Carbohydrate Polymers 102 (2014) 30–37

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Influence of Na^+, K^+ and Ca^{2+} on mechanical and structural properties of gels from chestnut and rice flours

M.D. Torres[a,*], A. Raymundo[b,c], I. Sousa[c]

[a] Departamento de Enxeñaría Química, Escola Técnica Superior de Enxeñaría, Universidade de Santiago de Compostela, Campus Vida, E-15782 Santiago de Compostela, Spain
[b] Núcleo de Investigação em Engenharia Alimentar e Biotecnologia – ISEIT Almada, Quinta da Arreinela de Cima, 2800-305 Almada, Portugal
[c] CEER – Biosystems Engineering, Instituto Superior de Agronomia/Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

A R T I C L E I N F O

Article history:
Received 26 July 2013
Received in revised form 5 November 2013
Accepted 8 November 2013
Available online 20 November 2013

Keywords:
Cation
Gluten-free
Gels
Rheology
Texture
Microscopy

A B S T R A C T

The addition of salts to foodstuffs to improve the mechanical characteristics and thereby increasing sample strength has been widely studied in gels of model systems, however has been investigated to a lesser extent in complex systems, such as gluten-free flour gels. The objective of this study was to evaluate the impact of cation concentration and ion valence of chloride salts (NaCl, KCl or $CaCl_2$, 0–2%, w/w) on textural, rheological and microstructural properties of selected gluten-free flour gels from blends of chestnut flour and whole, Agulha or Carolino rice flours. Firmness increased in the presence of $CaCl_2$. Storage and loss moduli increased slightly with the monovalent salts concentration, whereas a more pronounced rise was observed with the divalent salt addition. It was found that K^+ was more effective in modifying the rheology of studied gels than Na^+. Confocal microscopic images of gels revealed a microstructure consistent with the observed mechanical properties.

1. Introduction

Nowadays, gluten-free diets are gathering new followers and subsequent research focuses more strongly on new mixtures as a result of industrial demands for new gelling products with higher quality (Mariotti, Lucisano, Pagani, & Ng, 2009; Moreira, Chenlo, Torres, & Glazer, 2012), since many gluten-free products available on the market are based on formulations with low technological and nutritional characteristics (Marco & Rosell, 2008). In the last decade, the blending of chestnut flour (Cf) with rice flours (Rf) has been highlighted in various scientific investigations (Demirkesen, Mert, Sumnu, & Sahin, 2010; Moreira, Chenlo, & Torres, 2012; Sacchetti, Pinnavaia, Guidolin, & Dalla Rosa, 2004) as an interesting approach to produce gluten-free products. In this context, the present work is part of a research project that deals to develop alternative sweet/savoury gluten-free formulations based on gelled systems from blends of these two flours. In a previous work of the same authors (Torres, Fradinho, Raymundo, & Sousa, 2013), it was demonstrated that the gelling ability of a mixture of Cf and whole rice flour (Rw), Agulha rice flour (Ra) or Carolino rice flour (Rc) could be used as an interesting alternative to commercial gluten-free formulations, with subsequent multiple advantages from the economic and health hazard points of view. During the development of these formulations based on flour gelled systems, the understanding of how the complex mixture of flour and the ingredients commonly used in food formulations behave, under food processing conditions, plays an important role. In this sense, the same authors (Torres, Raymundo, & Sousa, 2013), in a parallel work, indicated that the presence of sucrose and xylitol strongly affected gluten-free gels structure and therefore rheology, texture and microstructure. Following our earlier works in this field, the effect of other ingredients, relevant for consumer acceptance, such as salts needs to be studied. Salt acts as a flavour enhancer, preserving agent, toughens the protein and can affect the colour, texture or structure of gels (Butow, Gras, Haraszi, & Bekes, 2002). Among the agents commonly used, salts of chloride such as sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$) can be remarked and are usually employed at concentrations below 2% (w/w) (Thrimawithana, Young, Dunstan, & Alanya, 2010) because of according to American Association of Cereal Chemists suggestions (AACC, 2000), low salt diet, especially in cereal foods, should be promoted.

The challenge is to understand the functions and interactions of each salt on gels, to improve the mechanical and structural properties and storage stability of the derived products (Moreira, Chenlo, & Torres, 2011). Formation of a continuous network in the gels can be promoted by the addition of salt, to screen the repulsions,

* Corresponding author. Tel.: +34 881816752; fax: +34 981 528 050.
E-mail addresses: lolytorresp@hotmail.com, mariadolores.torres.perez@usc.es (M.D. Torres).

0144-8617/$ – see front matter
http://dx.doi.org/10.1016/j.carbpol.2013.11.018

leading to a more aggregated structure (Harrington, Foegeding, Mulvihill, & Morris, 2009). It was reported that cations (Ca^{2+}, K^+, Na^+) affect the balance of attractive and repulsive forces between the molecules so that optimum gel strength will occur at certain levels of each cation (Lin & Lai, 2009; Pereira et al., 2013). Namely, Arda, Kara, and Pekcan (2009) demonstrated that the gels characteristics improve notably with the addition of KCl and $CaCl_2$. Other authors (Thrimawithana et al., 2010) indicated that the gel strength in the presence of Ca^{2+} cations was substantially greater than the strength of gels formed with K^+ cations at equivalent molar concentration or ionic strength, whereas smaller univalent cations, such as Na^+, featured a lower impact. Many authors (Nunez-Santiago & Tecante, 2007) have pointed out the importance of cation type and concentration as well as rate of cooling on the thermorheological properties of gels. It was reported that salts, depending on their nature and concentration, can cause either an elevation or a depression of the gelatinization temperature of chestnut flour pastes (Moreira et al., 2011). Salt effect on gelatinization is certainly different and more complex than the one of non-ionic solutes, as sucrose (Chiotelli, Pilosio, & Le Meste, 2002). It was found that salts addition can have an important role in the structure of the gel, which is noticeably dependent on the size and valence of the ion (McClements, 1999; Moritaka, Fukuba, Kumeno, & Nakahama, 1991). The presence of salts can also modify the thermal stability of proteins (Dickinson, 2006, 2010). It should be mentioned that the influence of ionic strength of salts on the stability of protein is complex and depends on the precise nature of the ions and the prevailing environmental conditions (McClements, 1999). Many food products have optimal ranges of rheology and texture, and it is important to stay within these limits even when the salt conditions are changed.

To date, much work has been accomplished on the gelling properties of foodstuffs in the presence of salts, as evidenced by many published papers (Harrington et al., 2009; Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Alonso, 2003; Montero & Pérez-Mateos, 2002; Pereira et al., 2013); nonetheless the impact on gluten-free flour gels has been studied to a lesser extent. The final mechanical and structural properties of these starchy materials are strongly dependent on processing temperature/time, cooling rates, salt content or pH conditions since they can affect the dynamic process of competition between phase separation and gel formation (Nunes, Raymundo, & Sousa, 2006a). Therefore, the main objective of the present work is to investigate the impact of cation concentration and ion valence of chloride salts (NaCl, KCl or $CaCl_2$) on the gel-forming kinetics, final mechanical properties and microstructure of selected gluten-free flour gels from blends of chestnut flour (Cf) and whole rice flour (Rw), Agulha rice flour (Ra) or Carolino rice flour (Rc). These gelling properties can be used to the development of alternative savoury gluten-free formulations with commercial applications. Texture properties of the final gels were obtained from the texture profile analysis. The rheological characteristics of these systems were evaluated using small amplitude oscillatory shear (SAOS) measurements, while the microstructure of the gels was analysed using confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

Two commercial gluten-free flours: chestnut flour (Cf) (Naiciña, Spain) and whole Agulha rice flour (Rw) (Próvida, Portugal) were analysed. Other two rice grain sources, Agulha rice and Carolino rice, were employed to obtain the corresponding flours, Ra and Rc, respectively, with comparative purposes. The two latter materials were kindly provided by the manufacturer (Novarroz, Portugal). Relevant physicochemical properties of these flours were reported in a previous work (Torres, Fradinho, et al., 2013). Namely, the moisture content values of studied flours were around $11.8 \pm 0.5\%$ dry basis (d.b.) and the average particle size around $93 \pm 2\ \mu m$. Sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$) were obtained from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO, USA). All chemicals used for the preparation of samples for confocal laser scanning microscopy (CLSM) assays were of analytical grade. Rhodamine B and fluorescein isothiocyanate (FITC) were purchased from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

2.2. Gel preparation

Gluten-free formulations developed in a previous work (Torres, Fradinho, et al., 2013) with blends of chestnut flour (Cf) and each of the three rice flours (Rw, Ra, Rc) at selected weight ratios (50/50) were used. Aqueous suspensions were prepared using demineralised water at levels that provides total flour content of 20 (%, w/w) for Rf samples and 30 (%, w/w) for CF samples. Formulations contained a mixture of both aqueous suspensions at 50/50 were subsequently prepared. Flours were dispersed together with different NaCl, KCl or $CaCl_2$ (0.5, 1.0, 1.5, 2.0% w/w) content by stirring at 800 rpm for 10 min on a magnetic hotplate stirrer (Arex, Velp Scientifica, Italy) at room temperature. Gels with and without salts were prepared following the protocol developed in a previous work (Torres, Fradinho, et al., 2013). Briefly, these suspensions were heated up to 90 °C and held at this temperature for 30 min. After heating, samples were placed in a fridge in order to allow full maturation of the gels, which were held at 5 °C for 24 h before performing texture and CLSM measurements. This time is enough to ensure the maturation of the gels structure without observing retrogradation phenomena (Torres, Fradinho, et al., 2013). Namely, in these gelled materials the content of water is very high and retrogradation, i.e. the realignment of amylose and amylopectin chains into more ordered structures can cause some syneresis, but this contraction of the gel matrix exudating the liquid phase was not noticed in these gelled systems, which means that retrogradation was not the dominant structural feature for us to consider. For rheological measurements, the aqueous suspensions were immediately loaded into the rheometer measuring system after 10 min stirring to promote gelatinization heating and cooling in situ, avoiding further perturbations of the gel matrix.

2.3. Colour properties

The colour measurements of all gels were carried out 24 h after samples preparation instrumentally using a Chroma Meter CR-400 (Minolta Co., Osaka, Japan) tristimulus colorimeter with standard illuminant D65 and a visual angle of 2°. The colour parameters (L^*, a^* and b^*) were assessed by CIELAB, where L^* defines the lightness and a^* (degree of redness or greenness) and b^* (degree of yellowness or blueness) are the chromaticity responsible parameters. At least fifteen measures were performed for each sample.

2.4. Texture analysis

Firmness measurements were made using a TA-XTplus (Stable Micro Systems, UK) texturometer with a load cell of 5 kg. Penetration tests were carried out in the gels contained in a cylindrical flask (2.5 cm diameter and 4.5 cm height) filled up to about 90% (i.e. 4 cm height of gel) using a cylindrical stainless steel probe of 11 mm diameter (5 mm of penetration and 2 mm/s of crosshead speed). All experiments were made 24 h after preparation of the gels in order to allow full maturation of the samples. Before performing texture measurements, gels were allowed to equilibrate

Table 1
The values of colour parameters (L^*, a^* and b^*) of gels prepared using blends (50/50) of chestnut flour (Cf) at 30% of total flour content and each of the three rice flours (Rw, Ra, Rc) at 20% of total flour content, w/w.[a]

Samples	Cf/Rw			Cf/Ra			Cf/Rc		
	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*
NaCl									
0	36.27 ± 0.12a	1.78 ± 0.02a	10.62 ± 0.08a	42.50 ± 0.18a	1.91 ± 0.01a	11.53 ± 0.11a	44.87 ± 0.10a	2.08 ± 0.02a	11.31 ± 0.15a
0.5	36.02 ± 0.05b	1.79 ± 0.01a	10.53 ± 0.09a	41.95 ± 0.06b	1.89 ± 0.02a	11.50 ± 0.05a	44.43 ± 0.09b	2.06 ± 0.03a	11.28 ± 0.05a
1.0	35.76 ± 0.09c	1.76 ± 0.02a	10.57 ± 0.07a	41.34 ± 0.04c	1.90 ± 0.03a	11.54 ± 0.03a	43.35 ± 0.08c	2.04 ± 0.02a	11.33 ± 0.07a
1.5	35.31 ± 0.12d	1.77 ± 0.02a	10.65 ± 0.08a	40.82 ± 0.03d	1.91 ± 0.02a	11.52 ± 0.10a	42.91 ± 0.06d	2.05 ± 0.01a	11.25 ± 0.05a
2.0	34.87 ± 0.13e	1.78 ± 0.01a	10.59 ± 0.08a	40.30 ± 0.04e	1.88 ± 0.03a	11.53 ± 0.04a	42.13 ± 0.07e	2.08 ± 0.01a	11.26 ± 0.06a
KCl									
0.5	36.00 ± 0.04b	1.78 ± 0.02a	10.63 ± 0.05a	41.92 ± 0.05b	1.92 ± 0.03a	11.50 ± 0.06a	44.40 ± 0.06b	2.07 ± 0.03a	11.30 ± 0.07a
1.0	35.73 ± 0.06c	1.77 ± 0.03a	10.58 ± 0.06a	41.30 ± 0.05c	1.90 ± 0.02a	11.43 ± 0.12a	43.33 ± 0.09c	2.09 ± 0.03a	11.28 ± 0.06a
1.5	35.29 ± 0.10d	1.77 ± 0.02a	10.62 ± 0.06a	40.78 ± 0.08d	1.91 ± 0.02a	11.52 ± 0.10a	42.90 ± 0.04d	2.06 ± 0.02a	11.27 ± 0.07a
2.0	34.54 ± 0.12e	1.76 ± 0.03a	10.60 ± 0.04a	40.24 ± 0.08e	1.90 ± 0.01a	11.50 ± 0.09a	42.10 ± 0.04e	2.08 ± 0.03a	11.31 ± 0.04a
$CaCl_2$									
0.5	35.70 ± 0.08c	1.79 ± 0.02a	10.60 ± 0.09a	41.28 ± 0.05c	1.90 ± 0.02a	11.49 ± 0.08a	43.29 ± 0.09c	2.06 ± 0.02a	11.28 ± 0.04a
1.0	33.28 ± 0.09f	1.76 ± 0.01a	10.58 ± 0.07a	39.70 ± 0.08f	1.91 ± 0.01a	11.48 ± 0.09a	41.50 ± 0.08f	2.04 ± 0.04a	11.25 ± 0.08a
1.5	31.52 ± 0.10g	1.78 ± 0.03a	10.57 ± 0.05a	37.49 ± 0.04g	1.92 ± 0.01a	11.51 ± 0.03a	39.71 ± 0.07g	2.08 ± 0.02a	11.30 ± 0.07a
2.0	30.42 ± 0.11h	1.78 ± 0.02a	10.61 ± 0.06a	36.58 ± 0.05h	1.93 ± 0.03a	11.50 ± 0.05a	38.53 ± 0.03h	2.08 ± 0.02a	11.33 ± 0.05a

Data are presented as mean ± standard deviation. Data values in a column with different superscript letters are significantly different at the $p \leq 0.05$ level.

at 20 °C for approximately 1 h in a temperature-controlled room. Each sample was measured at least in triplicate. Firmness (*N*) was defined as the maximum resistance to the penetration of the probe and was calculated as the height of the force peak during the first compression cycle (Bourne, 2002).

2.5. Rheological analysis

Small amplitude oscillatory shear measurements (SAOS) were performed on a RS-75 controlled stress rheometer (Haake, Germany). Serrated parallel plates (35 mm diameter and 0.500 mm gap) geometry was used to prevent wall slip effect as recommended by several authors (Barnes, Hutton, & Walters, 1989; Franco, Raymundo, Sousa, & Gallegos, 1998). Temperature was controlled by a DC5 circulating water bath (Haake, Germany) and measured with a thermocouple attached to the stationary element.

Before testing, aqueous suspensions were loaded carefully between the plates to ensure minimal structural damage, and held at rest for 10 min to allow temperature equilibration (20 °C). Paraffin oil was applied to the exposed edge of the samples to prevent evaporation of water during measurements.

At first, stress sweep tests were carried out both on suspensions and on gels in order to determine appropriate conditions for non-destructive testing. The stress range applied was of 0.1–1000 Pa at several frequencies (0.1, 1 and 100 Hz). Temperature, time and frequency sweep measurements were kept inside the viscoelastic region, in a stress range of 0.1–10 Pa for aqueous suspensions and 0.1–100 Pa for gels. All measurements were carried out at least in triplicate.

Thereafter, aqueous flour suspensions were heated up to 90 °C at 2 °C/min. After heating, time sweep tests were conducted at 90 °C for 60 min at 0.1 Hz. After thermal treatment, samples were cooled down to 5 °C at 1 °C/min. Both temperature sweep tests were performed at 0.1 Hz. A constant shear stress (2 Pa), within the linear viscoelastic region of the materials, was used in all the above tests. After cooling, time sweep tests were conducted at 5 °C, during a reasonable period of time (60 min) at 1 Hz. After this period, without disturbing the gel, frequency sweeps were performed at 20 °C, with oscillation frequencies over the range 0.01–100 Hz. A constant shear stress (30 Pa) within the linear viscoelastic region of the gels was used in the two latter tests. At least three replicates of each measurement were made and reproducible results were obtained.

2.6. Confocal laser scanning microscopy

Microstructure of the studied gels was determined from confocal laser scanning microscopy (CLSM) on a LSM-510 (Carl Zeiss, Germany), using 40×/UV/1.30 NA/oil objective lens. The light source was an argon laser. Following the procedure described by Nunes, Raymundo, and Sousa (2006b), fluorescent probe rhodamine B, added during the mechanical stirring of the mixtures (0.01 g rhodamine/500 g), was used for the non-covalent labelling of protein and starch. In contrast, salts were covalently labelled using fluorescein isothiocyanate (FITC) according to the method of Belder and Granath (1973) as described by Tromp, van de Veldea, van Riela, and Paquesa (2001). The excitation wave lengths were 488 and 568 nm, respectively, emission maxima were at 518 and 625 nm. A slice of gel was carefully cut from the flask to minimise structural damage, placed between a concave slide and a coverslip and sealed to prevent evaporation of water during tests.

2.7. Statistical analysis

The impact of NaCl, KCl and $CaCl_2$ addition on colour, texture and rheology of assayed gels was studied by means of one-factor analysis of variance (one-way ANOVA). In the cases where the analysis of variance indicated differences among means, post hoc analysis using a Scheffe test was performed to differentiate means with 95% confidence ($p < 0.05$). The statistical treatments were done using PASW Statistics (v.18, IBMSPSS Statistics, New York, USA).

3. Results and discussion

3.1. Colour properties

Table 1 shows the impact of NaCl, KCl and $CaCl_2$ on the colour parameters of selected gels prepared with Cf and Rw, Ra or Rc. Colour results pointed out that gels from tested mixtures became darker in the presence of the assayed salts. In this sense, the lightness (L^*) values drop statistically with increasing monovalent salts content, whereas the redness (a^*) and the yellowness (b^*) values remained practically constant. Similar behaviour was noticed for

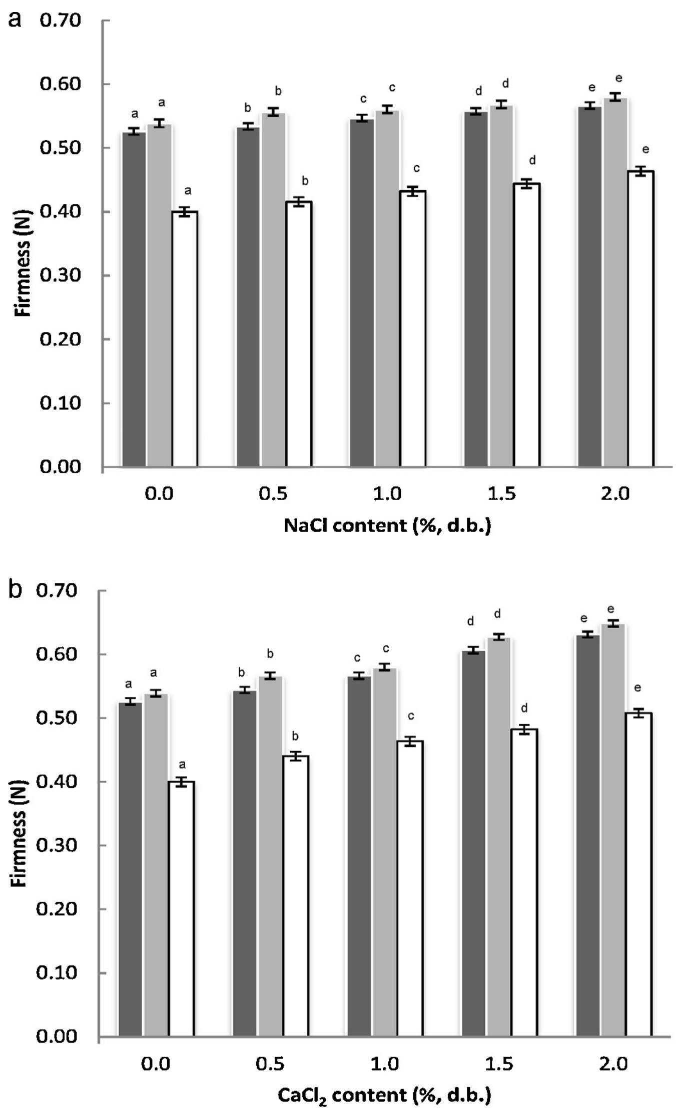

Fig. 1. Firmness values of gels prepared using mixtures (50/50) of chestnut flour (Cf) at 30% and each of the three rice flours (Rw, Ra, Rc) at 20% of total flour content in the presence of (a) KCl and (b) $CaCl_2$. Symbols: dark grey bars – Cf/Rw; light grey bars – Cf/Ra; white bars – Cf/Rc. Columns with different letters are significantly different at the $p \leq 0.05$ level. Data values for each flour were compared between data at different assayed concentrations.

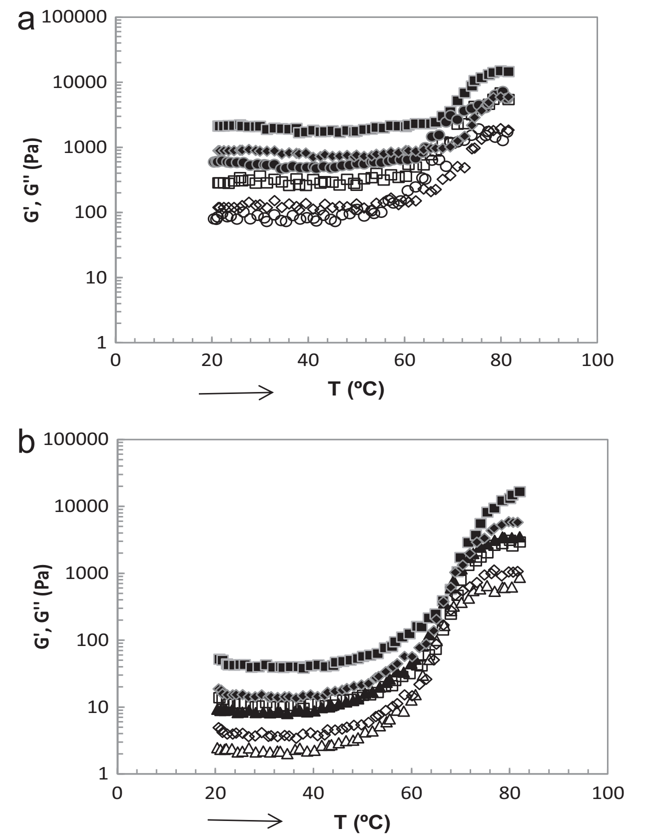

Fig. 2. Thermal profiles on heating (20–90 °C at 2 °C/min) of aqueous suspensions prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole (Rw) or (b) Carolino (Rc) rice flours at 20% of total flour content in the presence of KCl and $CaCl_2$ at 2.0% (w/w). Symbols: circles – Cf/Rw; triangles – Cf/Rc; diamonds – with KCl; squares – with $CaCl_2$; closed – storage modulus (G'); open – loss modulus (G'').

gels containing divalent salts, although these gels exhibited darker colour at equivalent salt content. This behaviour seems to be related to the high hydrophilic character of salts, which compete for available water with sugars and, therefore probably leading to a larger extension of caramelization and Maillard reaction. These results are in good agreement with the work reported by Moreira et al. (2011), who stated that the presence of salt helps to hold the colour properties and can modify the lightness of the final product.

It was noteworthy that Cf/Rc gels exhibited the least dark colour followed by Cf/Ra and Cf/Rw independently on the salt content, showing similar enhancement in the colour of the gels at the same salt addition. The obtained parameter values are consistent with those found for other chestnut (Torres, Fradinho, et al., 2013; Torres, Raymundo, et al., 2013) and rice (Song & Shin, 2007) flours.

3.2. Texture analysis

Fig. 1 displays the firmness values of selected gels prepared using Cf and Rw, Ra or Rc in the presence of (a) NaCl and (b) $CaCl_2$ at several concentrations. Texture results indicated that gels from tested mixtures containing chloride salts increased the firmness in a different way depending on the added salt. It was remarkable that the magnitude of this parameter rose linearly with increasing salt content. As comparing the effect of Na^+ and K^+ addition, it was noticed that K^+ was slightly more effective in modifying the firmness of tested gels (data not shown), which can be related to the higher size of the latter univalent cation (Thrimawithana et al., 2010). Latter authors indicated that ions with larger radius as K^+ have a more positive role in strengthening of the gel structure owing to the highest impact on the gel stabilization process. In the same line, the increase in the firmness values was significantly higher for gels containing $CaCl_2$, indicating the highest gel strength. The inspection of the experimental data showed that the firmness was related to the added type and concentration cation. Ambjerg-Pedersen and Jorgensen (1991) also found that the divalent cations promote intermolecular associations in starchy materials similar to studied flours. These results are consistent with those previously reported for other food gels (Pereira et al., 2013). These authors also found that $CaCl_2$ was the most effective salt for improving the gel strength, which was explained as a result of the establishment of cross-bridges between polymer networks forming the gel resulting from the presence of divalent ions Ca^{2+}.

Gels prepared using Cf/Rw and Cf/Ra, even in the presence of salts, exhibited close values of firmness. The lowest values of this parameter were achieved for gels from mixtures of Cf/Rc, even though its firmness is inside the range of variation of this characteristic for commercially available formulations (Batista, Nunes, & Sousa, 2002).

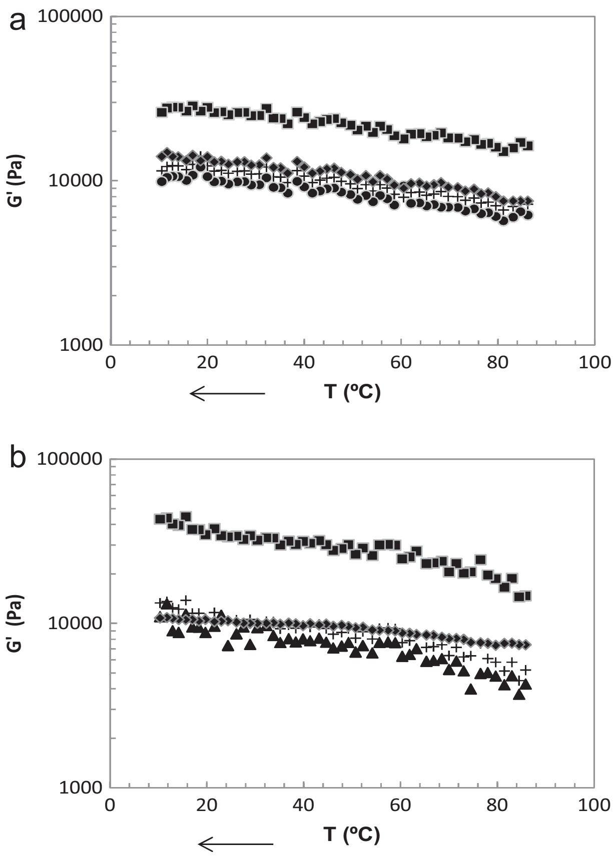

Fig. 3. Thermal profiles on cooling (90–5 °C at 1 °C/min) of gels from mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole (Rw) or (b) Carolino (Rc) rice flours at 20% of total flour content in the presence of NaCl, KCl and $CaCl_2$ at the highest assayed content. Symbols: circles – Cf/Rw; triangles – Cf/Rc; crosses – with NaCl; diamonds – with KCl; squares – with $CaCl_2$; closed – storage modulus (G').

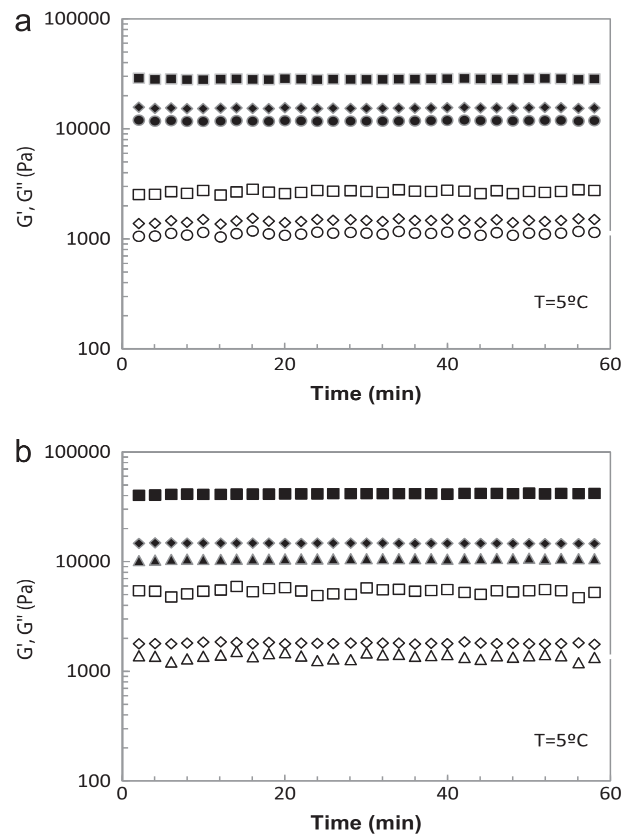

Fig. 4. Time sweeps (60 min at 5 °C) of gels prepared from (50/50) of chestnut flour (Cf) at 30% and (a) whole (Rw) or (b) Carolino (Rc) rice flours at 20% of total flour content in the presence of KCl and $CaCl_2$ at 2.0% (w/w). Symbols: circles – Cf/Rw; triangles – Cf/Rc; diamonds – with KCl; squares – with $CaCl_2$; closed – storage modulus (G'); open – loss modulus (G'').

3.3. Rheological analysis

Rheological results indicated that the thermal profiles on heating of Cf/Rw (Fig. 2a) gels were similar to those obtained for Cf/Ra (not shown), whereas Cf/Rc (Fig. 2b) gels exhibited a particular pattern. Latter sample showed the weakest gel properties, exhibiting lower values (about two decades) of G' and G'' below 70 °C. However, above this temperature a cross over point was observed, achieving similar values to Cf/Rw or Cf/Ra of both moduli at the highest temperatures. All gels exhibited G' larger than G'' (about one decade) in the whole temperature range. Both moduli remained practically constant during the initial heating. In the absence of salts, the first inflection point corresponding to the initial gelatinization temperature (T_0), was identified to be around 60.1 ± 1.2 °C, except for Cf/Rc where T_0 started earlier (45.5 ± 1.1 °C). Thereafter, G' and G'' began to increase rapidly with increasing temperature achieving a peak corresponding to the final gelatinization temperature (T_1) around 73.5 ± 1.4 °C for all samples. The tested salts delayed starch gelatinization of Cf/Rf gels by increasing the gelatinization temperature. The effect of chloride salts in delaying the starch gelatinization has been reported (Chiotelli et al., 2002; Preston, 1989) and different explanations for this phenomenon proposed. When salt is added to samples, it lowers water activity and increases the energy necessary for chemical and physical reactions involving water (Angioloni & Dalla Rosa, 2005).

The evolution of G' and G'' moduli with temperature for the above gels in the presence of KCl and $CaCl_2$ at 2.0% (w/w) is also presented in Fig. 2. Cf/Ra gels containing salts featured similar patterns to Cf/Rw (not shown). Storage and loss moduli were significantly enhanced in the presence of $CaCl_2$, whereas the same addition of monovalent salts involved a small increase in the values of both moduli. Particularly, gels containing NaCl exhibited the lowest enhancement of G' and G'' values (not shown), which is consistent with texture results. The salt effects are consistent with those previously reported (Moreira et al., 2011; Thrimawithana et al., 2010). These authors highlighted that salts interactions are greatly dependent on added type and content of the cation.

The thermal profiles on cooling of selected gels in the presence of NaCl, KCl and $CaCl_2$ are displayed in Fig. 3. During the cooling process down to 5 °C, G' increased progressively, achieving larger values (around two times higher) at the lowest temperatures, which indicated a moderate gel structure reinforcement. In the absence of salts, no significant differences were identified between assayed Cf/Rf mixtures. This pattern of structural development is consistent with the results previously featured for maize starch gels (Nunes et al., 2006b) and other starch pastes (Miles, Morris, Orford, & Ring, 1985). As it is well-known, both authors explained this behaviour by the combined effect of the gradual change of the swollen granules and the formation of an amylose gel network. The presence of KCl and $CaCl_2$ in gels from Cf/Rw (Fig. 3a) and Cf/Rc (Fig. 3b) showed a notably increase in the G' values in the whole temperature range, indicating a strength of the gels when salts were added. Again, gels containing NaCl exhibited the lowest enhancement of G' values (not shown). The highest enhancement in the magnitude of G' was observed for Cf/Rc gels containing $CaCl_2$ followed by Cf/Rw at the same conditions. Cf/Ra gels exhibited similar cooling curves to Cf/Rw samples (not shown).

The maturation kinetics of the studied gels (Fig. 4) were monitored through the evolution of the storage and loss moduli with time at 5 °C. In all cases, both moduli reached stability after 60 min of maturation, with steady values for G' around $(1.2 \pm 0.1) \times 10^4$ Pa and for G'' around $(1.1 \pm 0.1) \times 10^3$ Pa. Again, no statistical differences were observed between tested Cf/Rf gels in the absence of salts. Nevertheless, the presence of $CaCl_2$ in Cf/Rw (Fig. 4a) and Cf/Rc (Fig. 4b) gels increased notably the magnitude of both moduli in the whole temperature range. Specifically, this rise was around 3 times higher for Cf/Rw gels and about 4 times higher for Cf/Rc gels at the highest assayed $CaCl_2$ content. The addition of the monovalent salts showed similar trends, although with a lower (around 1.5 times with KCl and 1.2 with NaCl) enhancement in both moduli at the same salt content. The maturation curves for Cf/Ra gels were similar to those obtained for Cf/Rw samples (not shown). All these gels exhibited typical cooling and maturation curves of biopolymer gelation (Nunes, Raymundo, & Sousa, 2004). It is remarkable that in all cases a stable and fully developed structure was reached within the time of the experiment. The short time necessary to achieve stable gel structure represents an important advantage from an industrial point of view.

Representative mechanical spectra of these gels, obtained at the end of the run at 5 °C, are presented in Fig. 5. All gels showed a typical strong gel spectrum, i.e. G' was always larger than G'', at about one decade, and both moduli were almost frequency independent. This behaviour is in good agreement with the solid one previously reported for chestnut flour gels (Moreira, Chenlo, Torres, & Glazer, 2012; Torres, Fradinho, et al., 2013; Torres, Raymundo, et al., 2013) and rice flour pastes (Marco & Rosell, 2008). In the absence of salts, the studied gels exhibited qualitatively similar linear viscoelastic data, without notable variations in the values of both moduli throughout assayed angular frequency range. The presence of $CaCl_2$ rose significantly the values of G' and G'' moduli, whereas the same addition of NaCl or KCl implied a slight increase in the values of both moduli. Namely, the addition of NaCl involved the lowest increase in the magnitude of G' and G'' moduli. The largest increase in both moduli was achieved for Cf/Rc gels in the presence of $CaCl_2$ at 2%. It was noteworthy that G' and G'' increased almost linearly with increasing salt content independently on the

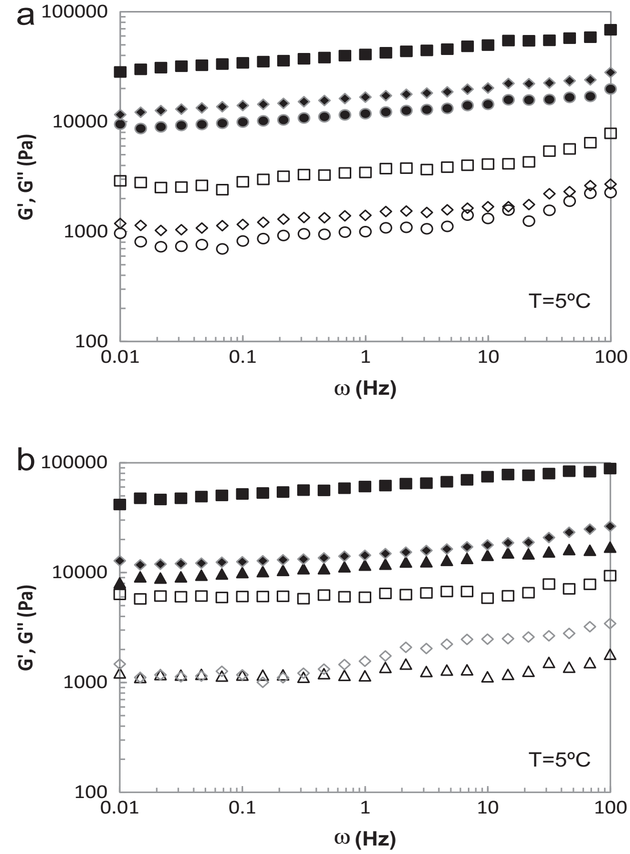

Fig. 5. Frequency sweeps (0.01–100 Hz at 20 °C) of gels from mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole (Rw) or (b) Carolino (Rc) rice flour at 20% of total flour content in the presence of KCl and $CaCl_2$ at 2.0% (w/w). Symbols: circles – Cf/Rw; triangles – Cf/Rc; diamonds – with KCl; squares – with $CaCl_2$; closed – storage modulus (G'); open – loss modulus (G'').

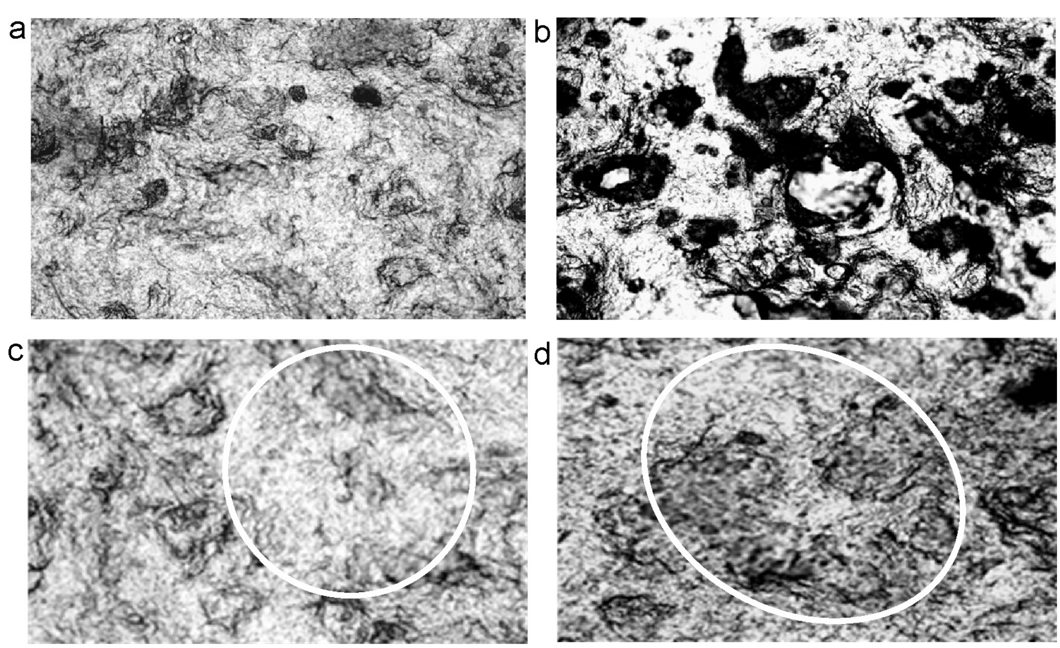

Fig. 6. CLSM images of gels prepared using mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole rice flour (Rw) or (b) Carolino rice flour (Rc) at 20% of total flour content in the presence of (c and d) $CaCl_2$ at 2.0% (w/w) at 20 °C.

ion valence of chloride salts. All gels exhibited stable and strong gel behaviour, being the least stable gels those prepared using Cf/Rc in the presence of monovalent salts. Even so, the obtained values are within those found for commercial formulations based on gelled systems (Batista et al., 2002). The results obtained for Cf/Rw, Cf/Ra and Cf/Rc gels in the presence of chloride salts from rheological testing at small amplitude oscillatory shear supported the information reached by texture measurements.

3.4. Confocal laser scanning microscopy

The impact of NaCl, KCl and $CaCl_2$ on the structure of assayed gels was investigated through confocal laser scanning microscopy at room temperature. Strong and stable starch/protein (grey/black areas) network for Cf/Rw (Fig. 6a) and Cf/Rc (Fig. 6b) gels in the absence of salts were clearly observed. Analysing CLSM images, it can be noticed that phase separation between starch and protein aggregates takes place. The presence of chloride salts enhanced the starch gel network. Particularly, CLSM images showed that starch aggregates in Cf/Rw (Fig. 6c) and Cf/Rc (Fig. 6d) gels are promoted in the presence of $CaCl_2$, leading to a more continuous and aggregated structure which was highlighted with white circles. The competition between the added salt and the starch by binding the water reduces the available water in the system and therefore promotes the concentration of starch in the solution, leading to a more strong gel structure. The largest enhancement was identified for Cf/Rc gels. Once more, Cf/Ra gels exhibited similar patterns to Cf/Rw. Gels containing monovalent salts showed similar behaviour to $CaCl_2$, but with less aggregated structures. This behaviour suggests cross-bridges between starch networks forming the gel resulting from the presence of Ca^{2+}. CLSM images gave evidence that the highest added salt promotes the highest enhancement of starch networking. These results are in good agreement with those previously reported by Maache-Rezzoug, Bouvier, Allaf, and Patras (1998), who highlighted that salts improve the mechanical properties of gels, increasing the starch chain association and giving a continuous mass. CLSM images of gels revealed a microstructure consistent with the observed mechanical properties, where the chain associations were mainly strengthened with the addition of $CaCl_2$, leading to a more aggregated structure.

4. Conclusions

The presence of chloride salts improved the mechanical properties of gluten-free gels and thereby the samples structure was reinforced. Gluten-free flour gelled formulations from blends of chestnut flour (Cf) and whole rice flour (Rw) or Agulha rice flour (Ra) depicted similar patterns, whereas those prepared with Carolino rice flour (Rc), exhibited particular properties. Firmness measurements indicated that formulations from blends (50/50) of chestnut flour at 30% and rice flours at 20% containing assayed salts can be an interesting alternative to obtain savoury gluten-free gelled products. Even though all samples exhibited stable and strong gel properties, rheological measurements revealed that the strength of the gels was enhanced in the presence of $CaCl_2$ followed by monovalent salts in the subsequent order, KCl and NaCl. The size of the ion impacted on the stabilization process, since ions with larger radius as K^+ have a more positive role in strengthening the gel structure. Microscopic analysis showed more aggregated structures of the studied gels in the presence of $CaCl_2$. These measurements allowed concluding that gels from Cf/Rc in the presence of $CaCl_2$ present an industrial advantage over Cf/Rw and Cf/Ra gels, since the first samples gave the strongest gels. $CaCl_2$ can be used as an adjuvant during the gelation process. These blends can be interesting gelled formulations for the gluten-free products market and can provide additional economic value to wastes or by-products as a strategy to add value to the chestnut and rice processing industry. These industries are relevant in countries like Portugal and Spain.

Acknowledgements

The authors wish to express their gratitude to the Research Group of Technologies for the Development of Industrial Bioproducts at the Universidade de Santiago de Compostela (Spain) for the image analysis. We also wish to thank to Novarroz (Portugal), who kindly provided rice materials.

References

AACC. (2000). *Approved methods of the American association of cereal chemistry* (10th ed.). Minnesota: American Association of Cereal Chemists.
Ambjerg-Pedersen, H. C., & Jorgensen, B. B. (1991). Influence of pectin on the stability of casein solution studied in dependence of varying pH and salt concentration. *Food Hydrocolloids*, *5*(4), 323–328.
Angioloni, A., & Dalla Rosa, M. (2005). Dough thermo-mechanical properties: Influence of sodium chloride, mixing time and equipment. *Journal of Cereal Science*, *41*, 327–331.
Arda, E., Kara, S., & Pekcan, Ö. (2009). Synergistic effect of the locust bean gum on the thermal phase transitions of k-carrageenan gels. *Food Hydrocolloids*, *23*, 451–459.
Barnes, H. A., Hutton, J. F., & Walters, K. (1989). *An introduction to rheology*. Netherlands: Elsevier Science Publishers BV.
Batista, P., Nunes, M. C., & Sousa, I. (2002). Physical characterisation of commercial dairy desserts. In F. Martinez Boza, A. Guerrero, P. Partal, J. Franco, & Munoz (Eds.), *Progress in rheology: Theory and applications*. Spain: Publicaciones Digitales.
Belder, A. N., & Granath, K. (1973). Preparation and properties of fluorescein labeled dextrans. *Carbohydrate Research*, *30*, 375–378.
Bourne, M. (2002). *Food texture and viscosity: Concept and measurement*. London, UK: Academic Press.
Butow, B. J., Gras, P. W., Haraszi, R., & Bekes, F. (2002). Effects of different salts on mixing and extension parameters on a diverse group of wheat cultivars using 2-g mixograph and extensigraph methods. *Cereal Chemistry*, *79*, 826–833.
Chiotelli, E., Pilosio, G., & Le Meste, M. (2002). Effect of sodium chloride on the gelatinization of starch: A multimeasurement study. *Biopolymers*, *63*, 41–58.
Demirkesen, I., Mert, B., Sumnu, G., & Sahin, S. (2010). Utilization of chestnut flour in gluten-free bread formulations. *Journal of Food Engineering*, *101*, 329–336.
Dickinson, E. (2006). Structure formation in casein-based gels, foams, and emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *288*, 3–11.
Dickinson, E. (2010). Flocculation of protein-stabilized oil-in-water emulsions. *Colloids and Surfaces B: Biointerfaces*, *81*, 130e140.
Franco, J., Raymundo, A., Sousa, I., & Gallegos, C. (1998). Influence of processing variables on the rheological and textural properties of lupin protein-stabilized emulsions. *Journal of Agricultural and Food Chemistry*, *46*, 3109–3115.
Harrington, J. C., Foegeding, E. A., Mulvihill, D. M., & Morris, E. R. (2009). Segregative interactions and competitive binding of Ca^{2+} in gelling mixtures of whey protein isolate with Na^+ k-carrageenan. *Food Hydrocolloids*, *23*, 468–489.
Lin, H. Y., & Lai, L. S. (2009). Isolation and viscometric characterization of hydrocolloids from mulberry (*Morus alba* L.) leaves. *Food Hydrocolloids*, *23*, 840–848.
Maache-Rezzoug, Z., Bouvier, J. M., Allaf, K., & Patras, C. (1998). Effect of principal ingredients on rheological behaviour of biscuit dough and on quality of biscuits. *Journal of Food Engineering*, *35*, 23–42.
Marco, C., & Rosell, C. M. (2008). Breadmaking performance of protein enriched, gluten-free breads. *European Food Research and Technology*, *227*, 1205–1213.
Mariotti, M., Lucisano, M., Pagani, M. A., & Ng, P. K. W. (2009). The role of corn starch, amaranth flour, pea isolate, and Psyllium flour on the rheological properties and the ultrastructure of gluten-free doughs. *Food Research International*, *42*, 963–975.
McClements, D. J. (1999). *Food emulsions: Principles, practice and techniques*. New York: CRC Press.
Medina-Torres, L., Brito-De La Fuente, E., Torrestiana-Sanchez, B., & Alonso, S. (2003). Mechanical properties of gels formed by mixtures of mucilage gum (Opuntia ficus indica) and carrageenans. *Carbohydrate Polymers*, *52*, 143–150.
Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. C. (1985). The role of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135*, 271–281.
Montero, P., & Pérez-Mateos, M. (2002). Effect of Na^+, K^+ and Ca^{2+} on gels formed from fish mince contained a carrageenan or alginate. *Food Hydrocolloids*, *16*, 375–385.
Moreira, R., Chenlo, F., & Torres, M. D. (2011). Effect of sodium chloride, sucrose and chestnut starch on rheological properties of chestnut flour doughs. *Food Hydrocolloids*, *25*, 1041–1050.
Moreira, R., Chenlo, F., & Torres, M. D. (2012). Rheology of gluten-free doughs from blends of chestnut and rice flours. *Food and Bioprocess Technology*, http://dx.doi.org/10.1007/s11947-012-0927-1

Moreira, R., Chenlo, F., Torres, M. D., & Glazer, J. (2012). Rheological properties of gelatinized chestnut starch dispersions: Effect of concentration and temperature. *Journal of Food Engineering*, *112*, 94–99.

Moritaka, H., Fukuba, H., Kumeno, K., & Nakahama, N. (1991). Effect of monovalent and divalent cations on the rheological properties of gellan gels. *Food Hydrocolloids*, *4*, 495–507.

Nunes, M. C., Raymundo, A., & Sousa, I. (2004). Effect of thermal treatment and composition on the mechanical properties of pea/kappa-carrageenan/starch desserts. In P. A. Williams, & G. O. Phillips (Eds.), *Gums and stabilisers for the food industry* (Vol. 12) (pp. 54–64). London, UK: Royal Society of Chemistry.

Nunes, M. C., Raymundo, A., & Sousa, I. (2006a). Gelled vegetable desserts containing pea protein, κ-carrageenan and starch. *European Food Research and Technology*, *222*, 622–628.

Nunes, M. C., Raymundo, A., & Sousa, I. (2006b). Rheological behaviour and microstructure of pea protein/k-carrageenan/starch gels with different setting conditions. *Food Hydrocolloids*, *20*, 106–113.

Nunez-Santiago, M. D. C., & Tecante, A. (2007). Rheological and calorimetric study of the sol–gel transition of k-carrageenan. *Carbohydrate Polymers*, *69*, 763–773.

Pereira, P. A. P., Souza, V. R., Teixeira, T. R., Queiroz, F., Borges, S. V., & Carneiro, J. D. S. (2013). Rheological behavior of functional sugar-free guava preserves: Effect of the addition of salts. *Food Hydrocoloids*, 404–412.

Preston, K. R. (1989). Effects of neutral salts of the lyotropic series on the physical dough properties of a Canadian red spring wheat flour. *Cereal Chemistry*, *66*, 144–148.

Sacchetti, G., Pinnavaia, G. G., Guidolin, E., & Dalla Rosa, M. (2004). Effects of extrusion temperature and feed composition on the functional, physical and sensory properties of chestnut and rice flour based snack like products. *Food Research International*, *37*, 527–534.

Song, J. Y., & Shin, M. (2007). Effects of soacking and particle sizes on the properties of rice flour and gluten-free rice bread. *Food Science and Biotechnology*, *16*, 759–764.

Thrimawithana, T. R., Young, S., Dunstan, D. E., & Alanya, R. G. (2010). Texture and rheological characterization of kappa and iota carrageenan in the presence of counter ions. *Carbohydrate Polymers*, *82*, 69–77.

Torres, M. D., Fradinho, P., Raymundo, A., & Sousa, I. (2013). Thermorheological and textural behaviour of gluten-free gels obtained from chestnut and rice flours. *Food and Bioprocess Technology* (in press)

Torres, M. D., Raymundo, A., & Sousa, I. (2013). Effect of sucrose, stevia and xylitol on rheological properties of gels from blends of chestnut and rice flours. *Carbohydrate Polymers* (in press)

Tromp, R. H., van de Veldea, F., van Riela, J., & Paquesa, M. (2001). Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. *Food Research International*, *34*, 931–938.