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Rheological and microstructural evolution of the most common gluten-free flours and starches during bread fermentation and baking

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Abstract

Mechanistic relations between the evolution of the starch/flour structure, dough rheology and bread quality were investigated using the most common flours and starches in gluten-free bread-making. Micrographs showed that the small wheat starch granules filled the spaces of the big granules, forming a uniform starch-hydrocolloid matrix. This granular advantage decreased the consistency and increased the uniformity of wheat-starch based doughs throughout fermentation, as shown by micrographs and the higher critical strain. The viscoelastic properties of the different doughs strongly influenced the bread volume and the crumb texture. Thus, starch-based breads showed higher specific volume and lower hardness, especially those made with wheat starch, whose lower pasting temperature also reinforced the continuous phase of the crumb. On the other hand, the large potato starch granules did not form a continuous starch-hydrocolloid matrix, resulting in breads with the lowest specific volume, elasticity, cohesiveness and resilience, and the highest hardness.

Keywords: starch, flour, gluten-free, bread, rheology, microstructure

1. Introduction

Gluten plays a principal role in bread development by giving cohesiveness and promoting the retention of the CO_2 produced during fermentation. Thus, gas expansion causes wheat breads to gain volume and attain acceptable crumb texture (Deora et al., 2014). Recently, the market of gluten-free breads has expanded and substantial efforts are underway to enhance their quality.

In wheat-containing doughs, rheological studies are crucial for understanding the functionality of flours and additives as well as predicting the dough machinability and bread quality (Stojceska and Butler, 2012). The small amplitude oscillatory shear (SAOS) technique is ideal to characterize the structural properties of viscoelastic materials (Morrison, 2001). In the last decade, creep-recovery has become another technique used to characterise the structural properties of viscoelastic doughs. It comprises a static rheological method in which an instantaneous stress is applied to the sample and the change in strain is measured over time. A creep phase is usually followed by a recovery phase in which the applied stress is removed (Steffe, 1996).

Studies connecting gluten-free dough rheology with the quality of the resultant bread are scarce. While it is true that numerous works include rheological analyses for dough characterization, mostly comprising SAOS and in lesser extent large deformation analyses (Masure et al., 2016), there are still no universal indicators that well correlate gluten-free dough rheology with the quality of the resultant bread. In some works, an increase in the bread volume was appreciated as viscoelastic moduli (G' and G'') decreased (Mancebo et al., 2015a,b; Rocha-Parra et al., 2015). However, studying different hydrocolloids, Mancebo et al. (2015b) observed that the creep-recovery technique could be more suitable than oscillatory shear tests to predict bread volume.

The absence of a reliable rheological indicator can be attributed to the diverse rheological evolution of the different doughs during processing (fermentation and baking). Rheological attributes of wheat doughs start changing at the beginning of fermentation. This is mainly due to: 1) the CO_2 expansion previously formed within the gas cells and, 2) the pH modification by means of such CO_2 and its influence on the gluten network (Pyler and Gorton, 2008). In gluten-free bread making, without a gluten network sensitive to acidification, non-studied similar rheological phenomena could be produced. As for the baking step, the fermented dough is exposed to heat transfer from the oven, resulting in a chain of phenomena governed by heat and moisture transfers (Le-Bail et al., 2011). At first the heat transfer results in an expansion of the gas cells contained in the fermented dough via: 1) increased CO_2 production by yeast (until yeast inactivation at 50-60°C), 2) gas expansion, 3) vaporization of the CO_2 and solubilized ethanol in the liquid phase of the dough and 4) moisture vaporization (Zhang, Lucas, Doursat, Flick and Wagner, 2007). However, when reaching a certain temperature, the hydrated starch gelatinizes and the protein coagulates, leading to crumb setting (Le-Bail et al., 2011), and therefore to an amorphous structure that covers the gas cells. In particular, wheat, corn, rice and potato starches have been reported to have gelatinization temperature ranges of 58-64, 62-72, 68-78 and 58-68°C, respectively (Biliaderis 2009). This amorphous matrix, formed mainly by gelatinized starch, will be further modified during cooling as starch retrogrades, influencing bread texture.

The most commonly used starches in gluten-free bread-making are maize starch and some starches from tubers, such as potato and tapioca (Masure et al., 2016), despite the growing prominence of the guaranteed gluten-free wheat starch in the last few years (Mancebo et al., 2015a). As for the flours, rice flour is the most commonly used, followed by maize flour, since they are the most highly produced and affordable cereals.

To the best of our knowledge, mechanistic studies, showing the influence of most commonly used starch-based ingredients in gluten-free bread-making on the interplay between dough rheology and bread quality, are scarce.

Starches and flours have extensive microstructural differences at granular structural scales, which can influence their capacity to generate gluten-free breads with high quality standards. However, use of scanning electron microscopy (SEM) as a tool to view gluten-free doughs and breads have been reported on very few occasions (de la Hera et al., 2013; Martinez et al., 2014; O´Shea et al., 2013; Peressini et al., 2011; Yano, 2010). In general, these studies are based on the use of a single gluten-free flour/starch or their combination, i.e., altogether during the mixing process. Nevertheless, comparative studies on the single effect of different starches and flours are scarce, and none of them include rheological and microstructural analysis.

The objective of this study was to obtain a comparative insight of the evolution of the most common flours and starches used in gluten-free bread making during fermentation and baking. In this way, changes produced in the doughs at large structural scales were pictured through SEM during fermentation and related to the evolution of the dough viscoelasticity (SAOS and creep-recovery). In addition, the development of the bread volume and crumb texture and microstructure during baking was also studied. We believed that results could show mechanistic correlations between the development of the starch/flour structure, dough rheology and bread quality, giving valuable information with the aim of predicting the quality of the resultant gluten-free bread.

2. Materials and methods

2.1. Materials

Coarse rice flour and maize flour were supplied by Harinera Castellana SL (Medina del Campo, Spain) and Maiceras Españolas, S.A. (Valencia, Spain), respectively. Wheat and potato starch were provided by Roquette (Lestrem, France) whereas Miwon maize starch (Daesang Co., Seoul, Korea) was purchase from the local market. The rest of ingredients used for bread-making were VIVAPUR 4KM HPMC (Hydroxypropyl Methylcellulose, JRS, Rosenberg, Germany), Saf-Instant dry yeast (Lesaffre, Lille, France), salt (Unión Salinera de España, Madrid, Spain), sucrose (Azucarera, AB, Madrid, España), sunflower ABRISOL (Ourense, Spain) and tap water.

Flour and starch composition was determined using the AACC methods (AACC, 2015) 44-15.02 (moisture content) and 46-30.01 (protein) with a Leco TruSpec device (Leco, St. Joseph, MI, USA). The most outstanding physical properties of the different flours and starches were also characterised to better understand the rheological and microstructural behaviour during fermentation and baking. Particle size was measured with a laser diffraction particle size analyser (Mastersizer 3000, Malvern Instruments, Ltd., Worcestershire, UK). The mean diameter of equivalent volume or mass d(4,3), which indicates the central point of the volume distribution of the particles, was recorded. Water binding capacity, defined as the amount of water retained by the flour/starch after being subjected to centrifugation, was measured as described in the method 56-30.01 (AACC 2015). The pasting properties were analysed using the standard method 61-02.01 (AACC, 2015) with a Rapid Visco Analyser (RVA-4) (Perten Instruments Australia, Macquarie Park, Australia). These analyses were carried out in duplicate. Data are shown in Table 1.

2.2. Methods

2.2.1. Dough preparation and bread-making

The following ingredients were used in bread-making: water (100 g/100 g flour or starch), instant dry yeast (3 g/100 g), salt (1.8 g/100 g), oil (6 g/100 g), HPMC (2 g/100 g) and white sugar (5 g/100 g). In all tests, the water temperature was held between 20 and 22 °C. Yeast was previously dissolved in the water before its incorporation. All the ingredients were mixed for 8 min in a Kitchen Aid 5KSM150 mixer (Kitchen Aid, Michigan, USA) with a dough hook (K45DH) at speed 2. Fermentation was performed at 30 °C and 80 % RH for 90 min. After fermentation, doughs were baked in an electric modular oven for 40 min at 190 °C. Bread-making was performed in duplicate.

For dough evaluation, 100 g of dough obtained after mixing, 45 min and 90 min of fermentation were placed in small aluminium moulds (140x40x35 cm, ALU-Schale, Wiklarn, Germany), introduced into polyethylene plastic bags and immediately frozen at -21°C. Doughs were kept in the freezer during 24 hours before rheological and microstructural analyses.

For bread characterization, 250g of dough obtained after mixing were placed in aluminium moulds (232x108x43.5 cm, ALU-Schale, Wiklarn, Germany) and then fermented and baked following the baking described above. Breadswere taken out from the oven after 20 and 40 min of baking. Subsequently, the loaves were removed from the moulds after a 60-min cooling period. They were then introduced into polyethylene plastic bags and stored at -21 °C during 24h until analysis.

2.2.2. Microstructural analysis of doughs and breads

Dough and bread photomicrographs were taken with Quanta 200FEI (Hillsboro, Oregon, USA) environmental scanning electron microscope (ESEM). Photomicrographs were taken in high vacuum mode. Crumbs pictures were taken from a perpendicular slant to the cell wall, i.e., showing the surface of a gas cell wall. Conversely, crust pictures were taken showing their lengthwise section, in other words, highlighting the thickness of the crust.

2.2.3. Rheological properties of doughs

Before conducting any rheological measurement, doughs were allowed to rest in the measurement position for 10 min as equilibration time, i.e., the necessary time to allow the stresses induced during sample loading to relax. The required equilibration time was selected according to previous time sweep tests carried out within the linear region (1 Pa) at 1 Hz and 25°C during 30 min. The time sweep test showed that in less than 10 min values of G' and G'' became independent of time. After adjustment of the gap, the excess dough was removed and the exposed edges of the samples were always covered with vaseline oil (Panreac Química S.A., Castellar del Valles, Spain) to avoid sample drying during measurements. In this study, yeast-containing doughs were analyzed after kneading (0min of fermentation), 45 and at 90 min of fermentation in order to include the effects of the gas volume and fermentation metabolites. All rheological tests were run in duplicate in a controlled stress a rheometer (Haake RheoStress 1, Thermo Fischer Scientific, Scheverte, Germany) with a titanium parallel plate geometry sensor PP60 Ti (60 mm diameter, and 3 mm gap).

2.2.3.1. Viscoelastic properties

Linear viscoelastic properties were studied by small amplitude oscillatory test (SAOS). Dynamic linear viscoelastic range was estimated by performing a stress sweep from 0.1 to 50 Pa at a frequency of 1 Hz.

Frequency dependence experiments were conducted from 10 to 0.01 Hz at 25 °C. The applied stress was always selected to guarantee the existence of linear viscoelastic response. At least two replicates of each oscillatory shear test were conducted.

2.2.3.2. Creep-recovery test

Creep tests were performed by imposing a sudden step shear stress in the linear viscoelastic region for 60 s. In the recovery phase, the stress was suddenly removed and the sample was allowed to rest for 180 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was performed in duplicate. Creep data were described in terms of creep compliance, J, which is defined as the strain divided by the stress applied (maintained constant during the creep test). Parameters readily available from the creep-recovery curves are the maximum creep compliance (J_{cmax}) and the maximum recovery compliance (J_{rmax}) measured at the end of the creep and recovery phase, respectively. The steady-state compliance (J_e) was calculated by subtracting J_{rmax} from J_{cmax}.

2.2.4. Bread properties

Bread volume was determined using a laser sensor with the Volscan Profiler (Stable Micro Systems, Godalming, UK). The volume measurements were performed on two loaves from each sample of each batch. The specific volume was calculated as the ratio of bread volume to its mass.

Crumb texture was measured with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) equipped with the “Texture Expert” software. A 25-mm diameter cylindrical aluminium probe was used in a “Texture Profile Analysis” (TPA) double-compression test to penetrate up to 50 % of the sample depth at a test speed of 2 mm/s, with a 30 s delay between the two compressions. Firmness (N), elasticity, cohesiveness and resilience were calculated from the TPA curve (Gomez et al. 2007). Texture analyses were performed on 30 mm slices. Analyses were performed on two slices from two loaves from each batch (each formulation). Each batch was made in duplicate (2×2×2).

2.2.5. Statistical analysis

Differences between the parameters for the flours were studied by analysis of variance (ANOVA). Fisher’s least significant difference (LSD) was used to describe means with 95% confidence intervals. The statistical analysis was performed with Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA).

3 Results and discussion

3.1. Microstructural and rheological evolution of doughs during fermentation

3.1.1. Microstructural evolution of doughs

Environmental scanning electron microscopy was used as a tool to investigate some of the phenomena occurring during fermentation in the different doughs, which could support some of the results observed later in the rheological study. In this study, only micrographs of doughs at time 0 and after 90 minutes of fermentation are shown (Fig. 1). In all micrographs, different starch granules appeared loose and embedded in a continuous phase together with the hydrocolloid. Nevertheless, flour-based doughs displayed the contour of large particles covered by starch granules, indicating that flour particles may not have been fully disrupted during the kneading process. In fact, some authors observed that the integrity of maize (de la Hera et al., 2012) and rice (Martinez et al., 2014) flour particles is not fully disrupted during kneading in gluten-free bread-making. Among starches, significant differences were also observed, highlighting the visual effect of the small wheat starch granules filling the spaces of the big granules as well as the large starch granules in doughs made with potato starch. It was assumed that the presence of a bimodal size distribution in wheat starch could be beneficial for packaging and building purposes and therefore for making the continuous starch-hydrocolloid matrix more uniform. On the other hand, it was also expected that the large potato starch granules would be less prone to pack with themselves resulting in a less uniform continuous phase. Thus, the morphological structure of these starches was expected to influence the specific volume of breads.

As the course of fermentation proceeded, in general the hydrocolloid-starch matrix (continuous phase) started to present small ruptures, which were especially noticeable in doughs made with maize and potato starch. The CO_2 expansion within the gas cells (Masure et al., 2016) could weaken the hydrocolloid network in which starch granules or flour particles are embedded, making the dough less consistent as the fermentation proceeds. However, doughs made with wheat starch did not show a significant number of discontinuities, probably as a consequence of the positive interaction between small and large starch granules, which could reinforce the system.

3.1.2. Dynamic linear viscoelastic range

Critical amplitudes of the shear stress (σ_c) and strain (γ_c) for the onset of the non-linear response were estimated from the normalized plot of G' and G'', taking as reference the average of their initial values at the lower torques reached by the rheometer (Table 2). Doughs made with flours, both maize and rice flours, showed a much higher σ_c than doughs made with starches. However, no clear differences were observed for γ_c, highlighting only the higher critical amplitude of the shear strain for wheat starch dough. As shown in Fig. 1., doughs made with wheat starch were more uniform, probably as a consequence of the positive packing properties of their granules, i.e., small granules filling the interstitial spaces of large ones. This could bring about doughs with higher resistance to strain during the strain sweep. On the other hand, the higher σ_c of doughs made with flours suggested more resistance to the applied stress than those made with starch. As seen in Table 1, maize and rice flours have an important fraction of protein compared to starches. However, maize and rice storage proteins are entrapped in protein bodies that need to be disrupted and freed during mixing to be functional

(Taylor et al., 2015). This disruption of the protein bodies has only been observed in maize under conditions when high mechanical energy (specific mechanical energy of ≥100 kJ/kg) was applied using extrusion cooking (Batterman-Azcona et al., 1999) or roller flaking (Batterman-Azcona and Hamaker, 1998). However, Gayral et al., (2016) reported that the protein included in the starch channels of flours that contain proteins could strengthen protein adhesion to the granule surface fostering granule-granule associations. Therefore, we believe that the high stability to shear stress of flour-based doughs can be attributed to the intrinsic size of the flour particle and its resistance to disruption compared to starch granules (Fig. 1).

As for the fermentation time, σ_c did not show significant differences, whereas only flours at time 0 of fermentation showed a significantly higher critical strain (γ_c) than after 45 and 90 min of fermentation, indicating that the dough structure can be broken with lower strains once fermentation starts. The CO_2 expansion previously formed within the gas cells could weaken the hydrocolloid network in which starch granules or flour particles are embedded, as seen in Fig. 1, causing the dough to be less resistant to strain as fermentation proceeds. This behaviour was similar in all doughs indicating no interactions between the type of starch and the fermentation time.

3.1.3. Mechanical spectra

The above interpretation is more clearly supported by the analysis of the mechanical spectra (Fig. 2). The plateau relaxation zone was observed in the analysed frequency window for doughs made with flours, both maize and rice. This region is characterised by the fact that G' is higher than G'', with both moduli depending on frequency but following a different pattern (Martinez et al., 2015a). This region is also characteristic of the occurrence of physical entanglements in polymeric materials (Ferry, 1980). In this case, it may be attributed to the packing effect of CO_2 bubbles surrounded by starch granules and flour particles as well as to the contribution of the network formed between hydroxypropyl methylcellulose (HPMC) macromolecules and starch granules. A different behaviour was found for doughs made with starch, since a crossover between G' and G'' was observed at low frequencies. This crossover corresponds to the end of the plateau region and to the beginning of the terminal zone of the relaxation spectrum. In solid foams, such as doughs/breads, when the average size of the starch granules (sub-micron scale) is at least one order of magnitude than the droplet of the discontinuous phase (millimetre scale gas cells), Pickering stabilization could be observed, as Dickinson (2012) suggested with starch particles in food emulsions. This suggests that the dispersed particles in the continuous phase would accumulate at the gas-continuous phase interphase to form a mechanical (steric) barrier that protects the gas cells against coalescence. In other words, the smaller particle size of starch granules compared to flour particles could increase the Pickering stabilization of the dough, shifting the plateau relaxation zone to lower frequencies (i.e., the terminal zone to higher frequencies). This transition occurred at higher frequencies for potato starch dough. Potato starch has a B-type crystalline polymorphism (Perez et al., 2009), characteristic of the absence of pores in the granular surface that leads to granules with low water absorption capacity (see also Table 1). In addition, potato starch granules are larger than the cereal ones (Table 1, Fig. 1). These structural differences could change the behaviour of the continuous phase of the dough compared to the rest of the starches (yielding a narrower plateau region) through a lower granule packing as well as a lower density of entanglements among biopolymer molecules in the continuous phase. These explanations would also explain the higher loss tangent values for potato doughs, indicating lower dough elasticity (Table 2).

It is noteworthy that as fermentation proceeded, the crossover was shifted to lower frequency values (widening the plateau region), which likely depended on the Pickering stabilization of the dough by the particles suspended in the continuous phase. This would suggest a gradual increase of the CO_2 bubble packing and a lower intensity of HPMC-starch entanglements throughout fermentation.

As for the individual contribution of the viscoelastic moduli, flour-based doughs showed higher viscoelastic moduli than the starch doughs, indicating a higher consistency of doughs made with flours. This phenomenon could be attributed to the larger particle size and the protein adhesion (Gayral et al., 2016), which is in agreement with what was mentioned before. This could foster granule-granule interactions within the flour particle (contours of large particles covered by starch granules are observed in Fig. 1), reinforcing the flour particle during kneading and therefore raising the individual contribution of viscoelastic moduli of the dough. Differences were also observed among the different starches, highlighting that wheat starch-based doughs had lower viscoelastic moduli (less consistency). Wheat starch possesses lower water absorption capacity than maize starch (Table 1). This, along with its bimodal size distribution, could promote greater continuity of the continuous phase and density of the dough structure. In other words, smaller granules would fit into the spaces between the larger ones, bringing about a gluten-free dough with lower consistency. Micrographs observed in Fig. 1 also depict this occurrence. It is noteworthy that the small wheat starch granules would be more prone for Pickering stabilization of the CO_2 bubbles of the dough. This property should be taken into account for attaining breads with high specific volume, as will be shown later in this study.

As predicted, the dough viscoelasticity also changed during the course of fermentation, decreasing over time. This suggests a decrease in dough elasticity with fermentation, which is in agreement with the observed shift of the crossover to lower frequencies. As mentioned, this can suggest a gradual increase of the CO_2 bubble packing and a lower intensity of HPMC entanglements (see also Fig. 1).

3.1.4. Creep-recovery test

The ability of doughs to recover some structure by storing energy was analysed by applying an instantaneous stress and measuring the change in strain over time (Fig. 2). This was performed as a secondary analysis for the dough elasticity. The creep recovery curves of gluten-free doughs exhibited a typical viscoelastic behaviour combining both viscous fluid and elastic responses (Lazaridou et al., 2007). Doughs made with flours exhibited lower compliance values in both creep and recovery phases. This occurrence is in agreement to what was observed in another study comparing rice flour with other starches (Mancebo et al., 2015a) and in the mechanical spectra of the current work. Again, this would indicate higher dough consistency (Edwards et al., 2003). Among starches, wheat starch displayed higher compliance values than maize starch at the three fermentation times, which is in agreement with the low consistency (low viscoelastic moduli) of wheat starch doughs observed in the mechanical spectra. As for the potato, a different trend was exhibited, with the highest compliance values at time 0 of fermentation. However, in this case, and converse to the rest of the samples, a strong increase of the compliance as the fermentation proceeded was not observed. This event could be due to the large size of potato granules with the absence of superficial pores, which could make dough less efficient in terms of granule packing and forming a continuous phase.

An additional parameter that can be extracted from the creep recovery test is the difference between the compliance value at the terminal region of the curve, where dough recovery has reached equilibrium, and the maximum compliance reached at the end of the creep phase, called steady state compliance (J_e) (Lazaridou et al., 2007). This value is an indicator of the elasticity of the dough. In Fig. 2, higher steady state compliance is observed for doughs made with wheat starch, which could be explained through the mechanisms discussed in the previous sections.

3.2. Physical and microstructural evolution of breads during baking

3.2.1. Microstructural evolution of bread crumb

During baking, the structural and physical properties of bread change, wherein semisolid dough transforms to bread with soft inner crumb and crispy outer crust. The magnitude of these transformations in gluten-free breads will especially depend on the starch properties. The crumb development of the different breads during baking was visually monitored through SEM (Fig. 4). In all the samples, images were taken perpendicularly to the cell walls to observe their surface. All pictures showed the presence of a continuous matrix formed by the starch and hydrocolloid, but in contrast to dough micrographs, the granules were more tightly compacted. Numerous physiochemical and biological transformations, mainly CO_2 release, gas volume expansion, water evaporation and starch gelatinization, take place during bread-baking process (Chhanwal and Anandharamakrishnan, 2015). Doughs made with starches presented a more uniform continuous phase than flour-based crumbs, especially those made with wheat starch. It seems that the building and packing features of the bimodal sized wheat starch together with its lower pasting temperature (Figs. 1, 2, 3) contributed to create a continuous phase that, after gelatinization, will lead to a continuous crumb structure (precursor for an acceptable crumb cohesiveness and resiliency). It is noteworthy that the large starch granules observed in the potato sample still looked perfectly rounded, indicating that they probably were not fully gelatinised during baking. As the course of baking progressed, the temperature increase initiated water evaporation and carbon dioxide release, which resulted in oven spring during initial baking stage. Carbon dioxide release triggered the upper expansion of the top crust and concurrently the development of crumb. Structural changes occur during the whole bread-baking process and they comprise mainly solidification and expansion. The network-like structure of bread crumb is predominantly due to starch gelatinization (Zhou and Therdthai, 2007), as shown in Fig. 4.

The development of the crust microstructure during baking was also studied. The doughs made with flours exhibited a structure formed by the starch granules surrounded by a protein matrix in which intact flour particles were still visible. On the other hand, the crust section of doughs made with starch appeared slightly less uniform. Micrographs also showed that starch did not gelatinize, forming a compact external layer. In the crust, water evaporates quickly, leaving the starch with no available water for gelatinization. In addition, steam was not applied at the beginning of baking, which was already reported to promote starch gelatinization in the crust (Altamirano-Fortoul et al., 2012; Le-Bail, et al., 2011). However, significant changes were not visible and a clear trend was not observed (Supplementary material).

3.2.2. Physical properties of breads

The effect of the type of starch source and the baking time on the specific volume and crumb texture is shown in Table 3. Breads made with flours had less specific volume than those made with starch. This could be related to the high consistency of flour-based doughs, i.e., high viscoelastic moduli and low maximum creep compliance (Martinez et al., 2015b). As mentioned, it can be attributed to the bigger particle size and the presence of a protein layer observed in Fig. 1. In particular, breads made with maize flour exhibited the lowest specific volume, which could be due to the higher water absorption capacity of maize flour compared to rice flour (Table 1). Meanwhile, starch-based breads showed a higher specific volume, especially wheat starch-based breads, followed by the bread made with maize starch. This is in agreement with the previous results obtained in the rheological analysis, where wheat starch-based doughs had lower consistency, i.e., lower viscoelastic moduli (Fig. 2), better packing properties and capacity to form a uniform continuous matrix in the dough (Figs. 1, 4). In addition, the lower pasting temperature indicates that wheat starch starts to gelatinize earlier, leaching amylose that could increase the viscosity and elasticity of the continuous starch-hydrocolloid continuous phase (Table 1). Also in good correlation with the rheological and microstructural analysis, potato starch-based breads had the lowest specific volume among the starch-based breads. This occurrence can be attributed to the large granular size of potato starch, which prevents the starch from forming an acceptable continuous phase with the rest of the dough/crumb components.

Specific volume was inversely correlated with crumb hardness. This reciprocal relationship has been reported in previous studies on gluten-free bread (Gallagher et al., 2003), and it was attributed to the lower resistance to dough deformation, with a higher percentage of air content. In general, starches showed a softer crumb with higher elasticity and resilience than flours. Again, wheat starch crumbs showed the best textural properties (lower hardness and higher elasticity, cohesiveness and resilience), likely attributed to the contribution of the wheat starch structure..

The development of the volume and textural parameters of breads along the course of fermentation is also shown (Table 3). Crumb elasticity, cohesiveness and resilience were not changed from 20 min to the end of fermentation. According to the results, it seems that some attributes of crumb structure are formed at the early stage of the fermentation and then they remain constant. However, bread volume increased over fermentation, leading to softer crumbs, indicating that some changes occur during the entire baking process. These changes produced in the structure of bread crumb are predominantly due to starch gelatinization (Zhou and Therdthai 2007).

4 Conclusions

Changes produced during the fermentation and baking of gluten-free breads depended on the structure and morphology of starch granules and flour particles. In general, results showed that the large and compact flour particles partially maintained their integrity during the kneading process causing doughs to be more consistent and resistant to shear stress. This led to breads with lower volumes and textural properties. On the other hand, the granular morphology, size, water absorption capacity and pasting temperature affected the way the starches interacted. In this way, the bimodal size distribution of wheat starch was more prone to form a uniform continuous starch-hydrocolloid matrix which was further enhanced during baking as a consequence of the low pasting temperature of wheat starch, entailing earlier amylose leaching. This led to a dough with low consistency but high capacity to retain CO_2 during fermentation, resulting in breads with the highest specific volume and the best textural parameters (low hardness and high elasticity, cohesiveness and resilience). These mechanistic relations between the development of the starch/flour structure, dough rheology and bread quality during bread-making will provide useful information for the gluten-free bread-making industry.

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References

AACC, (2015). Approved Methods of the American Association of Cereal Chemists, Methods 44-15.02 (Moisture), 46-30.01 (protein), 56-30.01 (water binding capacity), 61-02.01 (Rapid Visco Analysis), eleventh ed. American Association of Cereal Chemists, St. Paul, MN.

Altamirano-Fortoul, R., Le-Bail, A., Chevallier S., & Rosell, C.M. (2011). Effect of the amount of steam during baking on bread crust features and water diffusion. *Journal of Food Engineering*, 105, 379-385.

Batterman-Azcona, S. J., & Hamaker, B. R. (1998). Changes occurring in protein body structure and α-zein during cornflake processing. *Cereal Chemistry*, 75, 217-221.

Batterman-Azcona, S. J., Lawton, J. W., & Hamaker, B. R. (1999). Effect of specific mechanical energy on protein bodies and α-zeins in corn flour extrudates. *Cereal Chemistry*, 76, 316-320.

Biliaderis, C. (2009). Structural transitions and related physical properties of starch. In J. BeMiller, R. Whistler, (Eds.), Starch. Chemistry and Technology (pp. 149-191). Academic Press, New York. USA. Chhanwal, N., & Anandharamakrishnan, C. (2015). Temperature- and moisture-based modeling for prediction of starch gelatinization and crumb softness during bread-baking process. *Journal of Texture Studies*, 45, 462-476.

de la Hera, E., Martinez, M., & Gomez, M., (2013). Influence of flour particle size on quality of gluten-free rice bread. *LWT Food Science and Technology*, 54, 199-206.

de la Hera, E., Talegon, M., Caballero, P., & Gomez, M. (2012). Influence of maize flour particle size on gluten-free bread-making. *Journal of the Science of Food and Agriculture*, 93, 924-932.

Deora, N. S., Deswal, A., & Mishra, H. N. (2014). Alternative approaches towards gluten-free dough development: recent trends. *Food Engineering Reviews*, 6, 89-104.

Dickinson, E. (2012). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. *Trends in Food Science & Technology*, 24, 4-12.

Edwards, N. M., Mulvaney, S. J., Scanlon, M. G., & Dexter, J.E. (2003). Role of gluten and its components in determining durum semolina dough viscoelastic properties. Cereal Chemistry, 80, 755-763.

Ferry, J. D. (1980). Viscoelastic properties of polymers (3rd ed.). New York: John Wiley & Sons Inc.

Gallagher, E., Gormley, T. R., & Arendt, E. K. (2003). Crust and crumb characteristics of gluten-free breads. *Journal of Food Engineering*, 56, 153-161.

Gayral, M., Gaillard, C., Bakan, B., Dalgalarrondo, M., Elmorjani, K., Delluc, C., Brunet, S., Linossier, L., Morel, M-H., & Marion, D. (2016). Transition from vitreous to floury endosperm in maize (Zea mays L.) kernels is related to protein and starch gradients. *Journal of Cereal Science*, 68, 148-154.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten free formulations. *Journal of Food Engineering*, 79, 1033-1047.

Le-Bail, A., Dessev, T., Leray, D., Lucas, T., Mariani, S., Mottollese G., & Jury, V. (2011). Influence of the amount of steaming during baking on the kinetic of heating and on selected quality attributes of bread. *Journal of Food Engineering*, 105, 379-385.

Mancebo, C. M., Merino, C., Martínez, M. M., & Gómez, M. (2015a). Mixture design of rice flour, maize starch and wheat starch for optimization of gluten free bread quality. *Journal of Food Science and Technology*, 52, 6323-6333.

Mancebo, C. M., San Miguel, M. A., Martínez, M. M., & Gómez, M. (2015b). Optimisation of rheological properties of gluten-free doughs with HPMC, psyllium and different levels of water. *Journal of Cereal Science*, 61, 8-15.

Martínez, M., Oliete, B., Roman, L., Gomez, M. (2014). Influence of the addition of extruded flours on rice bread quality. *Journal of Food Quality*, 37, 83-94.

Martinez, M. M., Sanz, T., & Gomez, M. (2015a). Influence of wheat flour subjected to different extrusion conditions on the rheological behaviour and thermal properties of batter systems for coating. *LWT-Food Science and Technology*, 64, 1309-1314.

Martínez, M. M., Díaz, A., & Gómez (2015b). Effect of different microstructural features of soluble and insoluble fibres on gluten-free dough rheology and bread-making. *Journal of Food Engineering*, 142, 49-56.

Masure, H. G., Fierens, E., & Delcour, J. A. (2016). Current and forward looking experimental approaches in gluten-free bread making research. *Journal of Cereal Science*, 67, 92-111.

Morrison, F.A. (2001). Understanding Rheology. Oxford University Press, New York.

O'Shea, N., Doran, L., Auty, M., Arendt, E. & Gallagher, E. (2013). The rheology, microstructure and sensory characteristics of a gluten-free bread formulation enhanced with orange pomace. *Food and Function*, 4, 1856-1863.

Peressini, D., Pin, M., & Sensidoni, A. (2011). Rheology and bread-making performance of rice-buckwheat batters supplemented with hydrocolloids. *Food Hydrocolloids*, 25, 340-349.

Perez, S., Baldwin, P. M. & Gallant, D. J. (2009). Structural Features of Starch Granules I. In J. BeMiller, R. Whistler, (Eds.), Starch. Chemistry and Technology (pp. 149-191). Academic Press, New York. USA.

Pyler, E. J., & Gorton, L. A. (2008). Baking Science & Technology. Vol I: Fundamentals & Ingredients. Sosland Publishing company. Kansas City, MO

Rocha-Parra, A. F., Ribotta, P. D. & Ferrero, C. (2015). Apple pomace in gluten-free formulations: effect on rheology and product quality. *International Journal of Food Science and Technology,* 50, 682-690.

Steffe, J. F. (1996). Rheological Methods in Food Engineering. second ed. Freeman Press, East Lansing.

Stojceska, V., & Butler, F. (2012). Investigation of reported correlation coefficients between rheological properties of the wheat bread doughs and baking performance of the corresponding wheat flours. *Trends in Food Science & Technology*, 24,13-18.

Taylor, J. R. N., Taylor, J., Campanella, O., & Hamaker, B. R. (2015). Functionality of the storage proteins in gluten-free cereals and pseudo-cereals in dough systems. *Journal of Cereal Science*, 67, 22-34.

Wolter, A., Hager, A. S., Zannini, E., & Arendt, E. K. (2013). *In vitro* starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread. *Journal of Cereal Science*, 58, 431-436.

Yano, H. (2010). Improvements in the bread-making quality of gluten-free rice batter by glutathione. *Journal of Agricultural and Food Chemistry*, 58, 7949-7954.

Zhang, L., Lucas, T., Doursat, C., Flick, D., and Wagner M. (2007). Effects of crust constraints on bread expansion and CO_2 release. *Journal of Food Engineering,* 80, 1302-1311.

Zhou, W., & Therdthai, N. (2007). Three-dimensional modeling of a continuous industrial baking process. In D.W. Sun, (Ed.) Computational Fluid Dynamics in Food Processing (pp. 287-312). CRC Press, Boca Raton, FL.

Figure captions

Figure 1. Micrographs of doughs at the beginning (0 min) and at the end (90 min) of the fermentation.

Figure 2. Mechanical spectra of doughs after fermenting for 0 (clear grey lines), 45 (dark grey lines) and 90 min (black lines). G’ and G’’ are displayed with continuous and discontinuous lines, respectively.

Figure 3. Creep-recovery curves of doughs after fermenting for 0 (clear grey lines), 45 (dark grey lines) and 90 min (black lines).

Fig. 4. Micrographs of bread crumb from a slant perpendicular to the cell wall after 20 and 40 min of baking.

Supplementary material I. Micrographs of the crust section of breads after 20 and 40 min of baking.

Table 1. Composition and physical properties of the different flours or starches

Starch-based ingredient	Moisture (g water/100 g)	Protein (g protein/100 g)	D(4,3) (µm)	WBC (g water/g solid)	PT (°C)	PV (cP)	BR (cP)	FV (cP)
Maize flour	9.37	6.1	189.0	1.421	73.55	3535	1135	5472
Rice flour	8.70	7.8	205.0	1.291	70.20	3082	1482	3169
Maize starch	10.54	n.d.	17.5	1.337	75.20	4988	2207	4435
Wheat starch	11.10	n.d.	21.3	0.626	57.40	5697	2149	6329
Potato starch	14.66	n.d.	43.6	0.171	65.30	12143	9996	4111

D(4,3), De Brouckere mean diameter; WBC, Water binding capacity; PT, Pasting temperature; PV, Peak viscosity; BR, Breakdown; FV, Final viscosity

Table 2. Effect of the origin of the starch-based ingredient and fermentation time on the viscoelasticity of gluten-free doughs

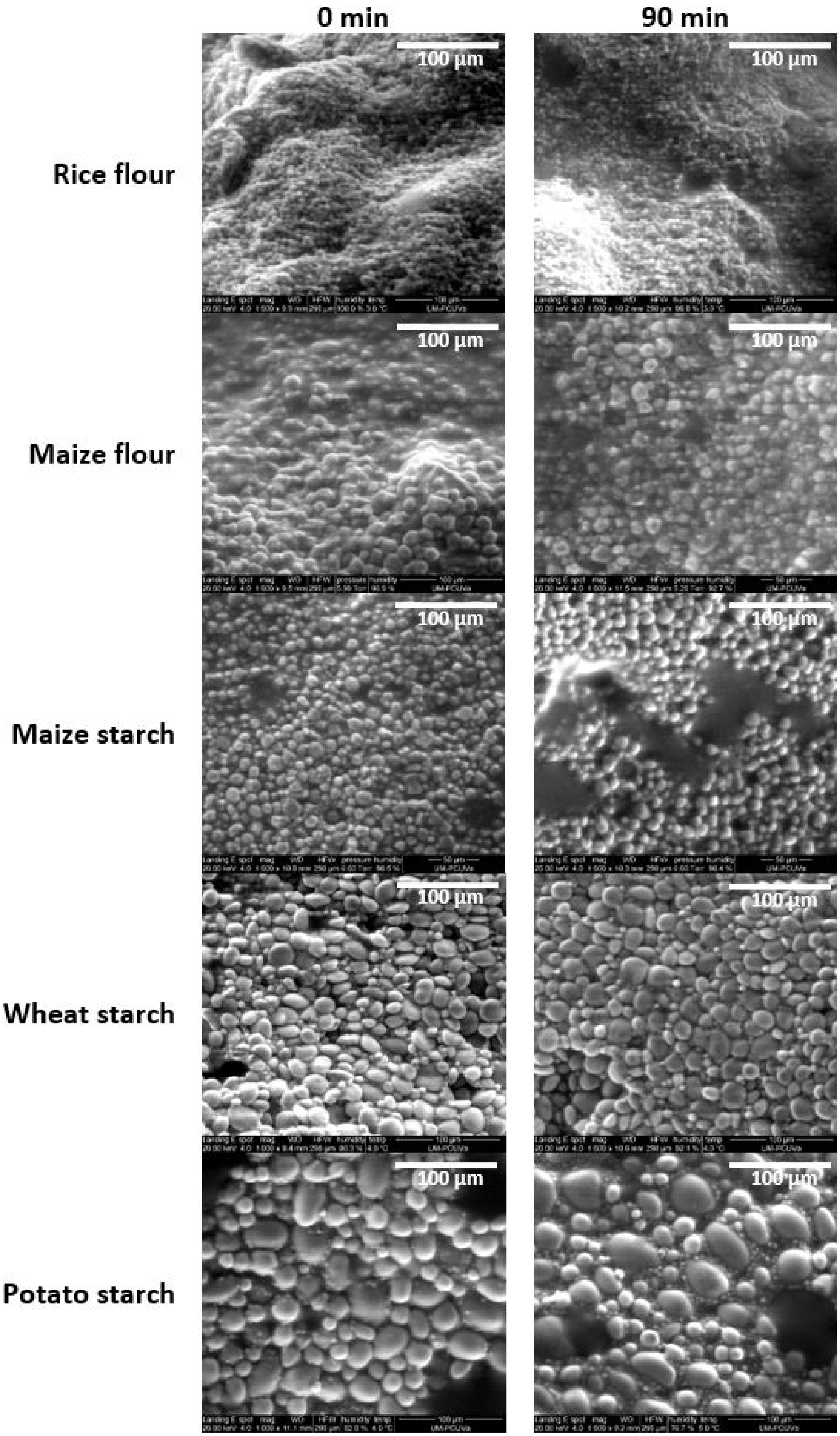
	Starch-based ingredient					**Fermentation time (min)**		
	Maize flour	**Rice flour**	**Maize starch**	**Wheat starch**	**Potato starch**	**0**	**45**	**90**
Critical Stress (Pa)	5,78bc	7,28c	1,08a	1,61ab	1,89ab	3,95a	2,91a	3,73a
Critical Strain	0,001839ab	0,001438ab	0,001100a	0,003862c	0,002232b	0,002803b	0,001798a	0,001681a
tan δ	0,535a	0,525a	0,723b	0,782b	0,957c	0,723ab	0,742b	0,648a

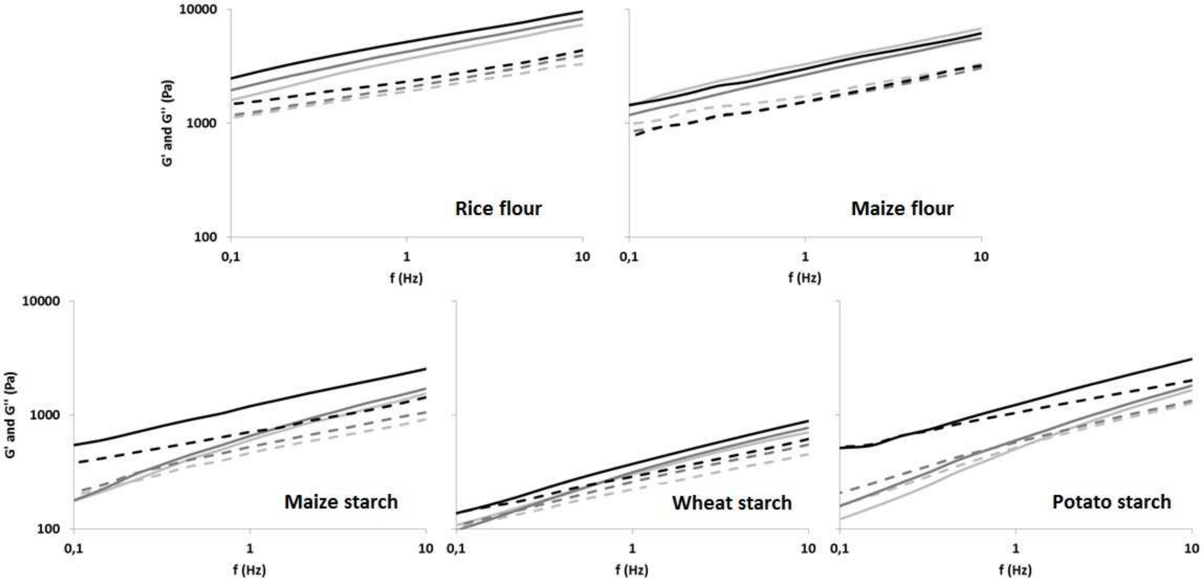
Values followed by the same letters within each parameter for each factor (starch-based ingredient and fermentation time) indicate no significant differences.
tan δ, loss factor

Table 3. Effect of the origin of the starch-based ingredient and the baking time on the volume and texture of gluten-free breads

	Starch-based ingredient					**Baking time (min)**	
	Maize flour	**Rice flour**	**Maize starch**	**Wheat starch**	**Potato starch**	**20**	**40**
Specific volume (mL/g)	2,18a	4,69b	7,14d	8,40e	6,64c	5,10a	6,52b
Hardness (N)	6,733b	0,732a	1,250a	0,957a	0,877a	1,71a	2,51b
Elasticity	0,750a	0,833b	0,955c	0,983c	0,956c	0,887a	0,904a
Cohesiviness	0,322a	0,576b	0,560b	0,681c	0,588b	0,545a	0,546a
Resilience	0,141a	0,327b	0,415c	0,568d	0,405bc	0,368a	0,374a

Values followed by the same letters within each parameter for each factor (starch-based ingredient and baking time) indicate no significant differences.


0 min
90 min
Rice flour
Maize flour
Maize starch
Wheat starch
Potato starch
100 µm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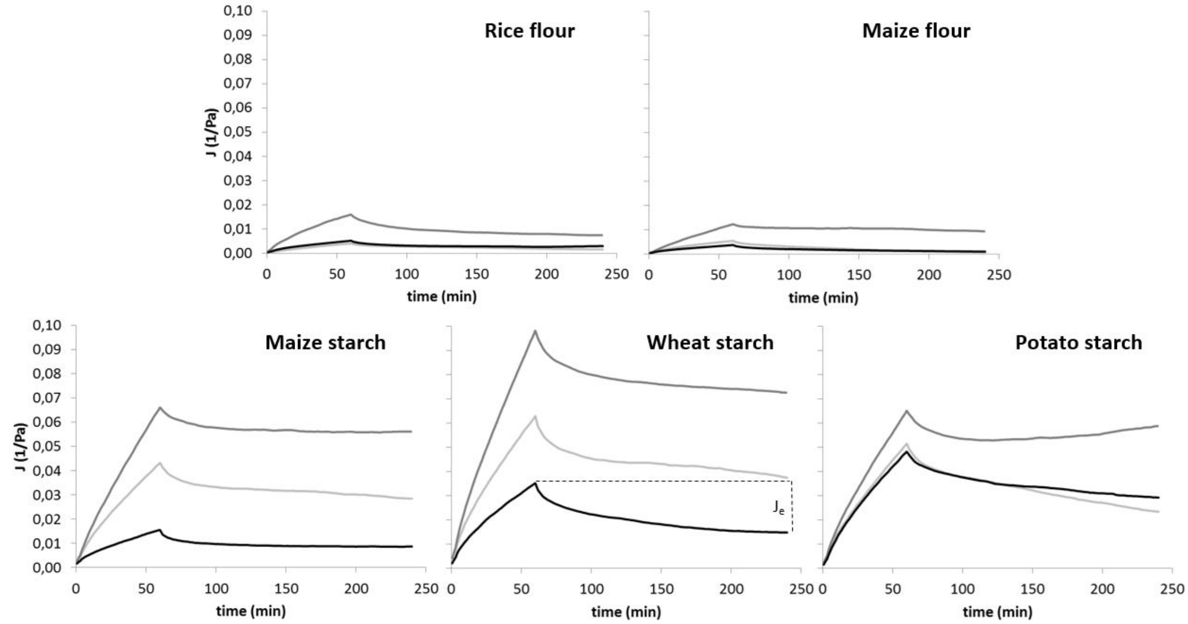
f (Hz)
Rice flour
Maize flour
Maize starch
Wheat starch
Potato starch


Rice flour
Maize flour
Maize starch
Wheat starch
Potato starch
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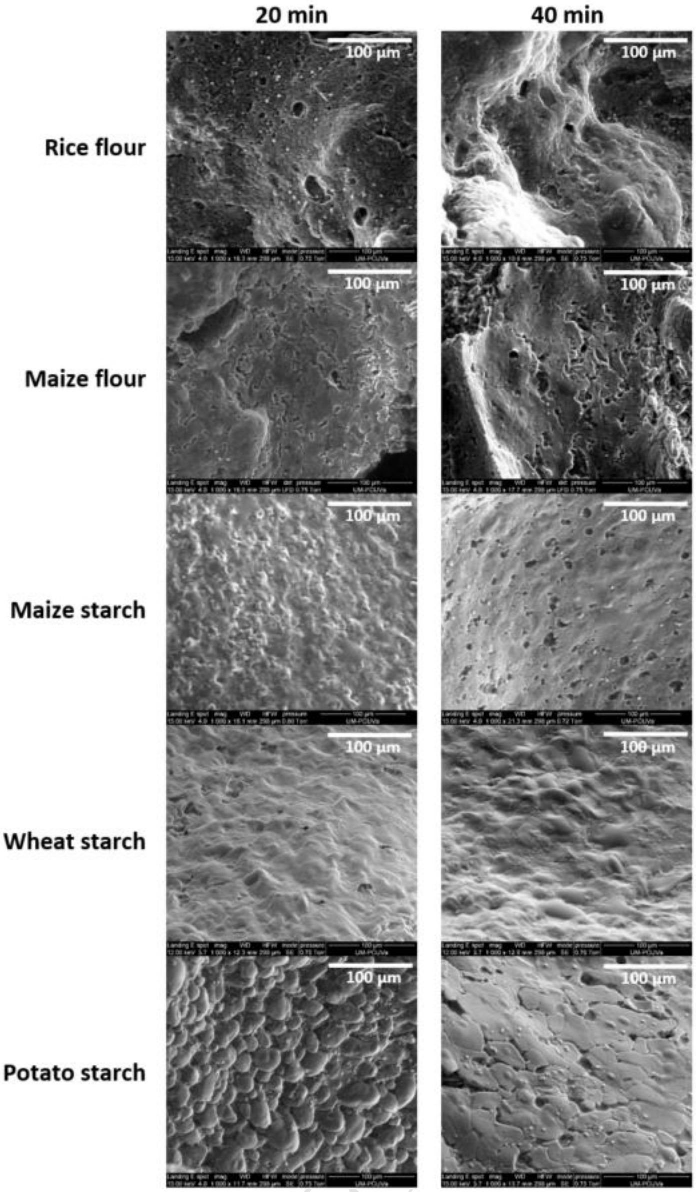
time (min)
0
50
100
150
200
250
J_e


20 min
40 min
Rice flour
Maize flour
Maize starch
Wheat starch
Potato starch
100 µm

Highlights

The rheological evolution of gluten-free doughs during fermentation was studied

The textural evolution of gluten-free breads during baking was studied

Mechanistic relations among starch, dough rheology and bread quality were obtained

Doughs with low consistency and uniform continuous phase provided high volume breads

Wheat starch was prone to form a continuous phase that increased bread quality