Characterization of chickpea flour-based gluten-free batters and muffins with added biopolymers: rheological, physical and sensory properties

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Running title

Chickpea flour-based muffins with biopolymers

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Summary

Chickpea flour (CF)-based muffin formulations were made with CF alone and with added biopolymers [whey protein (WP), xanthan gum (XG) and inulin (INL)] to evaluate their suitability to be a wheat flour (WF) substitute in muffins. Structural characteristics of the batters and muffins were studied by means of rheometry, microscopy, physicochemical properties, and texture and sensory analysis. Partial replacement of CF with XG, alone (at 0.5 and 1%) or blended with either WP or INL, significantly increased the batter viscoelasticity as denser matrices developed; moreover the muffins with XG added at 1% had similar hardness to wheat gluten muffins. The replacement of WF decreased the springiness, cohesiveness, chewiness, resilience and specific volume of the muffin. However, baked muffins with added XG also showed higher sensory sponginess and moisture associated with numerous air bubbles in the batter, and were perceived to be easier to swallow and to have better general appearance.

Keywords Chickpea flour, formulation, gluten-free, muffin, viscoelasticity, texture, descriptive sensory analysis.

Introduction

Traditionally, a muffin recipe is mainly composed of WF, sugar, vegetal oil, egg and milk (Sanz *et al.*, 2009). Today, there is an increasing number of people interested in wheat-free foods motivated by health concerns but also by the desire to avoid wheat in the diet (Matos *et al.*, 2014). Gluten-free muffin, cake or cupcake recipes contain rice flour as the principal ingredient (Gularte *et al.*, 2012a,b) or other starch sources, such as corn, potato or wheat (Ronda *et al.*, 2011). However, many gluten-free products available on the market are of low quality, exhibiting low volume, poor colour and crumbling crumb, besides with low protein and high fat contents (Matos *et al.*, 2014).

For that reason, in recent years the incorporation of starches, dairy proteins, hydrocolloids, fibres and emulsifiers in gluten-free flour is being investigated in order to mimic the properties of gluten and improve structure, mouth-feel, acceptability and shelf life. Incorporation of hydrocolloids such as gums and proteins from gluten-free sources has been suggested to improve gluten-free muffin quality characteristics (Singh *et al.*, 2015). Those authors showed that XG improved the quality characteristics of rice flour-based muffins. Also, the addition of casein significantly increased viscoelasticity in wheat-free muffins made with rice flour (Matos *et al.*, 2014). The incorporation of inulin in gluten-free layer cakes reduced batter viscosity, but a blend of oat and inulin (insoluble and soluble fibre) increased it, which was attributed to the high water retention capacity of the insoluble fibre (Gularte *et al.*, 2012a).

The United Nations has declared that 2016 will be the International Year of Pulses (IYP 2016). The hope of IYP 2016 is to position pulses as primary sources of protein and other essential nutrients, leading to dietary uptake. In fact, most health organizations encourage their frequent consumption (Gómez *et al.*, 2008), and nowadays strategies are

being investigated to increase daily consumption of legumes. Therefore, the enrichment of bakery products with legumes might be another way of increasing legume intakes and improving the nutritional value of gluten-free bakery products (Gularte *et al.*, 2012a). Nevertheless, the addition of legumes to bakery products could lead to modifications in their physical characteristics, such as texture and mouth-feel (Gómez *et al.*, 2008). The authors just cited studied the influence of total or partial replacement of WF with chickpea flour (CF) on quality characteristics of two kinds of cake, and found that the volume, symmetry and colour of the cakes decreased when the amount of CF increased. In contrast, Shevkani and Singh (2014) showed that gluten-free muffins comparable to those with WF can be prepared from corn starch by incorporating protein isolates from kidney bean and field pea, as legume protein isolates increase viscoelasticity of gluten-free muffin batters.

Nonetheless, scarce information is available on the use of legumes to prepare gluten-free bakery products. To the best of our knowledge, there is no research regarding the use of CF for making gluten-free muffins. Therefore, the first aim of this study was to research whether CF has the potential to be a WF substitute in muffins, evaluating the effect of three different biopolymers [protein: WP, gum: XG, and fibre: INL] and their blends on the rheological, physical and microstructural properties of gluten-free formulations, as well as on the physicochemical properties of the baked muffins made with complete replacement of WF with CF in order to try to mimic control WF-based muffins. A second aim was to evaluate the sensory properties of the baked muffins.

Materials and methods

Materials

Muffin ingredients were commercial WF and CF (11.5 and 9.9% moisture and 10.2 and 19.4% protein, respectively), both donated by García del Valle flour milling company (Soria, Spain), pasteurized liquid whole yolk (Ovopak, Seville, Spain), sucrose (AB Azucarera Iberia S.L., Madrid, Spain), salt (sodium chloride), ultra-high temperature whole milk (Pascual, Burgos, Spain), refined sunflower oil (Koipesol, Madrid, Spain), citric acid anhydrous and sodium hydrogen carbonate (Panreac Química S.L.U., Barcelona, Spain). Microparticulated WP concentrate (53% protein, SIMPLESSE 100 [E]) and XG (Keltrol F [E]) were donated by Premium Ingredients, S.L. (Girona, Spain), while INL was a “long-chain” INL with trade name Orafti®HP (BENEO-Orafti, Tienen, Belgium).

Gluten-free muffin making

One batter formulation was prepared as control with WF alone (Table 1). Then, CF-based batters were made without adding any biopolymer by replacing all of the WF with CF or by replacing part of the CF with WP, XG, INL and their blends. Samples were formulated and identified as shown in Table 1.

The batter was prepared as described by Martínez-Cervera et al. (2012a,b) with some modifications, using a KPM5 professional mixer (Kitchen Aid, St. Joseph, Michigan, USA). When added, WP and INL at 5, 10 and 15 g 100 g⁻¹ of CF were previously dissolved in the milk at 75 °C for 15 min, while XG (0.25, 0.50 and 1 g 100⁻¹ of CF) was incorporated as a dry powder. The batters were all kept at 25 °C for 60 min before measurements.

The batter (30 g) was dosed into each paper mould (55 mm diameter × 30 mm high). Twenty moulds were arranged in four rows of five muffins on a baking tray and baked for 25 min at 185 °C in a Memmert (UFE 400) electrical oven (Schwabach, Germany) preheated to that temperature for 45 min. Muffins were left to cool at room temperature for 1 h. Then they

were packed in coded polyethylene bags and stored at room temperature for an additional 24 h until determinations were conducted. Each batter/muffin formulation was prepared at least three times (three batches), on different days.

In all cases, three replicates of each instrumental measurement were carried out with samples prepared on three different days.

Rheological properties of muffin batter

Small-amplitude oscillatory shear (SAOS) tests were carried out with a Kinexus pro rotational rheometer (Malvern Instruments Ltd., Worcestershire, UK) and a Bohlin CVR 50 controlled stress rheometer (Bohlin Instruments Ltd., Cirencester, UK), using parallel-plate geometry (40 mm diameter and 1 mm gap). Samples were allowed to rest for 15 min before analysis to ensure both thermal and mechanical equilibrium, and they were covered with a thin film of Vaseline oil (PRS-Codex) to avoid evaporation. Temperature was controlled to within 0.1 °C by Peltier elements in the lower plates kept at 25 °C, except when non-isothermal heating processes were carried out.

To determine the linear viscoelastic (LVE) region, stress sweeps were run at 1 Hz with the shear stress of the input signal varying from 0.01 to 100 Pa. Critical values of shear stress ($\sigma_{\max}$) and shear strain ($\gamma_{\max}$), which can be taken as measurements of rheological stability (Mezger, 2011), maximum complex modulus ($G^*_{\max}$) and loss factor ($\tan \delta = G''/G'$) were obtained to limit the LVE range according to Campo-Deaño and Tovar (2009). Frequency sweeps were run subjecting the samples to stress that varied harmonically with time at frequencies (f) from 0.01 to 10 Hz. Strain amplitude was set at $\gamma = 0.1\%$, within the LVE range. Temperature sweep tests were performed from 25 to 95 °C at a linear heating rate

(1.6 °C min⁻¹). Frequency was set at 0.1 Hz, maintaining the shear stress signal at the minimum value provided by the Bohlin rheometer for parallel-plate geometry (2.98 Pa).

Physicochemical properties of muffin batter

Specific gravity (SG) was measured as the ratio of the weight of a standard container filled with batter to that of the same container filled with water (Martínez-Cervera *et al.* 2012a,b). Total soluble solids (TSS) content [g 100 g⁻¹ (w/w)] as measured by refractive index was determined with an Atago (Itabashi, Tokyo, Japan) dbx-30 refractometer. Moisture (%) was determined by drying samples in a Philips microwave oven (model M-718, 700 W).

Scanning electron microscopy of muffin batter

Microstructure was examined using a Jeol JSM-6500F (Jeol Ltd., Tokyo, Japan) field emission scanning electron microscope (SEM) with a resolution varying from 1.5 nm (15 kV) to 5.0 nm (1 kV). Samples were air-dried, then mounted and sputter-coated with Au (200 Å approx.) in an SPI diode sputtering system metallizer.

Instrumental texture of baked muffin

Texture measurements were carried out with a TA.HDPlus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) equipped with a 49 N load cell. Texture profile analysis (TPA) tests was performed at 25 °C, using a flat 100 mm diameter aluminium cylinder probe on the whole muffins after removing them from the mould. Muffins were double compressed up to 70% of the original height, with a rest period of 3 s between cycles. The trigger force

was 0.05 N and the test speed was 2 mm s⁻¹. Force-time curves gave the hardness, *Hard* (N), springiness, *Spr* (dimensionless), cohesiveness, *Coh* (dimensionless), chewiness, *Chew* (N), and resilience, *Res* (dimensionless). Definitions of textural parameters are given elsewhere (Gómez *et al.*, 2008; Singh *et al.*, 2015).

Physicochemical properties of baked muffin

Muffins were weighed before baking, after baking and cooling for 1 h at room temperature, and after packing and storing for an additional 24 h under the same conditions; the weight loss was calculated 1 h after baking and 24 h after packing. A digital calliper was used to measure collapses, i.e. difference in height (mm) of the muffins when they were extracted from the oven and either 1 h after baking or 24 h after packing. After weighing the muffins, their volume was determined 24 h after packaging by the rapeseed displacement method (Gras & MacRitchie, 1973), using glass beads (5-6 mm diameter) to prevent rapeseeds from sticking to muffins. The volume of the product was obtained directly from a 500 ml calibrated vessel, as indicated by the difference in the level of the glass beads in presence and absence of each muffin. In turn, the specific volume (cm³ g⁻¹) was evaluated by the ratio between the muffin volume and its weight (Gularte *et al.*, 2012a, b). Water activity (*a_w*) of the crumb was determined 24 h after packaging using a LabMaster-*a_w* (Novasina AG CH-8853, Lachen, Switzerland).

Sensory evaluation

Among the most widely used descriptive analysis is the quantitative descriptive analysis (QDA[®]) because it is based on the principle of a panellist's ability to verbalize perceptions

and to discriminate differences in sensory properties among samples of the specific product type for which they are to be trained (Jiménez *et al.* 2013). A panel of trained panellists ($n = 8$) with experience in evaluating chickpea products was used for QDA[®] of the muffins. Training sessions were conducted in accordance with Martínez-Cervera *et al.* (2011), and after ten 30-min sessions the panel reached a consensus, choosing the sensory attributes shown in Table S1. An experimental design of balanced complete blocks was employed and the muffins were rated in duplicate, in seven separate sessions, on an 8 cm unstructured scale. Muffins were presented in a randomized order in their moulds, with randomly assigned three-digit codes, and each panellist assessed four samples per session. Water was provided for rinsing the mouth between samples.

Statistical analysis

One-way analysis of variance was applied to study the effect of WF replacement with CF and biopolymers on each of the instrumental parameters and sensory attributes. Minimum significant differences were calculated using Fisher's least significant difference (LSD) tests with a 99% confidence interval for comparison of instrumental parameters and a 95% confidence interval for comparison of sensory attributes. A principal component analysis (PCA) of the QDA data was conducted, using correlation matrix and linear regression to correlate instrumental and sensory data. Data analyses were carried out with SPSS Statistics 19.0 (SPSS Inc., Chicago, IL) and XLSTAT 2009.4.03 (Addinosoft, Barcelona, Spain).

Results and discussion

Viscoelasticity of muffin batter

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249 Stress sweeps were carried out to determine the limit of the LVE range at 25 °C. As
250 can be seen in Table S2, 100%WF batter showed a significantly ($P < 0.01$) higher resistance
251 to the applied stress (higher $\sigma_{\max}$ value) and a higher degree of elasticity (lower $\tan \delta$ value)
252 than 100%CF. Furthermore, 100%WF proved to be more deformable than batter made with
253 CF alone (higher $\gamma_{\max}$ value), although differences were not significant. The addition of WP at
254 any concentration (5, 10 and 15%) and INL at 5 and 10% did not seem to make any important
255 structural change with respect to either 100%CF or 100%WF batters. However, batters with
256 added XG, alone (0.5% and 1% XG) or combined with WP or INL (10%WP-0.5%XG and
257 0.5%XG-10%INL), showed a significant increase in conformational stability (higher $\sigma_{\max}$
258 values) with respect to both 100%CF and 100%WF batters. Moreover, batters with added XG
259 were also more rigid (higher $G^*_{\max}$ values), this increase in rigidity being caused by an
260 increase in elasticity (G'), as indicated by their lower loss tangent values (Table S2). This
261 means that batters made with XG, especially at the highest concentration (1%XG batter),
262 were denser than both control and 100%CF batters.

263 Mechanical spectra of all the batters, obtained from frequency sweeps in the LVE
264 range at 25 °C, are shown in Fig. 1. In general, all batters showed the typical behaviour of
265 “soft” or “weak” gels, with considerable frequency dependence for both moduli throughout
266 the whole frequency range studied. Moreover, in all samples G' values were greater than G''
267 values, reflecting the viscoelastic behaviour of these batters, with solid-like characteristics,
268 and both moduli increased with increase in frequency. Similar behaviour was observed for
269 other gluten-free muffin batters (Martínez-Cervera *et al.*, 2012a,b; Matos *et al.*, 2014;
270 Shevkani & Singh, 2014; Singh *et al.*, 2015). To quantify the frequency dependence of G' and
271 G'' , both moduli were fitted to power law equations (Eqs. (1) and (2)):

272
$$G' = G'_0 f^{n'} \quad (1)$$

$$G'' = G''_0 f^{n''} \quad (2)$$

where G'_0 and G''_0 are elastic and viscous moduli at 1 Hz, respectively, and n' and n'' (both dimensionless) denote the rate of change of G' and G'' , respectively, with increasing f (Mezger, 2011).

As can be seen in Fig. 1, 100%CF batter showed a similar spectrum to that of the control batter (100%WF), although in 100%CF both moduli almost overlapped in the entire frequency range, converging at the lowest frequencies (~ 0.01 Hz), which indicates that the 100%CF batter has worse viscoelastic properties, as shown by its higher n' value (0.468 ± 0.015) (Table S3), in comparison with the control batter (0.354 ± 0.005), meaning that batter made with CF alone would be less time-stable.

Regarding the addition of the various biopolymers, replacement of CF with WP alone at any concentration (Figs. 1a,b), INL at 5 and 10% (Figs. 1e,f) and the blend of both ingredients (10%WP-10%INL; Figs. 1g,h) hardly modified the 100%WF and 100%CF spectra. Only INL at the highest concentration (15%INL) produced a slight improvement in the viscoelastic properties, as reflected by their significantly ($P < 0.01$) lower $\tan \delta$ value and higher moduli with respect to 100%WF and 100%CF batters (Table S3). Consequently, neither WP nor INL seemed to be appropriate ingredients to improve the viscoelastic characteristics of CF-based muffin batters.

In contrast, incorporation of XG alone at any concentration (Figs. 1c,d) or combined with either WP or INL (Figs. 1g,h) produced an increase in both moduli, which were approximately one order of magnitude greater than those of both 100%WF and 100%CF batters. Moreover, XG addition also seemed to stabilise the frequency dependence of both moduli. The significant improvement produced by XG can also be seen in Table S3, as shown by the significantly ($P < 0.01$): (1) higher G' and G'' values, (2) lower n' and n'' values, which means lower frequency dependence of both moduli, and (3) lower $\tan \delta$ values than gluten-

based batter (100% WF), which reflects a stronger, more elastic and more time-stable batter. Other authors have also found an increase in batter viscoelasticity (greater G' and G'' moduli) produced by incorporation of XG alone in WF dough (Upadhyay *et al.*, 2012) or in combination with jambolan fruit pulp in gluten-free eggless rice muffins (Singh *et al.*, 2015). The authors just cited indicated that the XG effect could be due to its ability to absorb water, which might mean that less free water would be available, increasing the batter viscoelasticity. In fact, this property has been attributed to the hydroxyl groups in the XG structure, which allow more hydrogen bonding with water molecules (Guarda *et al.*, 2004). These results agree with those obtained from the stress sweeps, where the presence of XG produced more rigid batters (high values of $G^*_{\max}$) with better viscoelastic stability (higher $\sigma_{\max}$ values).

The structural changes that occur during baking are also considered determining factors in bubble formation and stability, and therefore determine the final microstructure and texture of the baked product (Shelke *et al.*, 1990). In this regard, complex viscosity, $\eta^* = (G^*/\omega)$, and $\tan \delta$ values of 15%INL, 0.5%XG, 1%XG and their blends (10%WP-0.5%XG and 0.5%XG-10%INL) were plotted during heating from 25 to 95 °C together 100%WF and 100%CF batters (Fig. 2). Heating curves of batter made with CF alone and 100%WF batter were quite close to each other (Fig. 2a). Initially, at 25 °C both 100%WF and 100%CF batters showed near values of η^* (56.4 ± 7.3 and 46.8 ± 0.83 Pa s, respectively), which decreased progressively until they reached an inflexion point (T_g), approximately at 73.9 ± 3.5 °C for 100%WF and at 70.6 ± 0.21 °C for 100%CF, which is associated with starch gelatinization, protein coagulation and structure fixation (Lee *et al.*, 2004). This decrease might be related to a progressive loss of strength due to protein unfolding, resulting in a decrease in G' , as reported by Rosell and Foegeding (2007), or even to melting of the fat (Hesso *et al.*, 2015). However, Matos *et al.* (2014) reported that the decrease in modulus values up to the

temperature of 45 °C is related to the release of CO₂ formed in the batter, which is diffused into occluded air cells and expanded, and hence reduces batter density. As the decrease in η^* was much more evident in 100%CF than in 100%WF after 45 °C, and both batters had the same oil content, this could indicate that CF proteins may be more sensitive to heat than wheat proteins, losing batter strength and consistency to a higher degree. The lower carbohydrate content of CF might also influence the more significant η^* decrease (Gómez *et al.*, 2008).

After T_g , the η^* values of both batters increased until they reached a similar maximum value (407.4 ± 32.8 and 391.3 ± 46.0 Pa s for 100%WF and 100%CF, respectively). From ~72 to 95 °C, 100%CF batter also showed lower η^* values than the control, again indicating that chickpea protein might be less stable to heating. The incorporation of the various biopolymers and their blends into the 100%CF batter improved the viscoelastic properties of the batter during heating to different degrees and modified the T_g value. 15%INL produced η^* values slightly higher than those of 100%WF, while the incorporation of XG (individually or combined with INL or WP) produced a significant increase in the viscoelastic properties of the 100%CF batter during the whole heating period. As a result, η^* values were one order of magnitude greater or more than those of the control and 100%CF batters and remained practically stable until their inflexion points. Batter consistency is an important factor determining the final quality of muffins. Lower batter viscosities during heating have been related to final products with less volume (Shelke *et al.*, 1990), while higher viscosities improve both cake volume and crumb grain, preventing coalescence, migration and loss of air cells before the batter sets, giving the batter greater capacity to retain expanding nuclei and resist the settling of starch granules (Baixauli *et al.*, 2008).

Regarding the inflexion points (T_g), the incorporation of XG produced a delay in the T_g , which was $\sim 75.6 \pm 1.8$ °C for 0.5%XG, $\sim 79 \pm 2.3$ °C for 1%XG, 77.7 ± 0.1 °C for

10%WP-0.5%XG and 78.5 ± 5.0 °C for 0.5%XG-10%INL. However, the T_g for 15%INL was around 66.1 ± 4.8 °C. The delay produced by incorporation of XG would allow starch gelatinization to occur later, giving enough time for appropriate air and vapour expansion during heating and avoiding thermosetting (Martínez-Cervera *et al.*, 2012a,b).

All these observations were consistent with the $\tan \delta$ curves (Fig. 2b), showing a similar evolution of this parameter during heating in all the batters except the 100%CF batter. Accordingly, $\tan \delta$ values increased until they reached a maximum value, which corresponded to the inflexion point of the η^* curves, and after that there was a progressive decrease until 95 °C. In particular, 100%CF showed much higher values (around 1.5) until approximately 85 °C, indicating a decrease in the viscoelasticity of this batter produced by an increase in the viscous component. However, as occurred for η^* , the batters with XG showed the lowest $\tan \delta$ values (close to 0.5) during the whole heating, except for 0.5%XG batter, whose $\tan \delta$ values were very similar to those of 100%WF. Consequently, CF-based batters enriched with XG had a more elastic character during heating.

Physicochemical properties of muffin batter

No significant differences were found in SG values (Table S4) between the control (0.95 g l^{-1}) and 100%CF (0.96 g l^{-1}). In comparison with 100%CF batter, a significant decrease in SG was found when CF was replaced by WP at 10 and 15% levels, by INL at 5 and 10% levels, and by the 10%WP-10%INL blend, indicating higher incorporation of air into these batters as CF was replaced by these biopolymers. In contrast, a significant increase in SG was observed with XG alone at 0.5 and 1% levels (up to 1.01 g l^{-1}), which is not in agreement with the viscoelastic results, as these samples showed the highest batter density, and with WP alone at the lowest level (5%). In principle, a low SG value could indicate a higher number of gas

nuclei in the raw batter, which could encourage the formation of larger bubbles during baking and therefore result in greater height and volume (Martínez-Cervera *et al.*, 2012a,b), although many other factors also affect this quality parameter (Frye & Setser, 1991). Consequently, the various biopolymers have dissimilar functionality in terms of air incorporation or air retention capacity.

The TSS content of all the CF-based muffin batters was significantly higher than that of the control sample, which could be ascribed to their higher protein and sugar contents, depending on the biopolymer that was added (Table S4). Moisture content ranged between 27.7 and 32% for 10%WP-10%INL and control batters, respectively. Control batter had significantly ($P < 0.01$) higher moisture than CF-based batters, which may be associated with the higher moisture percentage of WF.

Muffin batter microstructure

Lastly, SEM images at $\times 300$ and $\times 1000$ magnification were taken of the 100%WF, 100%CF, 1%XG and 0.5%XG-10%INL batters (Fig. 3). The 10%WP-0.5%XG batters were almost identical to the 0.5%XG-10%INL batters (images not shown). In general, all the batters showed a continuous protein matrix from various sources (flour, egg and milk), in which fat globules and starch granules were mainly embedded, as reported by other authors on bakery product batters (Baixauli *et al.*, 2008; Hesso *et al.*, 2015).

The 100%WF and 100%CF batters (Figs. 3a-d) showed a similar number and distribution of air bubbles and fat globules, while starch granules cannot be detected in the control batter (Fig. 3b). However, starch contents of 39.1 and 78.8% w/w have been reported for CF (Kaur & Singh, 2007) and WF (Abebe *et al.*, 2015), respectively. Therefore, the absence of visible starch granules in 100%WF might be due to the effect of the different

particle size of WF (fine) vs. CF (coarse). All the CF-based batters (100%CF, 1%XG and 0.5%XG-10%INL) showed a rougher matrix (with presence of abundant starch granules of different sizes) (Figs. 3c–h) than the 100%WF matrix, which had a smoother appearance (Figs. 3a,b). This could be due to different starch and protein sources and especially to the higher protein and insoluble fibre contents of CF (de Almeida Costa *et al.*, 2006), which would produce visibly denser structures. The thickness of the CF-based matrices was more discernible in 1%XG and 0.5%XG-10%INL batters, as reflected by the higher $\sigma_{\max}$, $G^*_{\max}$ and viscoelastic moduli (G' and G'') and the lower $\tan \delta$ reported in the previous section. Moreover, the presence of XG in the batter produced a change in the size and number of fat globules and air bubbles, with the 1%XG and 0.5%XG-10%INL batters showing more fat globules of small and medium size (Figs. 3f,h, respectively) and numerous smaller air bubbles (Figs. 3e,g). Upadhyay *et al.* (2012) reported an inverse relation between bubble size and G' modulus in bread dough, while Ozkoc *et al.* (2009) suggested that the addition of hydrocolloids formed a thick layer on the surface of gas bubbles, reducing the opportunity for coalescence of individual gas cells.

In short, the SEM images corroborate that the addition of XG alone or in combination with INL or WP produced more compact and denser batters with a homogeneous distribution of a higher number of smaller bubbles, which could be expected to produce muffins of better quality. However, the SG values obtained were not in agreement with the SEM images of the batters.

Muffin textural properties

With respect to the TPA (Table S5), addition to CF of XG, either at 1% level or combined with INL, did not have effect on hardness, with a non-significant decrease for the

1%XG muffin compared with the control muffin. The lowest hardness was found in the 100%CF muffin, showing that gluten development is an important determinant of hardness (Sanz *et al.*, 2009). Hardness, springiness and cohesiveness are important textural properties related to consumer acceptance (Shevkani & Singh, 2014). Springiness is associated with a fresh, aerated product; therefore, high muffin quality will be associated with high springiness values (Sanz *et al.*, 2009). In this study, total or partial replacement of WF with CF, alone or with biopolymers and their blends, resulted in significantly reduced springiness. Muffins containing 15%INL showed the lowest springiness. Replacement of rice flour with INL also significantly reduced springiness in gluten-free layer cakes (Gularte *et al.*, 2012b). Moreover, Baixauli *et al.* (2008) previously found a decrease in springiness with progressive replacement of WF with a resistant starch type, Hi-Maize 260, which was related to a decrease in the number of muffin air bubbles and the existence of a denser matrix.

Total or partial replacement of WF with CF, alone or with biopolymers and their blends, also significantly reduced muffin cohesiveness, except for the 10%WP muffin. Among the various muffins, 15%INL muffins showed the lowest springiness, cohesiveness, chewiness and resilience values (0.197, 0.194, 1.3 N and 0.048 respectively). Chewiness values fell significantly as WF was replaced with CF, either alone or combined with the various biopolymers, indicating that, compared with the control, CF-based muffins were easier to chew. The same behaviour applied to resilience; the biggest effect was also found with 15% INL which caused a more significant decrease in this parameter, meaning that it took longer for the structure of the muffin to recover after compression.

Physical properties of the baked muffin

Physicochemical properties of the baked muffins are shown in Table 2. Weight losses of all muffins were greater after packing and storing for one day. 100%WF and 10%WP muffins showed the highest weight loss values either 1 h after baking or 24 h after packing. The lowest significant weight loss 1 h after baking corresponded to 100%CF muffin. There were significant differences in both weight losses between the 100%WF and 100%CF muffins. The former had higher weight loss values. One day after packing, all CF-based muffins incorporating XG had lower weight losses; this may be attributable to its high water-binding capacity (Singh *et al.*, 2015), which might have made free water unavailable to evaporate. Moreover, 1 h after baking, the 100%CF muffin had quicker collapse than the control, but after further time it stabilized, and there were non-significant differences between the collapse values of the two muffins 24 h after packing. Although muffins containing 0.25% of XG had the lowest collapse values, there were non-significant differences between the collapses 24 h after packing of 100% WF, 100%CF and all the muffins with added XG.

The greatest differences were observed in volume. Gómez *et al.* (2008) reported that the higher protein content and different amino acid composition of CF with respect to WF could affect the volume of the product. All CF-based muffins had significantly lower specific volume than the control. In spite of the increased viscoelasticity conferred by XG to the batters, the corresponding baked muffins had low volume. This result might also be explained by the higher fibre content of CF, resulting in less available water to develop the starch-protein network during baking, thus producing a decrease in muffin volume. A number of interrelated factors account for final volume in baking: the batter's rheological properties (affected by ingredients), the level of air incorporation, and the mixing and homogenization time and speed (Martínez-Cervera *et al.*, 2011). Finally, in comparison with the control, 15%WP muffin crumb had significantly ($P < 0.01$) higher water activity, which may be associated with higher water absorption owing to the higher protein content in this sample.

However, there were no significant differences in the water activity determined in the muffins prepared with WF and CF alone.

Therefore, partial replacement of CF with XG, alone (at 0.5 and 1%) or blended with both WP and INL, produced an increase in the compactness and a decrease in the air bubble size in the resulting batters. It could have been expected that higher batter density would result in higher muffin volume (Baixauli *et al.*, 2008). However, these microstructural changes were not associated to an improvement in the quality characteristics of the baked muffins. During baking process, baking powder generates gases, which should be retained in order to guarantee good muffin volume (Gómez *et al.*, 2008). These results indicated that the bubbles were not retained during baking. One important factor is the T_g of the flours. The starch gelatinization at higher temperatures would prevent the correct expansion of batters. According to Martínez-Cervera *et al.* (2013), the delay in T_g delayed batter thermosetting, which does not favoured bubble retention, although the incorporation of larger amount of leavening could be a possibility for counterbalancing the negative effect of XG on air retention capacity. In this study, gluten removal results in muffins of low quality characteristics, corroborating previous findings.

Sensory evaluation

Two-way ANOVA (samples and assessors) with interaction considering assessors as fixed effect was applied to the sensory scores obtained for each attribute evaluated (data not shown). Assessors were a significant source of variation in the case of some attributes, indicating poor level of agreement among the panel members regarding their evaluations and that additional training in the use of these descriptors could be needed. The trained panel's mean scores for the various descriptors chosen for these muffins are shown in Table S6.

Replacement of wheat flour with chickpea flour and the various biopolymers produced significant variations in all the attributes. A principal component analysis (PCA) was run on the data of the QDA[®] performed by the trained panel (Fig. 4). The biplot explains 52.61% of the variation, corroborating poor consonance between the panellists and thus a need for greater training in the evaluation of muffins. The first component (32.49%) shows positive correlation with the appearance attributes, while the second component (20.12%) is mainly related to taste attributes. Also, in the first dimension there is a separation of samples determined by the addition of XG, positioning these samples in the positive part of the first dimension. In the second dimension there is an opposition between the 100%WF and the 100%CF samples. The muffins with XG possessed the greatest cohesiveness, moisture, sponginess and springiness and were easiest to swallow, giving an impression of a more airy crumb with a more open type of structure, and they also had the highest water content, which could be explained by the ability of XG to hold water, enabling the crumb to recover its initial shape more rapidly and reflecting a better quality. Moreover, the blend of XG and INL produced the highest hardness, colour, height and positive general visual impression, whereas the muffins with a blend of XG and WP were perceived as being more adhesive. Muffins with added WP and INL, either alone or blended, were negatively correlated with the first component and they showed the highest score for difficulty in chewing, making them more difficult to crumble. The sample with 100%CF was perceived as having the greatest chickpea taste and stickiness; the latter may be a negative quality factor. This sample was located opposite the control muffin, which had the greatest typical muffin taste and after-taste. The incorporation of CF resulted in a chickpea-like taste, despite which it was not a driver of disliking for the panellists.

Conclusions

Total replacement of WF with CF and XG, alone (at 0.5 and 1%) or combined with INL or WP, produced a notable improvement in the viscoelastic properties of muffin formulations, either at 25 °C or during heating up to 95 °C, resulting in stronger, more elastic and more time-stable batters than wheat gluten batter with delayed starch gelatinization temperature. SEM images of CF-based formulations with added XG also revealed more homogeneous matrices with numerous small bubbles. In contrast, muffins baked from these batter formulations did not show better instrumental textural properties. Results also show that SG values increase slightly with increased XG content. According to these results, the increase in batter viscoelasticity due to the increase in XG cannot be associated with an increase in the number of air bubbles. Nevertheless, an improvement in sensory quality was perceived; CF-based muffins with added XG alone scored higher for cohesiveness, moisture, sponginess and springiness and were easier to swallow, whereas those made with XG and INL had higher hardness, colour, height and general visual acceptance scores. More research is needed to improve baking performance and muffin technological characteristics, but results show that incorporation of XG alone (at 0.5 and 1%) or combined with INL in CF-based batter could be a potential strategy for achieving good quality gluten-free muffins.

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Legend to Figures

Figure 1 Mechanical spectra for the control wheat flour (WF)-based batter and chickpea flour (CF)-based batters prepared with CF alone and with partial replacement of CF with whey protein (WP) alone at 5, 10 and 15% levels (a, b), with xanthan gum (XG) alone at 0.25, 0.5 and 1% levels (c, d), with inulin (INL) alone at 5, 10 and 15% levels (e, f) and with WP at 10% level blended with XG at 0.5% level, XG at 0.5% level blended with INL at 10% level and WP at 10% level blended with INL at 10% level (g, h). Mean values of nine measurements $\pm$ error bars.

Figure 2 Complex viscosity (η^*) (a), and loss tangent $\tan \delta$ (b) as a function of increasing temperature in the muffin control batter and batters with wheat flour (WF) replaced by chickpea flour (CF) and various percentages of biopolymers. Mean values of nine measurements.

Figure 3 SEM images for the control wheat flour (WF)-based batter (a, b) and chickpea flour (CF)-based batters prepared with CF alone (c, d), with partial replacement of CF with xanthan gum (XG) alone at 1% level (e, f) and with partial replacement of CF with XG at 0.5% level blended with inulin (INL) at 10% level (g, h) ($\times 300$ and $\times 1000$).

Figure 4 Two-dimensional PCA plot of the variations in quantitative descriptive analysis (QDA) among wheat flour (WF), chickpea flour (CF), and biopolymer-incorporated CF-based muffins. Identification of muffin samples as shown in Table 1.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Descriptive terms, scale end terms, and definitions and evaluation methods used in the quantitative descriptive analysis (QDA) of baked muffins rated by the trained panel.

Table S2 Effect of replacement of wheat flour (WF) with chickpea flour (CF) and various biopolymers on limit values of linear viscoelastic (LVE) range from stress sweep tests of the muffin batter at 25 °C.

Table S3 Effect of replacement of wheat flour (WF) with chickpea flour (CF) and various biopolymers on loss tangent and power law parameters from frequency sweep tests of the muffin batter at 25 °C.

Table S4 Effect of replacement of wheat flour (WF) with chickpea flour (CF) and various biopolymers on physicochemical properties of muffin batter at 25 °C.

Table S5 Effect of replacement of wheat flour (WF) with chickpea flour (CF) and various biopolymers on textural properties from TPA tests of the baked muffin at 25 °C.

Table S6 Mean values of the sensory attributes of baked muffins rated by the trained panel in the quantitative descriptive analysis (QDA[®]).

670 **Table 1** Formulations of CF-based gluten-free muffins (g 100 g⁻¹ flour or flour-biopolymer or flour-biopolymer blends)

Formulation	100%WF	100%CF	5%WP	10%WP	15%WP	0.25%XG	0.5%XG	1%XG	5%INL	10%INL	15%INL	10%WP- 0.5%XG	10%WP- 10%INL	0.5%XG- 10%INL
WF	100	0	0	0	0	0	0	0	0	0	0	0	0	0
CF	0	100	95	90	85	99.75	99.5	99	95	90	85	89.5	80	89.5
WP	0	0	5	10	15	0	0	0	0	0	0	10	10	0
XG	0	0	0	0	0	0.25	0.50	1	0	0	0	0.50	0	0.50
INL	0	0	0	0	0	0	0	0	5	10	15	0	10	10
Whole egg	81	81	81	81	81	81	81	81	81	81	81	81	81	81
Sucrose	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Salt	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Milk	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Oil	46	46	46	46	46	46	46	46	46	46	46	46	46	46
Citric acid	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Sodium hydrogen carbonate	4	4	4	4	4	4	4	4	4	4	4	4	4	4

671 WF: Wheat flour; CF: Chickpea flour; WP: Whey protein; XG: Xanthan gum; INL: inulin.

672

673 **Table 2** Effect of replacement of wheat flour (WF) with chickpea flour (CF) and various biopolymers
674 on physicochemical properties of the baked muffin at 25 °C

Type of formula	Weight loss 1 h after baking (g 100 g ⁻¹)	Weight loss 24 h after packing (g 100 g ⁻¹)	Collapse 1 h after baking (mm)	Collapse 24 h after packing (mm)	Specific volume 24 h after packing (cm ³ g ⁻¹)	Water activity 24 h after packing
100%WF	17.4±1.2 ^a	19.0±0.9 ^{a,b}	0.4±0.3 ^{b,c}	1.9±0.9 ^{a-d}	5.1±0.0 ^a	0.804±0.025 ^{b-d}
100%CF	13.1±0.8 ^g	16.8±0.8 ^{c,d}	1.1±0.3 ^a	1.4±0.3 ^{a-d}	2.5±0.0 ^{e,f}	0.836±0.013 ^{a,b}
5%WP	14.8±1.3 ^{b-f}	17.8±1.1 ^{b,c}	0.6±0.1 ^{a-c}	2.5±0.7 ^a	2.9±0.2 ^{c-e}	0.810±0.003 ^{a-d}
10%WP	17.0±0.8 ^a	19.9±0.8 ^a	0.3±0.3 ^{b,c}	1.1±0.6 ^{b-d}	3.3±0.2 ^{b,c}	0.785±0.004 ^d
15%WP	15.2±0.8 ^{b-d}	15.8±0.7 ^{e,f}	1.1±0.6 ^a	2.5±0.8 ^a	3.6±0.2 ^b	0.838±0.004 ^a
0.25%XG	15.0±0.7 ^{b-e}	15.1±0.7 ^{e,f}	0.1±0.1 ^c	0.8±0.4 ^d	2.9±0.1 ^{c-e}	0.793±0.009 ^{c,d}
0.5%XG	14.0±1.0 ^{c-g}	14.8±0.6 ^{e,f}	0.5±0.1 ^{b,c}	1.6±0.7 ^{a-d}	2.9±0.1 ^{c-e}	0.832±0.017 ^{a,b}
1%XG	13.5±0.8 ^{e-g}	14.3±0.4 ^f	0.5±0.2 ^{b,c}	2.2±1.3 ^{a-c}	3.0±0.1 ^{c,d}	0.832±0.006 ^{a,b}
5%INL	15.4±0.7 ^{b,c}	15.8±0.7 ^{d,e}	0.4±0.3 ^{b,c}	1.1±0.6 ^{b-d}	3.0±0.2 ^{c,d}	0.835±0.002 ^{a,b}
10%INL	13.3±0.7 ^{f,g}	14.1±0.5 ^f	0.8±0.2 ^{a,b}	1.0±0.2 ^{c,d}	2.8±0.2 ^{d-e}	0.820±0.014 ^{a-c}
15%INL	13.8±0.3 ^{c-g}	15.2±0.3 ^{e,f}	0.3±0.2 ^{b,c}	1.0±0.3 ^{c,d}	3.0±0.3 ^{c,d}	0.818±0.012 ^{a-c}
10%WP-0.5%XG	13.7±0.8 ^{d-g}	14.7±0.9 ^{e,f}	0.4±0.3 ^{b,c}	2.4±0.9 ^{a,b}	2.4±0.2 ^{f,g}	0.836±0.016 ^{a,b}
10%WP-10%INL	15.9±0.6 ^{a,b}	17.1±0.6 ^{c,d}	0.7±0.4 ^{a,b}	1.1±0.5 ^{b-d}	3.3±0.2 ^{b,c}	0.816±0.010 ^{a-d}
0.5%XG-10%INL	13.5±0.8 ^{e-g}	15.0±0.6 ^{e,f}	0.4±0.3 ^{b,c}	1.9±0.6 ^{a-d}	2.1±0.2 ^g	0.794±0.001 ^{c,d}

675 Values are given as mean ($n = 9$) ± standard deviation.

676 ^{a-g}Different letters in the same column mean significant differences ($P < 0.01$) among
677 samples according to LSD multiple range test.

678 WF: wheat flour; CF: chickpea flour; WP: whey protein; XG: xanthan gum; INL: inulin.

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