectively). This means, that in order to obtain a *Spirulina* gel with mechanical properties similar to the control a more extensive thermal treatment, involving higher temperatures and times of

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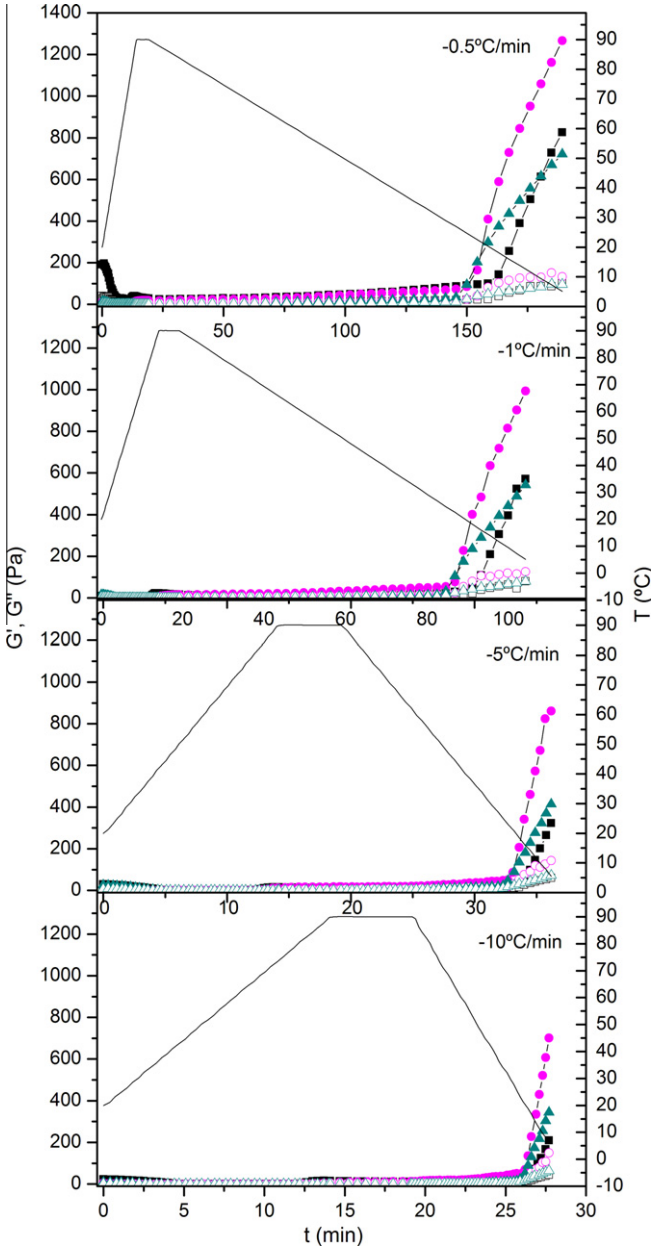

Fig. 5. Evolution of G' and G'', of pea/κ-carrageenan/starch suspensions (■) with *Haematococcus* (●) and *Spirulina* (▲), during thermal treatment (90 °C/5 min), performed at different cooling rates (−0.5, −1.0, −5.0 and −10.0 °C/min). G' (closed symbol), G'' (open symbol), T (line).

exposure, should be applied. It has been proposed by Batista et al. (2011) that *Spirulina* protein molecules (44.9% dw) compete for water binding sites, hindering the hydration of starch granules, since this microalga (Cyanobacteria) lacks a rigid cell-wall. It is thus possible to provide conditions for more extensive starch granules swelling and amylose release by increasing the intensity of the thermal treatment.

3.2. Effect of heating and cooling rates

Gel setting conditions play a major role on the development of the gel structure and resulting rheological properties, and are determined not only by the temperature/time applied but also by the heating and cooling rates implemented. To study the influence of these factors on pea/κ-carrageenan/starch systems with microalgal biomass, tests were conducted at 90 °C/5 min, applying different heating and cooling rates. Fig. 3 presents G' and G'' evolution of the samples that were submitted to the same heating and cooling rates: ±0.5 °C/min, ±1.0 °C/min and ±5.0 °C/min. For the slower heating/cooling rates (±0.5 °C/min, ±1.0 °C/min) an increase in T_{gel} was observed (Table 1) compared to the faster heating/cooling rate (±5.0°C/min): from 16.4 to 23.6 °C in the control; from 20.7 to 25 °C in *Haematococcus*, and from 22.9 to 35 °C in *Spirulina*. Although an increase of gel setting temperature is an advantageous feature, it should be kept in mind that the time length of the processing (5.3 and 2.7 h against 36 min) is a major drawback.

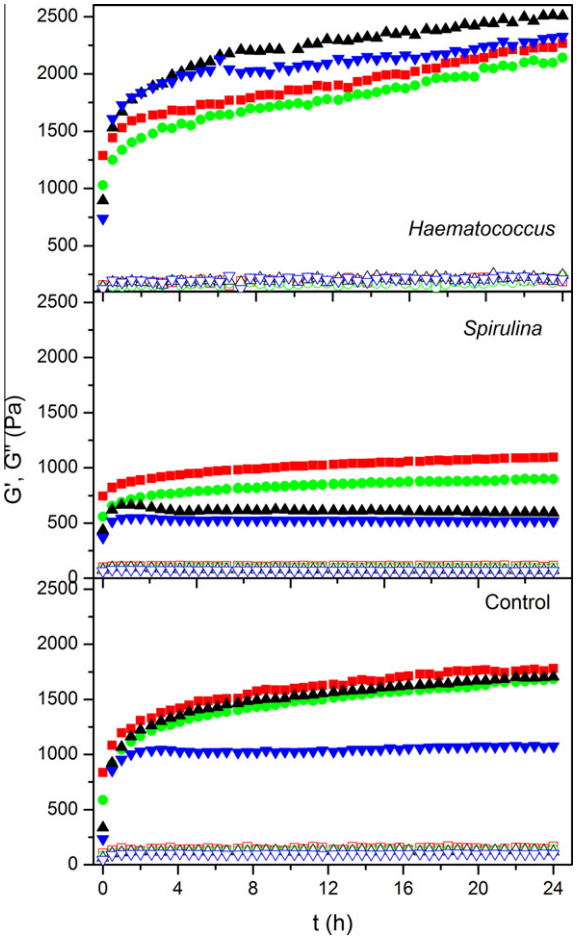

Fig. 6. Maturation kinetic curves at 5 °C, of pea/κ-carrageenan/starch gels with *Haematococcus* and *Spirulina*, after thermal treatment (90 °C/5 min) performed at different cooling rates, −0.5 °C/min (■), −1.0 °C/min (●), −5.0 °C/min (▲) and −10.0 °C/min (▼). G' (closed symbol), G'' (open symbol).

Maturation kinetic curves are presented in Fig. 4 and the respective fitting parameters derived from Eq. (2) are presented in Table 1. For the Control gel, higher G'_{24h} and G'_{eq} (~2300 and 2700 Pa) are observed for lower heating/cooling rates with only small differences between them. In the case of *Haematococcus* gels, the differences in G'_{24h} are less pronounced for the various rates (2510–2971 Pa) and in terms of calculated G'_{eq} these differences are even smaller, being the highest value attained for the faster rates (4116 Pa). Oppositely, *Spirulina* gels show major differences between each of the three rates, indicating that for these systems it is highly advantageous to use lower heating/cooling rates upon gel setting, in order to attain a higher structuring degree. In fact, at ±0.5 °C/min *Spirulina* gel attained higher G'_{24h} and G'_{eq} values than the control system (2871–3329 Pa against 2346–2763 Pa).

Many studies on biopolymers evidence the cooling rate influences on the mechanical properties of simple gels (e.g. Silva and Rao, 1995) as well as on phase separation process and physical

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properties of mixed systems (Lóren et al., 1999; Turgeon and Beaulieu, 2004). Considering that cooling rate plays a major role on the development of gel structure, larger than the heating rate does, it was decided to maintain the thermal treatment at 90 °C/5 min and using a constant heating rate of 5 °C/min while studying the effect of different cooling rates: −0.5, −1.0, −5.0 and −10.0 °C/min.

From Fig. 5 and Table 1 it can be observed that control gels presented a T_{gel} of 18.1–18.2 °C for lower cooling rates (−0.5 and −1 °C/min), and 16.4–16.6 °C for higher cooling rates (−5.0 and −10 °C/min). As previously discussed, samples with microalgae addition presented higher T_{gel} values which increased with decreasing cooling rates. For *Haematococcus* T_{gel} ranged from 18.7 to 22.5 °C and for *Spirulina* from 21.0 to 24.7 °C. At higher cooling rates the temperature decreases too rapidly (8.5 and 17 min for −5 and −10 °C/min, respectively), not allowing the solution to reach equilibrium at each temperature step, requiring lower temperatures for gel setting, since gelation time increases exponentially with temperature (Tosh and Marangoni, 2004).

Maturation kinetic curves are presented in Fig. 6, and the respective fitting parameters derived from Eq. (2) are listed in Table 1. *Spirulina* gels presented significantly smaller G'_{24h} and G'_{eq} (<1200 Pa) values, than the control gels (>1600 Pa, excepting for cooling rates of 10 °C/min). This is in accordance with previous studies (Batista et al., 2008) and could be related to a competitive interaction between the microalga and the other biopolymers present in the mixed gel system, namely starch, which is supported by the present heating curves results (Fig. 5). Denaturation of *Spirulina* components and gel formation has been reported as a complex phenomenon, as a consequence of the presence of protein-pigment (phycocyanin) complexes (Chronakis, 2001). Gels submitted to slower cooling rates generally presented higher G'_{24h} and G'_{eq} values. In fact, for systems cooled slowly, there is more time to achieve dynamic equilibrium of the gel network, i.e. gel structure maturation, leading to a more structured network on cooling (Nunes et al., 2006a). However, for the systems under study, the rheological properties (G'_{24h}, G'_{eq}) of the gels cooled at −1 and −5 °C/min are not substantially different. This presents an advantage considering that lesser time (17 min instead of 85 min) will be needed to achieve a similar final product. Gels subjected to the fastest cooling rates (−10 °C/min) showed much lower G'_{24h} values (control: 1081 Pa, *Spirulina*: 519 Pa). The maturation kinetic curve of these gels is also quite different, being not well adjusted to Eq. (2). In these cases, G' increases rapidly up to a maximum and then slightly decreases, reaching an almost constant equilibrium value. This behaviour resembles a structural relaxation phenomenon, especially if considering that the rapid cooling would not let the solution to reach equilibrium at each temperature step, as referred before (Tosh and Marangoni, 2004). Alternatively, in the case of *Haematococcus* gels, a general tendency for increasing G'_{eq} or G'_{24h} with decreasing cooling rates was not observed, inclusively the highest values were attained for the faster cooling rates. This suggests that the addition of *Haematococcus* promotes a structural reinforcement of the gel matrix that is independent of the cooling rate, under these gel setting conditions (90 °C/5 min, +5 °C/min). This structural reinforcement action has been suggested (Batista et al., 2011) to be related to its high fat content (41%), considering that fat droplets can act as active filler particles embedded in the gel matrix as observed for milk gelled systems (Houzé et al., 2005).

4. Conclusions

The linear viscoelastic properties of pea/κ-carrageenan/starch mixed gel systems was highly dependent on the gel setting conditions, including temperature/time of thermal processing and heating/cooling rates. Increasing temperature (70–90 °C, 5 min) resulted in more structured gels, while the effect of time (5–30 min, 90 °C) was less pronounced. Higher values of the viscoelastic functions were achieved upon heating and/or cooling the mixed biopolymer suspensions at lower rates. However, the time length required for the process, and the subsequent costs involved, should be considered.

The addition of microalgal biomass promoted some modifications on the gel structure, although the response to gel setting conditions followed in general the behaviour of the control gel. This is in agreement with previous studies on the interaction of *Haematococcus a*and *Spirulina* with pea protein, κ-carrageenan and starch (simple and mixed gel systems), where it was observed that the gelling mechanism is ruled by the biopolymers, while microalgae seem to be embedded in the gel network acting as active particle fillers (Batista et al., 2011). *Haematococcus* gels, which presented an attractive "*strawberry-pink*" colour, became highly structured (G' and G'' always higher than the control) and were less dependent on gel setting conditions. *Spirulina* gels presented lower values for viscoelastic functions than the control gel, which was probably related to the fact that starch gelatinization process was hindered. This drawback was overcome when using lower heating/cooling rates and more extensive thermal treatments.

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