Carbohydrate Polymers 98 (2013) 249–256

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Effect of sucrose, stevia and xylitol on rheological properties of gels from blends of 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

ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 10 June 2013
Accepted 15 June 2013
Available online 20 June 2013

Keywords:
Sweeteners
Gluten-free
Gels
Rheology
Texture
Microscopy

ABSTRACT

The development of high quality gluten-free products requires the understanding of the phenomena that dictate the ingredient interactions commonly used in foodstuff. In this work, the main objective was to develop alternative gluten-free gelled desserts from blends of chestnut flour (Cf) and whole (Rw), Agulha (Ra) or Carolino (Rc) rice flours. The impact of sucrose, stevia and xylitol on textural, rheological and structural properties of selected gels was investigated. Texture results indicated that studied gels in the presence of sucrose and xylitol decreased significantly the firmness. Rheological outcomes showed that the temperature ramps on heating of Cf/Rw gels were similar to those obtained for Cf/Ra, whereas Cf/Rc gels presented a particular pattern. The presence of sucrose resulted in a significant decrease in the values of storage and loss moduli. Confocal microscopic images showed that the sugar addition leads to a less aggregated structure with fracture lines well marked.

1. Introduction

In the last decades, gluten-free diets are gathering new followers and subsequent gel research focuses more strongly on new mixtures as a result of industrial demands for new products with higher quality (Moreira, Chenlo, Torres, & Glazer, 2012; Moroni, Bello, & Arendt, 2009). In the most of the available products on the market, low technological and nutritional quality is observed (Mariotti, Lucisano, Pagani, & Ng, 2009). A few studies indicated that the blending of chestnut flour (Cf) with rice flours (Rf) can be an interesting approach to produce gluten-free products (Demirkesen, Mert, Sumnu, & Sahin, 2010; Sacchetti, Pinnavaia, Guidolin, & Dalla Rosa, 2004). In a recent work (Torres, Fradinho, Raymundo, & Sousa, 2013), it was demonstrated that the gelation ability of a mixture of Cf and different types of Rf could be used as an interesting alternative to commercial gluten-free desserts, with subsequent multiple advantages from the economic and health hazard points of view. The development of these products requires the understanding of the phenomena that dictate the ingredient interactions commonly used in food formulations. Sugars are usually used in food gels as sweetening agents and affect notably the gel structure and texture (Bayarri, Durán, & Costell, 2004). Sucrose is probably one of the most studied sweeteners, since it plays an important role as an ingredient or preserving agent in many formulations and technological processes (Moreira, Chenlo, & Torres, 2011). Usually sugar provides good structures, flavour and texture to the product, contributes to moisture retention and prolongs freshness (Nip, 2007).

In some diets the energetic value of sucrose is considered an undesirable feature, leading to growing interest in low-calorie alternatives to sucrose (Bayarri, Izquierdo, & Costell, 2007). Attempts to provide sweetness for the diet have included several synthetic sweeteners which are often regarded either as having an undesirable aftertaste or as being linked to health concerns (Holm, Wendin, & Hermansson, 2009). Recent publications (Guggisberg, Piccinali, & Schreier, 2011; Basu, Shivhare, & Singh, 2013) have shown a strong increase in the attention to natural sweeteners like stevia and xylitol for its sweetening qualities. Stevia or steviol glycoside, which is up to 200–300 times sweeter than sucrose, can be isolated from dried leaves of shrub *Stevia rebaudiana* (Guggisberg et al., 2011). Medical research has shown benefits of stevia in treating obesity and high blood pressure. It is also considered as an effective sugar alternative for those on low-carbohydrate diets such as diabetics (Manisha, Soumya, & Indrani, 2012). Currently, some attempts for manufacturing gelled systems with stevia such as mango jams (Basu et al., 2013) or yoghurts (Guggisberg et al., 2011) were made with promising results. Xylitol is a polyol, with

* Corresponding author. Tel.: +34 881816752; fax: +34 981 528 050.
E-mail addresses: mariadolores.torres.perez@usc.es, lolytorresp@hotmail.com (M.D. Torres), araymundo@almada.ipiaget.org (A. Raymundo), isabelsousa@isa.utl.pt (I. Sousa).

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http://dx.doi.org/10.1016/j.carbpol.2013.06.018

a sweetening power similar to sucrose, found in fruits and vegetables (Prakash, Varma, Prabhune, Shouche, & Rao, 2011). The use of xylitol-containing products has resulted in defined reduction in teeth caries and represents interesting alternatives for high-caries-risk populations (Kandelman, 1997). Therefore, xylitol has important applications in pharmaceuticals and food industries (Sokmen & Gunes, 2006). Nevertheless, no information is at present available on the effect of xylitol on the viscoelastic properties of flour gelled systems.

Substitution of sucrose by other sweeteners can affect the system's viscoelasticity (Bayarri et al., 2004). When the sugar content of a product is changed, not only sweetness, but also food rheology and texture may be affected (Holm et al., 2009). From previous studies, it was obvious that sugars significantly influence the thermal properties of starchy materials, the effect depending on the sugar used, starch origin, and storage conditions (Tomasik, Wang, & Jane, 1995). In this sense, it was reported that addition of sucrose delayed the formation of gel networks of starch pastes from three waxy maize genotypes (Yuan & Thompson, 1998). Similar results were found for chestnut flour doughs in the presence of sucrose (Moreira et al., 2011). The presence of sugars can also modify the thermal stability of proteins (Fu & Rao, 2001). These authors explained the important role of the hydroxyl groups of sucrose in pectin gel formation and that sucrose may stabilise the structure or junction zones. Contrarily, other authors found that the addition of sugars can promote the protein weakening, indicating softening of the final products (Melander & Horváth, 1977). Many food products have optimal ranges of rheology and texture, and it is important to stay within these limits even when the sugar conditions are changed.

The development of alternative gluten-free desserts based on gelled systems from flours involves the understanding of how the complex mixture of flour and polysaccharides behave under food processing conditions. The final structure and textural properties of these systems are strongly dependent on processing temperature/time, cooling rates, polysaccharide content or pH conditions since they can affect the dynamic process of competition between phase separation and gel formation (Tolstoguzov, 1998; Nunes, Raymundo, & Sousa, 2006a). Hence, the objectives of the present work are to develop alternative gluten-free formulations from selected blends of chestnut flour (Cf) and whole rice flour (Rw), Agulha rice flour (Ra) or Carolino rice flour (Rc) and to investigate the effect of sucrose, stevia and xylitol on the gel-forming kinetics, final mechanical properties and microstructure of these systems using different experimental techniques.

2. Materials and methods

2.1. Raw materials

Two commercial gluten-free flours: chestnut flour (Cf) (Naiciña, Spain) and whole rice flour (Rw) (Próvida, Portugal) were studied. Other two rice grain sources, Agulha rice and Carolino rice, were used to obtain the corresponding flours, Ra and Rc, respectively, with comparative purposes. These materials were kindly supplied by the manufacturer (Novarroz, Portugal). Sucrose (table sugar), stevia (Raab Vitalfood GmbH, Germany) and xylitol (Raab Vitalfood GmbH, Germany) were employed. Xylitol powder used in this work has the same sweetness power as sucrose, whereas stevia powder is 200 times sweeter. All chemicals used for the preparation of samples for confocal laser scanning microscopy (CLSM) tests were of analytical grade. Rhodamine B and fluorescein isothiocyanate (FITC) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

Gels were prepared using chestnut flour (Cf) and each of the three rice flours (Rw, Rc, Ra) at several ratios (%) (Cf/Rf: 0/100, 20/80, 40/60, 50/50, 60/40, 80/20, 100/0). The formulations contained demineralised water at levels that provides total flour content of 20 and 30% (w/w). Flours were dispersed together with different sucrose content (5, 10, 15, 20, 25%, w/w), stevia or xylitol by stirring at 800 rpm for 10 min on a magnetic hotplate stirrer (Arex, Velp Scientifica, Italy) at room temperature. Equivalent sweetness of stevia and xylitol relative to tested sucrose concentrations were employed. Gels without and with sugars were prepared following the protocol developed in a previous work (Torres et al., 2013). Briefly, these suspensions were heated up to 90 °C and kept at this temperature for 30 min. Thereafter, samples were placed in a fridge in order to allow full maturation of the gels, which were kept at 5 °C for 24 h before performing texture and CLSM measurements. This time is enough to ensure the maturation of the gels structure. For rheological analysis, the aqueous suspensions were immediately loaded into the rheometer measuring system after 10 min stirring to promote gelatinisation heating and cooling in situ, avoiding further perturbations of the gel matrix.

2.3. Colour characterisation

The colour of all gels was measured instrumentally in a Chroma Meter CR-400 (Minolta Co., Osaka, Japan) tristimulus colourimeter with standard illuminant D65 and a visual angle of 2°. The colour parameters (L^*, a^* and b^*) were evaluated 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 ten measures were performed for each sample. Original colour parameters L_0^*, a_0^*, b_0^* were employed to calculate the total colour difference (ΔE^*) using the following expression,

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_2^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

2.4. Texture characterisation

Firmness was determined from the texture profile analysis (TPA) using a TA-XTplus (Stable Micro Systems, UK) texturometer with a load cell of 5 kg, being the force resolution around of 0.1 g. Penetration measurements 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). The experiments were performed 24 h after preparation of the gels in order to allow full maturation of the samples. Gels were allowed to equilibrate at 20 °C for approximately 1 h in a temperature-controlled room before performing any measurements. Each formulation was measured at least in triplicate. Firmness (N) was considered 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 characterisation

Small amplitude oscillatory shear measurements were carried out on a RS-75 controlled stress rheometer (Haake, Germany) with temperature controlled by DC5 circulating water bath (Haake, Germany) and measured with a thermocouple attached to the stationary element. 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). Aqueous suspensions

Table 1
L^*, a^* and b^* colour values of gels prepared using mixtures (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^*
Sucrose									
0	36.27 ± 0.12^{f}	1.78 ± 0.02^{a}	10.62 ± 0.08^{a}	42.50 ± 0.18^{f}	1.91 ± 0.01^{a}	11.53 ± 0.11^{a}	44.87 ± 0.10^{f}	2.08 ± 0.02^{a}	11.31 ± 0.15^{a}
5	38.55 ± 0.11^{e}	1.69 ± 0.01^{b}	9.86 ± 0.10^{b}	43.65 ± 0.11^{e}	1.85 ± 0.02^{b}	10.94 ± 0.04^{b}	45.33 ± 0.08^{e}	2.01 ± 0.01^{b}	10.96 ± 0.03^{b}
10	40.87 ± 0.09^{d}	1.56 ± 0.03^{c}	9.51 ± 0.03^{c}	44.84 ± 0.08^{d}	1.73 ± 0.02^{c}	10.68 ± 0.03^{c}	46.32 ± 0.10^{d}	1.93 ± 0.03^{c}	10.71 ± 0.06^{c}
15	42.41 ± 0.12^{c}	1.48 ± 0.01^{d}	9.23 ± 0.05^{d}	45.52 ± 0.05^{c}	1.66 ± 0.01^{d}	10.29 ± 0.06^{d}	47.61 ± 0.12^{c}	1.86 ± 0.02^{d}	10.33 ± 0.08^{d}
20	43.16 ± 0.04^{b}	1.41 ± 0.01^{e}	8.94 ± 0.03^{e}	46.16 ± 0.04^{b}	1.58 ± 0.01^{e}	9.95 ± 0.05^{e}	48.74 ± 0.05^{b}	1.76 ± 0.01^{e}	9.99 ± 0.06^{e}
25	45.98 ± 0.04^{a}	1.36 ± 0.02^{f}	8.73 ± 0.04^{f}	47.50 ± 0.05^{a}	1.52 ± 0.01^{f}	9.69 ± 0.06^{f}	50.22 ± 0.11^{a}	1.68 ± 0.03^{f}	9.74 ± 0.05^{f}
Stevia									
0.025	36.32 ± 0.10^{f}	1.79 ± 0.01^{a}	10.58 ± 0.12^{a}	42.33 ± 0.23^{f}	1.90 ± 0.01^{a}	11.30 ± 0.08^{a}	44.89 ± 0.10^{f}	2.08 ± 0.02^{a}	11.35 ± 0.09^{a}
0.050	36.73 ± 0.31^{f}	1.78 ± 0.01^{a}	10.38 ± 0.21^{a}	42.69 ± 0.31^{f}	1.89 ± 0.02^{a}	11.26 ± 0.12^{a}	44.97 ± 0.08^{f}	2.07 ± 0.02^{a}	11.28 ± 0.11^{a}
0.075	37.03 ± 0.41^{f}	1.76 ± 0.02^{a}	10.22 ± 0.12^{a}	42.83 ± 0.14^{f}	1.89 ± 0.01^{a}	11.10 ± 0.09^{a}	44.99 ± 0.04^{f}	2.05 ± 0.01^{a}	11.12 ± 0.07^{a}
0.100	37.79 ± 0.32^{e,f}	1.73 ± 0.03^{a,b}	10.06 ± 0.23^{a,b}	43.09 ± 0.21^{e,f}	1.88 ± 0.03^{a,b}	11.02 ± 0.14^{a,b}	45.09 ± 0.05^{e,f}	2.01 ± 0.04^{a,b}	11.06 ± 0.13^{a,b}
0.125	38.24 ± 0.22^{e}	1.70 ± 0.02^{b}	9.96 ± 0.05^{b}	43.44 ± 0.11^{e}	1.85 ± 0.01^{b}	10.87 ± 0.08^{b}	45.13 ± 0.03^{e}	2.00 ± 0.02^{b}	10.99 ± 0.06^{b}
Xylitol									
5	38.33 ± 0.11^{e}	1.70 ± 0.02^{b}	9.84 ± 0.04^{b}	43.68 ± 0.05^{e}	1.86 ± 0.01^{b}	10.90 ± 0.03^{b}	45.54 ± 0.09^{e}	2.00 ± 0.01^{b}	10.94 ± 0.02^{b}
10	40.57 ± 0.18^{d}	1.66 ± 0.03^{b}	9.58 ± 0.05^{c}	44.80 ± 0.09^{d}	1.77 ± 0.02^{c}	10.78 ± 0.07^{b,c}	46.02 ± 0.10^{d}	1.95 ± 0.01^{c}	10.83 ± 0.08^{b,c}
15	40.95 ± 0.14^{d}	1.58 ± 0.02^{c}	9.55 ± 0.11^{c}	44.99 ± 0.04^{d}	1.76 ± 0.02^{c}	10.73 ± 0.03^{c}	46.41 ± 0.10^{d}	1.93 ± 0.02^{c}	10.75 ± 0.04^{c}
20	42.21 ± 0.22^{c}	1.48 ± 0.01^{d}	9.25 ± 0.05^{d}	45.31 ± 0.12^{c}	1.75 ± 0.05^{c,d}	10.38 ± 0.09^{d}	47.53 ± 0.12^{c}	1.86 ± 0.02^{d}	10.42 ± 0.08^{d}
25	42.63 ± 0.21^{c}	1.47 ± 0.02^{d}	9.18 ± 0.03^{d}	45.58 ± 0.06^{c}	1.68 ± 0.02^{d}	10.27 ± 0.07^{d}	47.72 ± 0.13^{c}	1.85 ± 0.03^{d}	10.29 ± 0.06^{d}

[a] Data are presented as mean ± standard deviation. Data values in a column with different superscript letters are significantly different at the $p \le 0.05$ level. Equivalent sweetness of stevia and xylitol relative to tested sucrose concentrations were employed.

were loaded carefully between the plates to ensure minimal structural damage, and held at rest for 10 min before testing to allow temperature equilibration (20 °C). The exposed edges of the sample were covered with paraffin oil to prevent evaporation of water during measurements.

Firstly, samples were tested over a range of stresses to determine appropriate conditions for non-destructive testing. Stress sweep tests were performed both on suspensions and on gels. The stress range applied was of 0.1–1000 Pa at several frequencies (0.1, 1 and 100 Hz). Temperature, time and frequency sweep tests 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. At least two replicates of each measurement were made.

Subsequently, aqueous 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 in order to ensure the complete gelatinisation of the samples. After thermal treatment, samples were cooled down to 5 °C at 1 °C/min. All 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 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 carried out 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. All measurements were made at least in triplicate and reproducible results were achieved.

2.6. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was performed on a LSM-510 (Carl Zeiss, Germany), using 40×/UV/1.30 NA/oil objective lens. The light source was an argon laser. According to 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. Contrary, sugars 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 cut with care from the flask to minimise structural damage, placed between a concave slide and a coverslip and then sealed to prevent evaporation.

2.7. Statistical analysis

The influence of sucrose, stevia and xylitol content on textural and rheological properties was evaluated by means of one-factor analysis of variance (one-way ANOVA). When the analysis of variance indicated differences among means, post hoc analysis using a Scheffe test was carried out to differentiate means with 95% confidence ($p < 0.05$). All statistical treatments were done using PASW Statistics (v.18, IBMSPSS Statistics, New York, USA).

3. Results and discussion

3.1. Colour characterisation

The colour parameters of selected gels prepared with Cf and Rw, Ra or Rc in the presence of sucrose, stevia or xylitol at several concentrations are presented in Table 1. Colour results indicated that gels from tested mixtures became lighter when sugars were added. Namely, the lightness (L^*) values rose significantly with increasing sucrose content, whereas the redness (a^*) and the yellowness (b^*) values decreased. Similar behaviour was noticed for xylitol samples, although these gels exhibited darker colour at equivalent sweetness of xylitol relative to tested sucrose concentrations. In contrast, stevia content was too low to influence significantly the colour parameters values, only slight modifications at the highest sweetener content can be observed. Overall, colour differences when compared with Cf/Rf gels without additives might be classified as distinct ($1.5 < \Delta E^* < 3$) for samples in the presence of xylitol to very distinct ($\Delta E^* > 3$) for samples in the presence of sucrose according to Drlange classification (Drlange, 1994). These results are consistent with the work reported by Manisha et al. (2012), who also identified colour change (increased lightness and decreased yellowness) of cakes in the presence of several sweeteners.

Colour characteristics of tested gels were also notably affected by Cf/Rf ratio (not shown). The lightness values decreased significantly with increasing Cf amount; subsequently, yellowness,

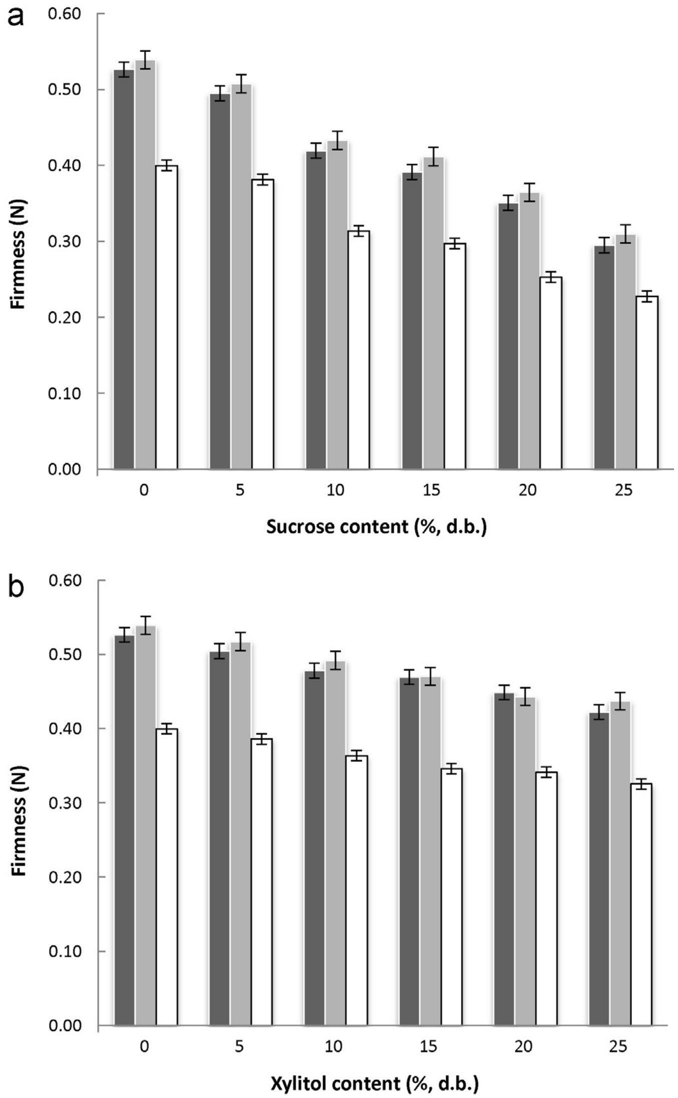

Fig. 1. Firmness values of gels prepared using mixtures (50/50) of chestnut flour (Cf) at 30% and each of the three rice flours (Rw, Ra, Rc) at 20% of total flour content in the presence of (a) sucrose and (b) xylitol. Symbols: dark grey bars – Cf/Rw, light grey bars – Cf/Ra, white bars – Cf/Rc. *d.b., dry basis.

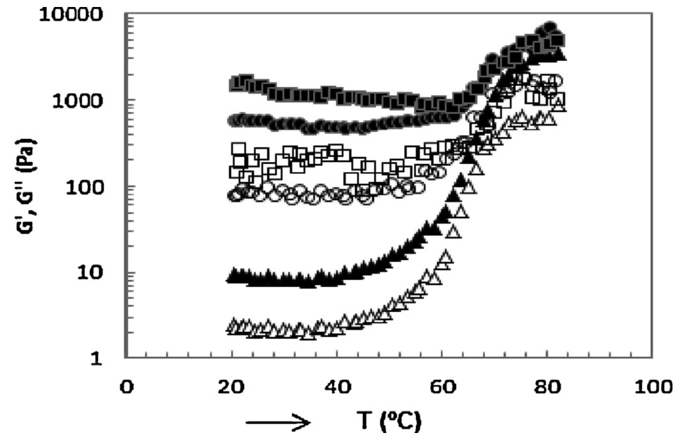

Fig. 2. Temperature ramps on heating (20–90 °C at 2 °C/min) of aqueous suspensions prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and each of the three rice flours (Rw, Ra, Rc) at 20% of total flour content. Symbols: circles – Cf/Rw, squares – Cf/Ra, triangles – Cf/Rc, closed – storage modulus (G'), open – loss modulus (G'').

and redness values increased. The magnitudes of these parameters increased significantly with increasing Cf until Cf/Rf reached 50/50%. Above this ratio, the aforementioned colour parameters did not exhibit significant differences. The colour parameters showed that Cf/Rc gels were the least dark followed by Cf/Ra and Cf/Rw independently on the assayed ratio and sugar content, indicating improvement in colour of the gels. The obtained parameters values are within the range of values measured in other rice (Song & Shin, 2007) and chestnut (Torres et al., 2013) flours.

3.2. Texture characterisation

Fig. 1 shows the firmness values of selected gels prepared using Cf and Rw, Ra or Rc in the presence of (a) sucrose and (b) xylitol at several concentrations. Firmness values of studied gels were similar to those obtained for commercially available desserts (Batista, Nunes, & Sousa, 2002). Texture results indicated that gels from tested mixtures containing sucrose and xylitol decreased significantly the firmness. It was noteworthy that the magnitude of this parameter decreased linearly with increasing sucrose and xylitol content. The drop in the firmness values was higher for gels containing sucrose. This behaviour is related to the sucrose structure, since the largest and complex molecules tend to further disperse between starch/proteins aggregates, leading to gels with lower firmness values (Maache-Rezzoug, Bouvier, Allaf, & Patras, 1998; Manisha et al., 2012). Stevia content was too low to influence the firmness values. These results are consistent with those previously reported for mixed gels prepared with rice starches and several types of polysaccharides (Huang, Kennedy, Li, Xu, & Xie, 2007).

The firmness of the gels was related not only to the type and concentration of the sugars, but also to the Cf/Rf ratio. Namely, Cf/Rf gels showed a drop in the firmness values with increasing Cf content (not shown). It was noticed that in terms of firmness Cf/Rf (0/100) gels at 30% (d.b.) were comparable to Cf/Rf (100/0) gels at 20% (d.b.). Gels prepared using assayed blends at (50/50) showed the best characteristics in terms of firmness when compared with commercial formulations (Batista et al., 2002). At the same assayed Cf/Rf ratio, Cf/Rw and Cf/Ra gels showed close values of firmness. Cf/Rc gels presented the lowest values, even though its firmness is inside the range of variation of this characteristic for commercial desserts (Batista et al., 2002).

3.3. Rheological characterisation

The evolution of storage (G') and loss (G'') moduli with temperature for selected gels from Cf/Rw, Cf/Ra and Cf/Rc is depicted in Fig. 2. Temperature ramps on heating indicated that all assayed samples exhibited G' larger than G'' (about one decade) in the whole temperature range. Both moduli remained practically constant during the initial heating. The first inflection point corresponding to the initial gelatinisation temperature (T_0) was identified around 60.1 ± 1.2 °C, except for Cf/Rc where T_0 started earlier (45.5 ± 1.1 °C). These results are in close agreement with those reported for pure gels from the same flours (Torres et al., 2013). Thereafter, G' and G'' began to increase rapidly with increasing temperature achieving a peak corresponding to the final gelatinisation temperature (T_1) around 73.5 ± 1.4 °C for all samples. Once the final gelatinisation temperature was reached, slight differences on the viscoelastic properties were noticed for all the tested gels. This behaviour is consistent with that previously found for pure chestnut and rice flours in a previous work of the same authors (Torres et al., 2013).

Rheological outcomes showed that the temperature ramps on heating of Cf/Rw gels were similar to those obtained for Cf/Ra,

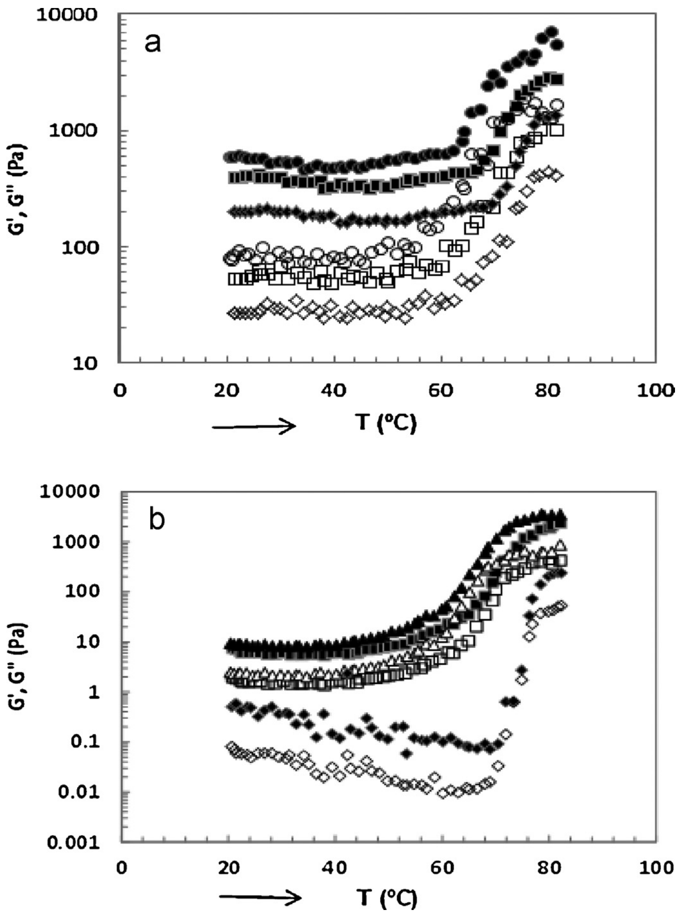

Fig. 3. Temperature ramps on heating (20–90 °C at 2 °C/min) of aqueous suspensions prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole rice flour (Rw) or (b) Carolino rice flour (Rc) at 20% of total flour content in the presence of sucrose and xylitol at 25% (w/w). Symbols: circles – Cf/Rw, triangles – Cf/Rc, diamonds – with sucrose, squares – with xylitol, closed – storage modulus (G'), open–loss modulus (G'').

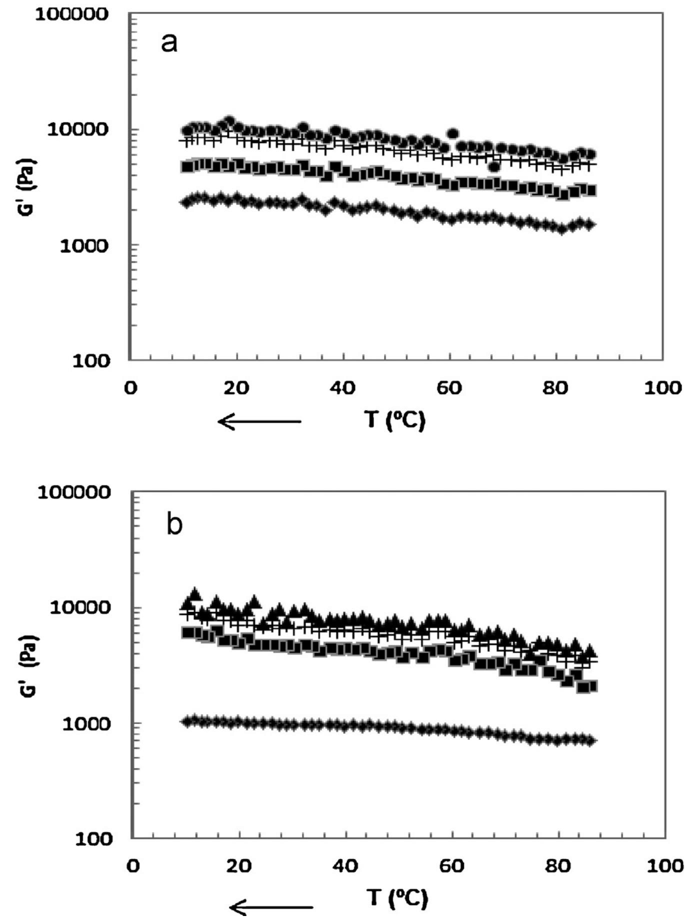

Fig. 4. Temperature ramps on cooling (90–5 °C at 1 °C/min) of gels prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole rice flour (Rw) or (b) Carolino rice flour (Rc) at 20% of total flour content in the presence of sucrose, xylitol and stevia at the highest assayed content. Symbols: circles – Cf/Rw, triangles – Cf/Rc, diamonds – with sucrose, squares – with xylitol, crosses – with stevia, closed – storage modulus (G').

whereas Cf/Rc gels exhibited a particular pattern. Specifically, this 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. The strongest gel network was rapidly formed upon heating above 70 °C for Cf/Rc gels. The promotion on the gel properties was related to the largest amounts of soluble components present in Rc systems (Torres et al., 2013).

Fig. 3 shows the temperature ramps on heating of selected gels prepared using (a) Cf/Rw and (b) Cf/Rc (50/50) in the presence of sucrose and xylitol at 25% (w/w). These gels were chosen as representative examples of all studied systems. Cf/Ra samples in the presence of sugars again featured similar patterns to Cf/Rw (not shown). The addition of sucrose resulted in a significant decrease in the values of storage and loss moduli, whereas the same addition of xylitol involved a small decrease in the values of both moduli. No significant differences were noticed in the presence of stevia (not shown). In these systems protein is present at levels of 7% (Moreira, Chenlo, & Torres, 2012). It is well known that proteins, even at low levels, can be responsible for important structural effects. It is noteworthy that G' and G'' moduli for Cf/Rc in the presence of sucrose gradually decreased below 65 °C as temperature increased, indicating softening of the gels. These changes can be explained by protein weakening promoted in the presence of sucrose, since it tends to disperse the protein molecules, decreasing of the inter-chain hydrophobic interactions of proteins (Melander & Horváth, 1977; Rosell, Collar, & Haros, 2007). The sugar effects are consistent with those previously reported (Gallagher, O'Brien, Scannell, & Arendt, 2003; Moreira et al., 2011). Both authors highlighted that sugar can cause a sample softening, due partially to the competition between the added sugar and the polymers: starch and proteins for the available water in the system. The tested sweeteners also delayed starch gelatinisation by increasing the gelatinisation temperature. This behaviour was explained as result from the reduction of the water activity of the system due to the action of sugars as well as related to interactions among the sugars and the starch chains (Mezreb, Goullieux, Ralainirina, & Queneudec, 2006; Sumnu, Ndife, & Baymdirh, 1999).

Fig. 4 depicts the temperature ramps on cooling of selected gels in the presence of sucrose, xylitol and stevia in order to understand the role of each sugar on the gel network of the assayed mixtures. During the cooling process down to 5 °C, G' increased progressively, achieving larger values (around two times) at the lowest temperatures, which revealed a moderate gel structure reinforcement. No significant differences were identified between assayed Cf/Rf mixtures in the absence of sugars. This pattern of structural development is consistent with the results previously reported for maize starch gels (Nunes et al., 2006b) and other starch pastes (Miles, Morris, Orford, & Ring, 1985), who 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

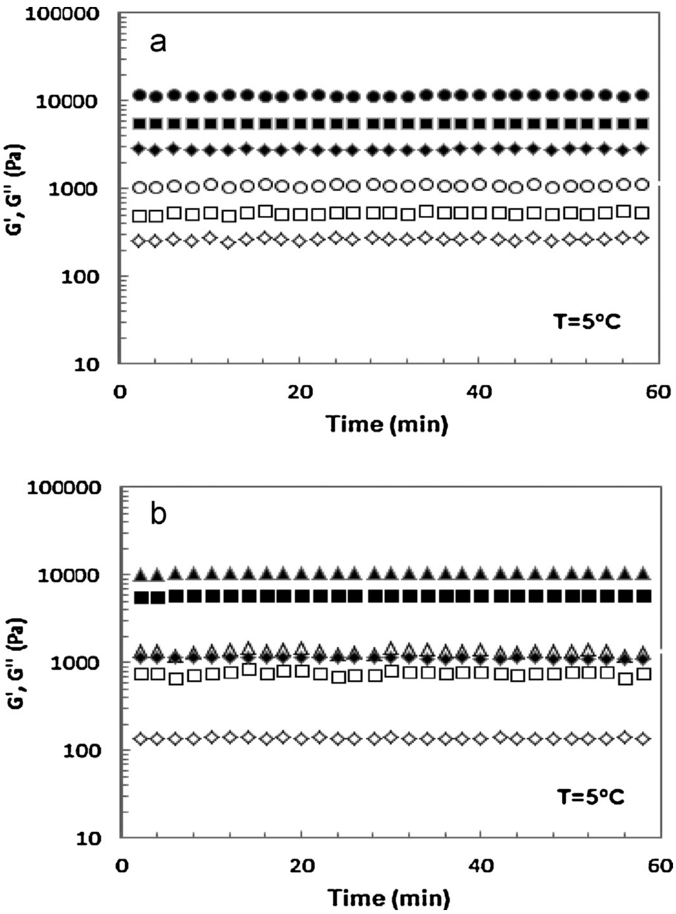

Fig. 5. Time sweeps (60 min at 5 °C) of gels prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole rice flour (Rw) or (b) Carolino rice flour (Rc) at 20% of total flour content in the presence of sucrose and xylitol at 25% (w/w). Symbols: circles – Cf/Rw, triangles – Cf/Rc, diamonds – with sucrose, squares – with xylitol, closed – storage modulus (G'), open – loss modulus (G'').

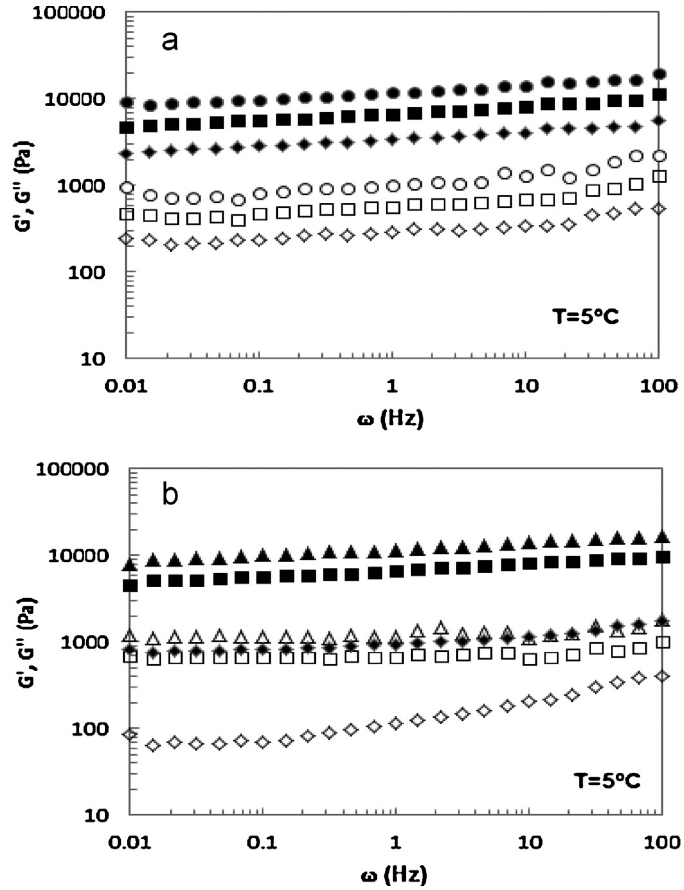

Fig. 6. Frequency sweeps (0.01–100 Hz at 20 °C) of gels prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole rice flour (Rw) or (b) Carolino rice flour (Rc) at 20% of total flour content in the presence of sucrose and xylitol at 25% (w/w). Symbols: circles – Cf/Rw, triangles – Cf/Rc, diamonds – with sucrose, squares – with xylitol, closed – storage modulus (G'), open – loss modulus (G'').

sucrose and xylitol in gels prepared with Cf/Rw (Fig. 4a) and Cf/Rc (Fig. 4b) showed a notably drop in the G' values in the whole temperature range, indicating a softening of the gels when sugars were added. Cf/Ra gels exhibited similar cooling curves to Cf/Rw samples (not shown). The highest G' values reduction was achieved for Cf/Rc gels followed by Cf/Rw gels in the presence of sucrose. As it is was expected, no significant variations were observed when stevia was added.

The maturation kinetics of studied gels were monitored through the evolution with time of G' and G'' moduli at 5 °C (Fig. 5). Both moduli reached stability after 60 min 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 found between assayed gels in the absence of sugars. However, the addition of sucrose in gels prepared using Cf/Rw (Fig. 5a) and Cf/Rc (Fig. 5b) reduced notably the values of both moduli in the whole temperature range. Namely, this drop was around 4 times for Cf/Rw gels and about one decade for Cf/Rc gels at the highest assayed sucrose content. Similar trends were observed in the presence of xylitol, although with a lower reduction (around two times) in both moduli at equivalent xylitol content. Cf/Ra gels exhibited similar maturation curves to Cf/Rw samples (not shown). Again, no statistical differences were identified when stevia was added. The cooling and maturation behaviour exhibited is typical of biopolymer gelation (Nunes, Raymundo, & Sousa, 2004). All these gels reached a stable and fully developed structure within the time of the experiment. The short time necessary to achieve stable gel structure is an important advantage from an industrial point of view.

Fig. 6 shows the mechanical spectra of these gels, obtained at the end of the run at 5 °C. All gels exhibited a typical strong gel spectrum in the absence of sugars, i.e. G' was always larger than G'', at about one decade, and are both almost frequency independent. This tendency is consistent with the solid behaviour found for chestnut flour gels (Moreira, Chenlo, Torres, & Glazer, 2012; Moreira, Chenlo, Torres, & Prieto, 2010) and rice flour pastes (Marco & Rosell, 2008). In the absence of sugars, all gels presented qualitatively similar linear viscoelastic data, without notable variations in the values of both moduli throughout assayed angular frequency range. Nevertheless, the sucrose addition decreased significantly the values of G' and G'' moduli, whereas the same addition of xylitol involved a smaller reduction in the values of both moduli. No significant differences were observed in the presence of stevia (not shown). It is remarkable that the highest reduction in both moduli was observed for Cf/Rc gels in the presence of sucrose. Particularly, G'' modulus exhibited a pronounced drop at the lowest angular frequency values. All gels exhibited stable and strong gel behaviour, being the least stable gels those prepared using Cf/Rc in the presence of sucrose. Even so, the achieved values are within those reported for commercial dairy desserts (Batista et al., 2002). Overall, the output data obtained from small deformation rheology were in close agreement with those found from texture measurements of the same gels.

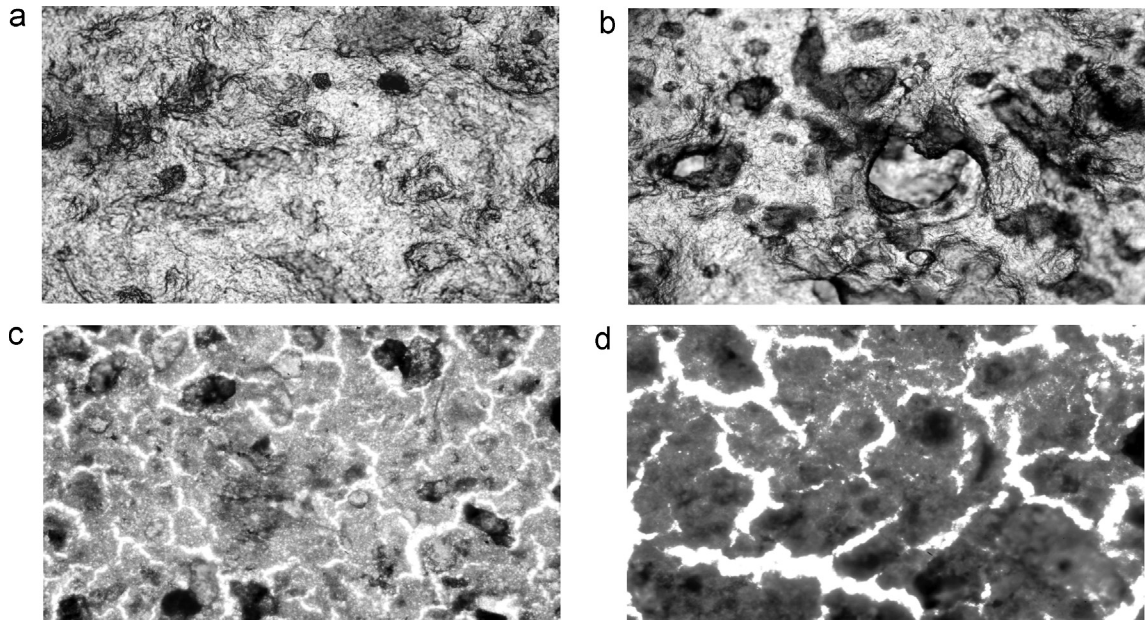

Fig. 7. CLSM images of gels prepared with mixtures (50/50) of chestnut flour (Cf) at 30% and (a) whole rice flour (Rw) or (b) Carolino rice flour (Rc) at 20% of total flour content in the presence of (c and d) sucrose at 25% (w/w) at 20 °C.

3.4. Confocal laser scanning microscopy

The influence of sucrose, xylitol and stevia on the structure of tested gels was investigated through confocal laser scanning microscopy at room temperature. Microscopic observations clearly show strong and stable starch/protein (grey/black areas) network for Cf/Rw (Fig. 7a) and Cf/Rc (Fig. 7b) gels in the absence of sugars. Analysing CLSM images, it can be observed that phase separation between starch/protein aggregates and sucrose (white areas) takes place. Namely, CLSM images showed that chain associations in Cf/Rw (Fig. 7c) and Cf/Rc (Fig. 7d) gels are reduced in the presence of sucrose, leading to a less aggregated structure. The competition between the added sugar and the available water in the system also promotes the weakening of the gel structure. The largest phase separation was identified for Cf/Rc gels. Once more, Cf/Ra gels exhibited similar patterns to Cf/Rw. The addition of xylitol showed similar behaviour to sucrose, but with more aggregated structures. Contrary, as expected the addition of stevia did not affect the gel structural properties (data not shown). CLSM images gave evidence that lower sugar content slows down the phase separation process in favour of starch/protein networking. These results are in good agreement with those previously reported by Maache-Rezzoug et al. (1998), who highlighted that sugar makes the product fragile, since it tends to disperse the protein and starch molecules, thereby preventing the formation of a continuous mass. CLSM results supported the information obtained for Cf/Rw, Cf/Ra and Cf/Rc gels in the presence of sugars by means of rheological and texture measurements.

4. Conclusions

The presence of sucrose and xylitol strongly affected gluten-free gels structure and therefore rheology, texture and microstructure, whereas the stevia content was too low to influence significantly gels properties. Gluten-free flour gels from blends of chestnut flour (Cf) and whole rice flour (Rw) or Agulha rice flour (Ra) exhibited similar patterns, whereas those prepared with Carolino rice flour (Rc), showed particular properties. Texture measurements suggested that formulations prepared using blends (50/50) from chestnut flour at 30% and rice flours at 20% in the presence of assayed sugars can be an interesting alternative to obtain gluten-free gelled products. Although all samples showed stable and strong gel properties, rheological measurements indicated that the addition of sucrose involved a significant weakening of the gels, whereas equivalent additions of stevia and xylitol resulted in a smaller softening of these systems. Microscopic observations showed less aggregated structures of the studied gels in the presence of sucrose. These measurements allowed to conclude that gels from Cf/Rw and Cf/Ra in the presence of sucrose present an industrial advantage over Cf/Rc gels, since the latter samples gave the most fragile gels. From the textural and structural point of view, replacement of sucrose by low-calorie sweeteners such as xylitol or stevia in tested gels can be considered as an interesting approach for the development of alternative gluten-free formulations based on gelled systems, providing additional economic value to wastes or by-products coming from chestnut and rice processing industry. Further work will be necessary to evaluate these gels on the basis of sensory characterisation.

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. The authors also wish to thank to Novarroz (Portugal), who kindly provided rice materials.

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