Wageningen Academic Publishers

Production and characterisation of gluten-free chestnut sourdough breads

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Received: 13 April 2015 / Accepted: 25 August 2015

RESEARCH ARTICLE

Abstract

The impact of sourdough amount (20, 40 and 60%) on the rheological behaviour of gluten-free chestnut-rice dough formulations and quality parameters (pH, total titratable acidity, firmness, volume and crumb microstructure) of the corresponding breads was studied. The addition of sourdough led to decreases in complex modulus indicating a softening of the dough samples. Higher levels of sourdough addition had a detrimental effect on the volume and texture of breads. X-ray micro computed tomograph images showed that gluten-free chestnut-rice breads prepared with a sourdough incorporation of 20% had the most homogenous structure with high number of smaller pores. Inferior crumb structures were observed with the further addition of sourdough. Confocal laser scanning microscopy revealed that the proteins in breads prepared with sourdough were smaller due to their degradation into smaller peptides by the sourdough fermentation. These changes resulted in an improvement of the bread quality.

Keywords: confocal laser microscopy, firmness, rheology, volume, X-ray μCT

1. Introduction

Celiac disease, which is an autoimmune disorder, occurs in genetically vulnerable individuals exposed to cereal gluten proteins, in particular wheat proteins. This gluten-sensitive enteropathy is controlled by a combination of genetic and environmental risk factors; hence a permanent withdrawal of gluten from celiac patients' diet is required throughout their life-span. Since wheat gluten is able to create a continuous protein network which helps to retain gas produced from yeast fermentation and oven rise, many gluten-free baked products on the market are of poor quality with low volume, poor texture and flavour as well as fast staling. Thus, gluten replacement is still remaining to be one of the most challenging tasks for cereal technologist and scientists (Moore *et al.*, 2008).

Sourdough, a mixture of flour and water fermented with lactic acid bacteria and/or yeast, are applied to enhance the quality of wheat breads (Moroni *et al.*, 2009). In order to meet the growing interests of celiac patients for high quality and healthier products, this old biotechnological process has recently been applied as a new approach in gluten-free bread-making. It has been hypothesised that the problems associated to gluten-free dough and bread features may be overcome by the application of sourdough fermentation. The rheological properties of gluten-free dough as well as the quality of gluten-free breads in terms of volume, texture, crumb structure, flavour and nutritional value may be improved by the application of this technology (Moroni *et al.*, 2011; Schober *et al.*, 2007; Üçok and Hayta, 2015; Zannini *et al.*, 2012). In addition, the positive effect of sourdough on the shelf life by retarding the staling process as well as by protecting gluten-free bread from mould and bacterial spoilage have also been demonstrated by researchers (Moore *et al.*, 2007).

According to FAO statistics, the Republic of Korea and China are the top producers of chestnut with its production about 43% of the world's chestnuts. Other major chestnutproducing countries are Italy and Turkey with its production about more than 25% of the world's chestnuts (http://faostat.fao.org/site/339/default.aspx). Chestnut flour comprises high quality proteins with essential amino acids (4-7%), relatively high amount of sugar (20-32%), starch (50-60%), dietary fibre (4-10%), a low amount of fat (2-4%) and also some important vitamins such as vitamins E, C, B group and minerals such as potassium, phosphorous, magnesium, calcium, copper, iron, manganese and sulphur (Chenlo *et al.*, 2007; Demirkesen *et al.*, 2010). In addition, it has some important phenolics compounds (gallic and ellagic acid) that have shown to be positive health effects upon consumption (Blaiotta *et al.*, 2012). Beside chestnut flour's health and nutritional benefits, the ingredients of chestnut flour may positively influence functional properties of dough (Chenlo *et al.*, 2007; Demirkesen *et al.*, 2010; Moreira *et al.*, 2010, 2013). While the fibre content of chestnut flour may provide emulsifying, stabilising, texturizing and thickening properties to dough, the sugar content of chestnut flour may improve the colour and flavour properties of gluten-free products (Demirkesen *et al.*, 2010). However, it has been reported that high amounts of chestnut flour might deteriorate the product quality giving low specific volume, firmer texture, dark colour and bitter taste (Demirkesen *et al.*, 2010). It hypothesised that these drawbacks associated with using only chestnut or rice flour can be overcome by the potential synergistic effect of using of chestnut and rice flour together in the formulation (Demirkesen *et al.*, 2010, 2013, 2014a; Moreira *et al.*, 2012). It has been observed that gluten-free breads prepared using rice-chestnut flour mixtures have higher volume and softer texture compared with those prepared with only chestnut or rice flour (Demirkesen *et al.*, 2010, 2011). Since the higher fibre content and larger starch granules of chestnut flour contribute towards the stabilisation of gas bubbles, breads prepared with the blend of chestnut and rice flours have shown better crumb structures (Demirkesen *et al.*, 2013). In a recent study, it has also been observed that staling of gluten-free rice breads was delayed by the replacement of rice flour with chestnut flour (Demirkesen *et al.*, 2014a). The main objective of this study was to assess the effect of different amounts of sourdough addition on the rheological behaviour of gluten-free chestnut-rice dough formulations as well as on the quality parameters (pH, total titratable acidity (TTA), texture, volume, crumb microstructure) of chestnut rice breads.

2. Materials and methods

Materials

Chestnut flour was supplied by Kafkas Pasta Şekerleme San. & Tic. A.Ş. (Karacabey, Bursa, Turkey). Bob's Red Mill Organic Brown Rice Flour (Milwaukie, OR, USA) was obtained from a local market. Sugar (sucrose), salt, vegetable oil (Market Pantry® vegetable oil, MN, USA) and instant yeast (Red Star Yeast & Products, Milwaukee, WI, USA) were purchased from local markets. A gluten-free commercial starter named brown rice sourdough starter (*Lactobacillus plantarum*) specifically designed for use in gluten-free sourdough breads was used. Emulsifier DATEM (diacetyltartaric acid esters of monoglycerides) was obtained from Danisco Co., (Copenhagen, Denmark). Xanthan and guar were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Table 1. Formulations used to prepare different gluten-free bread types containing different amounts of sourdough (20, 40 and 60% SD). Percentages of ingredients are based on 100 g of flour weight.[1]

Ingredients	C1	C2	20% SD	40% SD	60% SD
Chestnut flour	0	30	24	18	12
Rice flour	100	70	56	42	28
Sourdough			40	80	120
Water	163	163	143	123	103
Sugar	8	8	8	8	8
Salt	2	2	2	2	2
Oil	8	8	8	8	8
Yeast	2	2	2	2	2
X-G gum blend	0.5	0.5	0.5	0.5	0.5
Emulsifier DATEM	0.5	0.5	0.5	0.5	0.5

[1] C1 = control samples prepared from only rice flour without addition of sourdough; C2 = control samples prepared by the replacement of 30% rice flour with chestnut flour without addition of sourdough; SD = samples prepared by the replacement of 30% rice flour with chestnut flour and the addition of different percentages of sourdough. Sourdough had a dough yield of 200, i.e. 1 part water and 1 part flour; X-G = xanthan-guar gum.

Methods

Sourdough preparation

The starter strain (0.6 g) was added to a mixture containing water (100 g), rice flour (70 g) and chestnut flour (30 g) thereby giving a dough yield of 200 (Table 1). The resultant dough was mixed thoroughly by hand for 2 min, poured into large beakers, covered and then incubated in a proofing cabinet (InterMetro Industries Co., Wilkes-Barre, PA, USA) at 30 °C and 85% relative humidity for 72 h. Different amounts of the resultant sourdough (20, 40 and 60%) was added to bread formulations.

Bread making procedure

Gluten-free bread formulations used in this study are presented in Table 1. The amount of water (at 30 °C), which was determined by conducting preliminary experiments to determine optimum absorption, added to the dough was 163% on flour basis. Both gum blend (xanthan-guar gum) and emulsifier (DATEM) were used at the level of 0.5% in the dough formulation. The blend of xanthan-guar was prepared by mixing equal amounts of each gum and before adding the gums into the dough mixture, each gum (0.5%) was dispersed in half of the water to be added in the dough formulation using a high-speed homogeniser (Brinkmann Instruments, Westbury, NY, USA).

During preparation of the bread formulations, first remaining dry ingredients (chestnut flour, rice flour, instant yeast, sugar, salt and emulsifier) were mixed thoroughly, and then the melted shortening was added. Finally the gum suspension and water were added slowly and mixed for 3 min at 85 rpm and then 2 min at 140 rpm using a mixer (5K45SS; Kitchen Aid, Elkgrove Village, USA). After complete mixing, fermentation was done at 35 °C and 85% relative humidity in a proofing cabinet (InterMetro Industries Co.) for 40 min. Following fermentation, the fermented dough samples (200 g each) were baked in a rotatory electric oven (National Mfg. Co., Lincoln, NE, USA) at 200 °C for 30 min. Gluten-free breads prepared with only rice flour and the replacement of rice flour with 30% chestnut flour without addition of sourdough were selected as controls based on the preliminary study (Demirkesen *et al.*, 2010).

Rheological measurements

For the rheological experiments, gluten-free dough samples were prepared without adding any yeast to the dough formulation to avoid interference of bubble formation in the measurements. The rheological measurements were conducted using a rheometer (model ARG-2; TA Instruments, Newcastle, DE, USA) and a parallel plate geometry (40 mm diameter and 2 mm gap) at 25 °C. The dough sample was placed between the plates and the edges were carefully trimmed with a spatula; oil was added to the sample edges prevent sample dehydration. For the relaxation of the residual stresses, the dough was rested at room temperature for 20 min before testing. Frequency sweep experiments were carried out at 0.5% strain (within the lineal viscoelastic region) at frequencies ranging from 0.1 to 10 Hz. Results were expressed in terms of complex modulus (G^*) values. All the rheological experiments were performed at least twice and their averages were reported in the study.

pH and total titratable acidity in gluten-free sourdoughs and breads

The TTA and pH values were determined in sourdough, bread batters and breads. The pH values were measured from an aliquot of 10 g of sourdough, bread batters and bread crumb suspended with 100 ml distilled water. For the determination of TTA, these suspensions were titrated against 0.1 N NaOH to a final pH valve of 8.5. TTA was expressed as the amount of 0.1 N NaOH/10 g consumed in ml. Each analysis was done in triplicate and their averages are reported in the study.

Firmness

Baked loaves were allowed to cool for 1 h before obtaining measurements using a TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA). Samples of cubic shapes with dimensions of 25 × 25 × 25 mm were taken from the centre of bread and were compressed to 25% of thickness with a cylindrical probe (diameter a 25.4 mm) (approved method 74-09; AACC, 2000). The firmness (which was taken as the force required for compression of the sample) of the crumb was calculated from a force-distance graph. The measurements were done in duplicate.

Specific bulk volume

The specific volume, which is the ratio between bread volume and weight of loaves, was expressed in cm^3/g by using the rapeseed displacement method after 20 min cooling at 25 °C. Then, specific volume was calculated as the volume/mass ratio (cubic centimetre per gram) of bread according to (approved method 10-05; AACC, 2000). The measurements were done in duplicate.

Analysis of bread microstructure

X-ray micro computed tomography analysis

The X-ray micro computed tomograph (X-ray μCT) MicroCT 40 (Scanco Medical Inc., Wayne, PA, USA) was used to determine the porous structure of gluten-free bread crumbs prepared with/without the addition of different amounts of sourdough. Based on the study of Kelkar *et al.* (2011), the parameters selected for foods were found to be most favourable at 45 kVp and 177 μA intensity. The largest sample cell having 40.5 mm diameter and medium resolution was used for scanning the crumb samples. Each of the bread samples was cut to fit into the sample cell with minimal damage to the structure using a serrated cutter and then cotton was placed on top and bottom of the sample in the cell to avoid movement of the sample in the test tubes. Finally, the sample cell was covered with a paraffin film to prevent any possible moisture loss. To create a tomogram, a sample was put on a rotary stage and when the X-rays pass through the sample, the stage is rotated. Each sample was scanned to obtain 100 slices of 0.040 mm thickness each.

Image analysis

Image J software (http://rsbweb.nih.gov/ij/) was used to assess the scanned images of the samples. In order to eliminate possible artefacts, the scans were segmented to obtain similar sized rectangular region of interest from the middle part of the sample. Thresholding operation was done using the Otsu's algorithm to convert the grayscale into a binary image (foreground image (air) and background image (bread)) (Demirkesen *et al.*, 2014b). Porosity values of the bread crumbs was calculated from the ratio of the number of foreground (air) voxels divided by the total number of voxels in the image using Image J. The shape descriptors plugin was used to obtain the number of pores, aspect ratio, roundness and the average size of the pores. Because of the resolution of the μCT, only pores greater than 0.05 cm^2 were considered. Crumb structures of bread samples were examined by calculating porosity, the number of pores and mean roundness values by Image J. The equations for the roundness and aspect ratio are given below (Russ, 2004):

$$\text{Roundness} = \frac{4 \times \text{area}}{\pi \times \sqrt{(\text{major axis})}} \tag{1}$$

$$\text{Aspect ratio} = \frac{\text{major axis}}{\text{minor axis}} \tag{2}$$

where the major axis is the length and the minor axis is the width of the pores. Aspect ratio and roundness value of 1 indicates a perfect circle.

Confocal laser scanning microscopy

Samples were cut from bread samples while they were still in a frozen stage using cold razor blades and placed on long cover glasses (24 × 60 mm, No. 1.5; Sigma-Aldrich, St Louis, MO, USA). Staining was performed following the method of Lee *et al.* (2001). Briefly, a 0.05% fluorescein isothiocyanate (FITC; Sigma-Aldrich) solution in 0.5 mM sodium hydroxide with pH=8 was added to the samples and allowed to stain in the dark for 60 min.

An Olympus FluoView FV1000 inverted confocal microscope (Olympus, Tokyo, Japan) was used for the analysis of the samples. FITC was excited using an argon laser at 488 nm using an optimally configurable laser power of 7% and an emission wavelength of 520 nm was set by the software. Samples were analysed using a PlanApo N 60× NA 1.4 objective (Olympus America Inc., Center Valley, PA, USA) for oil immersion.

Statistical analysis

One-way ANOVA was used to determine whether gluten-free formulations affected pH, TTA, firmness, specific volume, and crumb microstructure of breads significantly ($P \leq 0.05$). If significant difference was found, means were compared by using the Tukey multiple comparison test ($P \leq 0.05$) by using MINITAB statistical software (release 16.1.1; Minitab Inc., State College PA, USA).

3. Results and discussion

pH and total titratable acidity of sourdough, dough and bread

Data on acidification of fermented sourdough, gluten-free bread doughs and breads prepared with/without the additions of different amounts of sourdough are reported in Table 2. The lowest pH and the highest TTA values were obtained from fermented sourdough samples ($P \leq 0.05$). Conversely, in absence of sourdough, control dough and bread samples had the highest pH and the lowest TTA values ($P \leq 0.05$). The similar findings have been reported by Crowley *et al.* (2002) and Moroni *et al.* (2011). As illustrated in Table 2, increasing amounts of sourdough in both dough and bread samples resulted in a progressive reduction of pH values and increase in TTA values, which is in agreement with the study of Crowley *et al.* (2002) and Moroni *et al.* (2011).

Rheological measurements

The complex modulus (G*) values of dough samples prepared with/without the addition of different amounts of sourdough is presented in Figure 1. All dough samples showed an elastic like structure with elastic modulus significantly higher than viscous modulus, thus complex modulus values are close to the storage modulus values. Control chestnut-rice dough samples had the highest complex modulus over the whole frequency range. It has been suggested that rheological properties of dough samples are affected by the high fibre content and fibrous structure of dough prepared with chestnut flour (Demirkesen *et al.*, 2010). Entanglement of fibres in chestnut flour appears to be reason for the highest complex modulus (G*) values of chestnut flour containing dough samples. As indicated in Figure 1, the addition of sourdough decreases values

Table 2. pH and total titratable acidity (TTA) values of doughs and breads prepared with the addition of different amounts of sourdough (SD).[1,2]

Gluten-free formulations	Dough		Bread	
	pH	TTA	pH	TTA
C1	6.72±0.005a	3.01±0.017d	6.55±0.038a	2.69±0.059c
C2	6.41±0.082a	2.82±0.029d	6.44±0.020a	2.97±0.040c
20% SD	5.32±0.210ab	5.41±0.012bc	5.42±0.063b	5.23±0.079b
40% SD	4.87±0.012ab	7.37±0.058b	4.94±0.041bc	6.97±0.094b
60% SD	4.61±0.089c	8.72±0.011b	4.59±0.037c	8.38±0.105a
100% SD	3.82±0.091c	14.54±0.153a	nd	nd

[1] Formulations having different letters within a column are significantly different ($P \leq 0.05$).
[2] C1 = control samples prepared from only rice flour without addition of sourdough; C2 = control samples prepared by the replacement of 30% rice flour with chestnut flour without addition of sourdough; SD = samples prepared by the replacement of 30% rice flour with chestnut flour and the addition of different percentages of sourdough; nd = not determined.

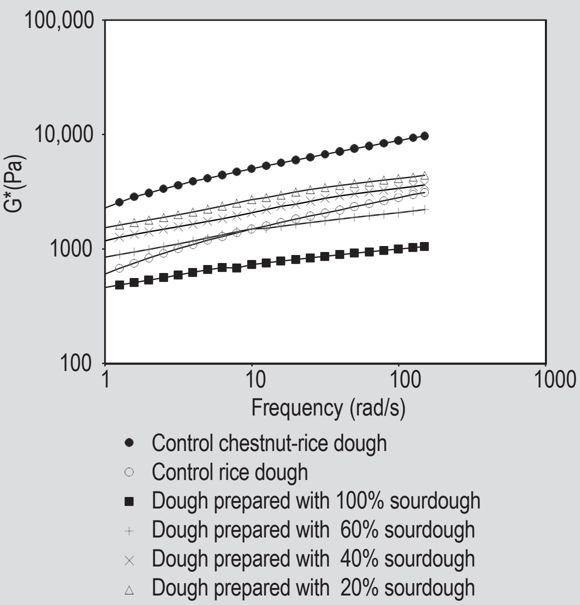

Figure 1. Complex modulus (G*) of gluten-free dough samples prepared with/without the addition of different amounts of sourdough.

of complex modulus (G*) of the dough samples. The addition of higher amount of sourdough resulted in further decreases in the complex modulus (G*). The decreases in complex modulus (G*) of dough samples indicated that the addition of sourdough promotes softening of dough. Protein breakdown and consequently the release of small polypeptide during fermentation have also been identified as the major cause for the weakening of the proteins-proteins and proteins-starch interactions resulting in weakening the elasticity of doughs. In addition, the decreases of the complex modulus of the gluten-free dough samples might be related to the reduced water holding capacity of hydrolysed proteins, which is induced by organic acids and enzymes released during the sourdough fermentation. Thus, in the lack of sourdough fermentation, hence reduced degradation of starch and protein components, generally higher complex modulus (G*) values are obtained from the control chestnut-rice dough samples. Drastic decreases in the degree of elasticity by sourdough fermentation were also stated in other studies (Clarke *et al.*, 2002; Galle *et al.*, 2012; Houben *et al.*, 2010). It has also been reported that mild acid hydrolysis of starch during sourdough fermentation may influence the network connectivity in an opposite way and the acidification could have a rapid effect on the strength of the batter by increasing G*. Since a softening of dough was observed in this study, it may be stated that the effect of the acidification on dough strength was masked by the extensive hydrolytic events in sourdough (Moroni *et al.*, 2011).

Firmness and specific volume of breads

Firmness and specific volume of breads prepared with/without the addition of different amounts of sourdough are illustrated in Figures 2 and 3. Gluten-free bread formulations significantly affected firmness and specific volume values of breads ($P \leq 0.05$). As illustrated in Figures 2 and 3, control chestnut-rice breads exhibited significantly lower hardness and higher specific volume values as compared to control rice breads ($P \leq 0.05$). This might be attributed to the fact that the viscosity and viscoelastic properties of rice dough were not sufficient to allow bubble stability during the fermentation and baking processes. On the other hand, the higher fibre content of chestnut flour enhanced the viscoelastic properties of dough resulting in entrapment of more air bubbles during fermentation and

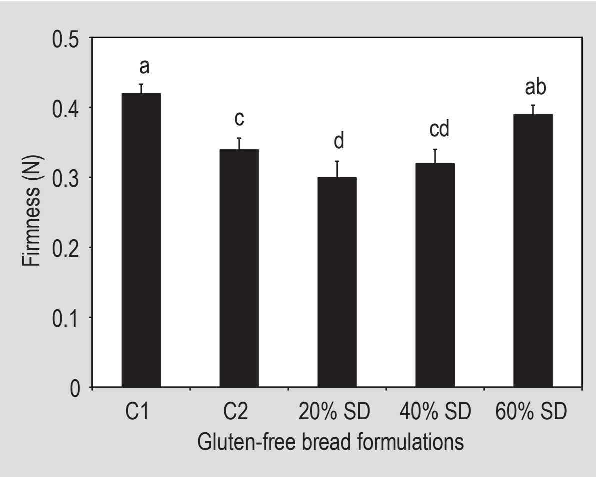

Figure 2. Firmness values of gluten-free dough samples prepared with/without the addition of different amounts of sourdough. C1 = control rice bread; C2 = control chestnut-rice bread; 20% SD = bread prepared with addition of 20% sourdough; 40% SD = 40% of added sourdough; 60% SD = bread prepared with addition of 60% sourdough. Bars with different letters are significantly different (*P*≤0.05).

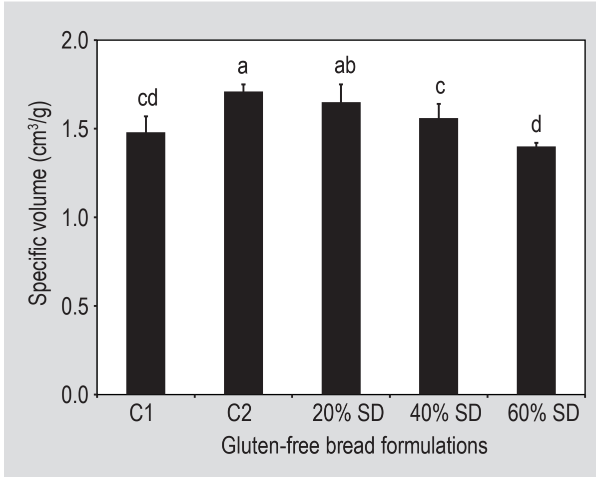

Figure 3. Specific volume values of gluten-free dough samples prepared with/without the addition of different amounts of sourdough. C1 = control rice bread; C2 = control chestnut-rice bread; 20% SD = bread prepared with addition of 20% sourdough; 40% SD = 40% of added sourdough; 60% SD = bread prepared with addition of 60% sourdough. Bars with different letters are significantly different (*P*≤0.05).

baking process (Demirkesen *et al.*, 2010). Thus, significantly higher loaf volume and softer texture were obtained from gluten-free control breads in the presence of chestnut flour. As indicated in Figures 2 and 3, addition of sourdough improved the quality of gluten-free breads, especially their texture, up to 40%. Therefore, the highest specific volume and the lowest firmness values were obtained from breads with the addition of 20% sourdough. However, further addition of sourdough appears to negatively affect the quality of gluten-free breads. Softer dough formation with the addition of certain amount of sourdough might have facilitated greater bubble expansion upon the fermentation process. The improvement of specific volume and texture of gluten-free breads by addition of sourdough (20%) might be related to the improvement of the capacity of dough to retain CO_2. Conversely, addition of higher levels of sourdough had a detrimental effect on volume and increased the firmness of breads. Several previous studies also showed that sourdough addition might enhance specific volume and texture of breads when it is used at a certain content (Crowley *et al.*, 2002; Novotni *et al.*, 2012).

X-ray micro computed tomography analysis

2D and 3D X-ray μCT images of gluten-free bread samples prepared with/without the addition of different amounts of sourdough is presented in Figure 4. As depicted in Figures 4A, B, C and D control rice and control chestnut-rice bread samples exhibited different crumb structures. The differences in the crumb structures are related to the type (open versus closed) and the distribution of pores, as well as the effect of pore size on moisture diffusivity and the differences in viscosity and viscoelastic properties of dough samples. Pores within the food materials can be categorised as closed pores (closed from all sides) and open pores where air and water vapour typically can penetrate them (Datta *et al.*, 2007; Demirkesen *et al.*, 2014b). In the study of Wang *et al.* (2011) has been stated that non-uniform distribution of open pores and extensive distribution of closed pores in breads might result in the total or partial collapse of breads which largely affect the appearance and texture of crumb structure of breads (Datta *et al.*, 2007; Demirkesen *et al.*, 2014b). As indicated in Figures 4A and B, control rice breads show a heterogeneous crumb structure with their higher amounts of open pores. On the other hand, the crumb structures of control chestnut-rice breads were more homogenous with a lower number of open pores. Pore size has also been found to affect the microstructural properties of breads due to its relationship with mass transfer processes (Adedeji and Ngadi, 2009; Datta, 2007; Demirkesen *et al.*, 2014b; Kassama and Ngadi, 2005;). While, larger and interconnected pores trigger faster transport processes resulting in the flowing of fluids mostly outside of the solid, in the presence of smaller pores, fluid flows inside the solid matrix. Therefore, non-uniform distribution of higher amount of open and larger pores in the control breads prepared with rice flour might cause loss of more moisture, leading to an undesirable crumb appearance. Conversely, for control chestnut-rice breads, the presence of relatively higher number of small pores promotes the presence of more gas bubbles and moisture in their closed pores resulting in finer crumb structure. The important relationship between the viscosity and viscoelastic properties of dough and crumb structure of breads has also

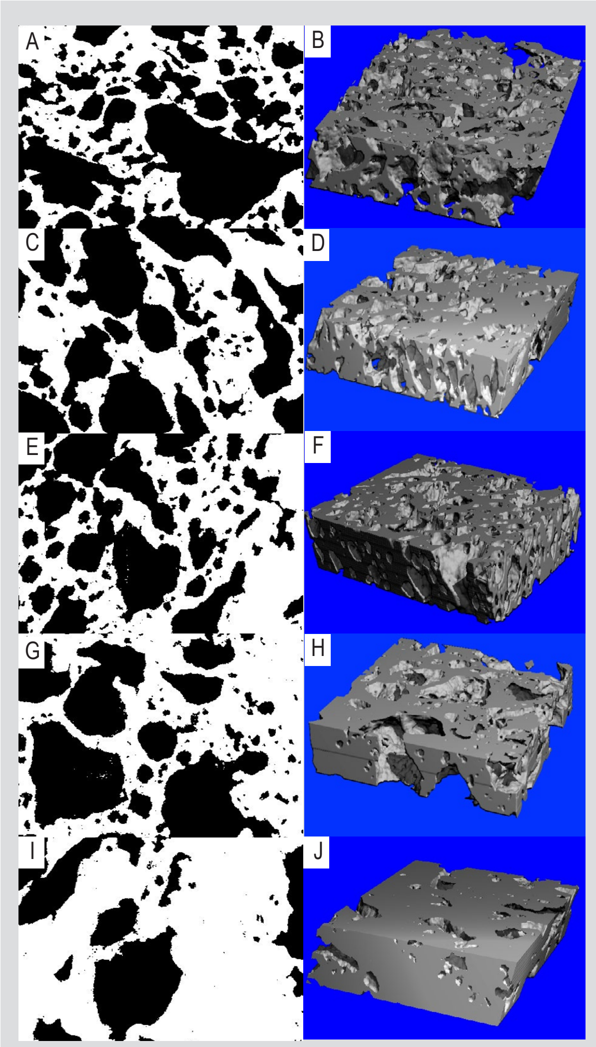

Figure 4. 2D and 3D X-ray micro computed tomograph (μCT) images of gluten-free bread samples prepared with/without the addition of different amounts of sourdough. (A) and (B) Control rice bread; (C) and (D) control chestnut-rice bread; (E) and (F) bread prepared with addition of 20% sourdough; (G) and (H) bread prepared with addition of 40% sourdough; (I) and (J) bread prepared with addition of 60% sourdough. Pictures on left side represent 2D and on right side represent 3D X-ray μCT images.

been reported by Demirkesen *et al.* (2013). In the case of rice dough, the viscosity and viscoelastic properties of dough were not high enough to provide sufficient bubble stability for the fermentation and baking processes (Demirkesen *et al.*, 2013). On the other hand, as it appears to indicate Figure 1, the higher fibre content of chestnut flour may increase the viscosity and viscoelastic properties of dough during mixing and fermentation processes resulting in larger entrapment of air bubbles (Demirkesen *et al.*, 2013). Therefore, the control chestnut-rice breads presented a more uniform structure with smaller and higher number of closed pores (Figures 4C and D). The 2D and 3D X-ray μCT images of gluten-free bread samples prepared with the addition of sourdough are presented in Figures 4E-J. It is observed that the addition of certain level of sourdough (20%) provided a more homogenous crumb structure to gluten-free breads (Figures 4C and F). However, addition of higher levels of sourdough (40 and 60%) worsened bread structure leading to very denser crumb (Figures 4G-J). In the study of Crowley *et al.* (2002) was demonstrated that high level of sourdough incorporation in wheat breads resulted in heterogeneous and coarser crumb structure appearance with very large pores.

Porosity, number of pores, averages size of pores, aspect ratio and roundness values of pores in gluten-free bread samples prepared with/without addition of different amounts of sourdough were also determined by using X-ray μCT (Table 3 and 4). While the gluten-free formulation significantly affected porosity, number of pores and averages size of pores ($P \leq 0.05$), the differences in aspect ratio and roundness values of pores for gluten-free breads prepared with different formulations were found to be not statistically different. It has been suggested that porosity measurement alone does not provide accurate information about distribution of pores; hence it cannot solely be used to define pore structure. Nevertheless, X-ray μCT provides wide-ranging inside information about homogeneity of crumb structure by allowing quantification and qualification of crumb structure images. As illustrated in Table 3, among all breads, the highest porosity values were obtained from control rice breads. It has been stated that 99% of bread's total porosity are resulting from a wide-range of interconnected and open pores (Wang *et al.*, 2011). Thus, the highest porosity values of control rice breads might be caused by their high number of the open pores (Figures 4A and B). The highest average areas of pores observed for control rice breads might also be caused by the interconnection between all gas cells (open pores) (Table 4, Figures 4A and B). These results also indicate the presence of a non-uniform crumb structure with very large pores of control rice breads (Figures 4A and B). Control chestnut-rice breads had the lowest average pores area and the highest number of pores. In addition, as compared to control rice breads, control chestnut-rice breads had lower porosity values than that might have resulted from their lower number of open pores (Table 3, Figures 4C and D). As indicated in Figures 4E-J, generally, more opened structures were obtained from chestnut-rice breads with the addition of sourdough. In addition, reductions in the number of pores and enlargements of pore areas were observed for gluten-free chestnut-rice breads prepared with sourdough incorporation. This result was in agreement with the studies of Crowley *et al.* (2002) and Moroni *et al.* (2011). Among gluten-free breads prepared with the addition of different amounts of sourdough, the most homogenous structure with a high number of smaller pores was observed in gluten-free breads prepared with

20% of sourdough addition (Figures 4E and F). Denser crumb structures were observed in gluten-free breads with higher addition of sourdough. That can be visually observed in Figures 4G-J. Inferior crumb structures have been also observed in wheat breads containing high levels of sourdough (Crowley *et al.*, 2002).

The relationship between the width of the pore to its height which are referred as the aspect ratio was found to be under 2 for all bread samples. This result shows the ellipsoidal nature of the pores (Ishida *et al.*, 2001). The roundness of pores in gluten-free bread samples prepared with/without sourdough incorporation was ranged between 0.558 and 0.636 indicating their non-circular shape. This finding is in agreement with those presented by Demirkesen *et al.* (2013).

Table 3. Porosity of the gluten-free bread samples prepared with the addition of different amounts of sourdough.[1,2]

Gluten-free bread samples	Porosity
C1	0.617a
C2	0.523b
20% SD	0.444bc
40% SD	0.419c
60% SD	0.154e

[1] Formulations having different letters are significantly different ($P \leq 0.05$).
[2] C1 = control samples prepared from only rice flour without addition of sourdough; C2 = control samples prepared by the replacement of 30% rice flour with chestnut flour without addition of sourdough; SD = samples prepared by the replacement of 30% rice flour with chestnut flour and the addition of different percentages of sourdough.

Confocal laser scanning microscopy

2D images of gluten-free bread samples are shown in Figure 5. From the firmness and specific volume results, the most improved quality appear to be in control chestnut-rice breads and breads prepared with the addition of 20% sourdough. Thus, the microstructure of gluten-free control chestnut-rice breads and breads prepared with the addition of 20% of sourdough and stored at 0 and 96 h were studied by confocal laser scanning microscopy. The starch granules appeared as a shady diffuse green as they absorbed the dye to a lesser degree than protein. As indicated in Figure 5A, the brighter protein areas appeared to be more frequent for control breads and the starch granules appeared more swollen. However, protein regions in sourdough containing breads appear to be smaller (Figure 5B), which indicated that the protein particles in sourdough containing breads were degraded over the fermentation process. The larger aggregation upon sourdough formation which have been found in other studies (Moore *et al.*, 2007) was not observed in present study. In contrast, because of the degradation of proteins into smaller peptides, proteins in sourdough containing breads can no longer cross-link and therefore do not aggregate upon baking as demonstrated by Schober *et al.* (2007). This finding might also prove that why lower G* values were obtained from sourdough containing breads (Schober *et al.*, 2007). It was also suggested that the enhancement of bread quality with sourdough incorporation might be due to less interface of protein with the starch gel upon heating process. This might be the reason for the improvement of bread quality with addition of 20% sourdough. In both type of breads after 4 day storage, starch granules appeared more swollen than in fresh breads (Figures 5C and D), which probably due to the water movement to adsorb to the starch and the protein matrix appeared coarser.

Table 4. Quantification of porous structure per slice thickness of the gluten-free bread samples prepared the addition of different amounts of sourdough.[1,2]

Gluten-free bread samples	Number of pores per area	SE	Aspect ratio of the pore	SE	Roundness of the pore	SE	Average size of the pore (cm^2)	SE
C1	2.71c	12.0	1.80a	3.5	0.599a	0.9	0.518a	2.8
C2	17.43a	20.2	2.05a	1.6	0.558a	0.4	0.065d	0.1
20% SD	4.69b	15.7	1.90a	2.2	0.562a	0.6	0.112c	0.4
40% SD	4.46b	14.2	1.76a	3.1	0.622a	0.8	0.162b	0.5
60% SD	2.27c	7.6	1.74a	4.4	0.636a	1.2	0.105cd	0.5

[1] Formulations having different letters are significantly different ($P \leq 0.05$). Analysis was done in duplicate. Pores >0.05 cm^2 are considered. Values are means of 100 slices. SE = standard error of 100 slices.
[2] C1 = control samples prepared from only rice flour without addition of sourdough; C2 = control samples prepared by the replacement of 30% rice flour with chestnut flour without addition of sourdough; SD = samples prepared by the replacement of 30% rice flour with chestnut flour and the addition of different percentages of sourdough.

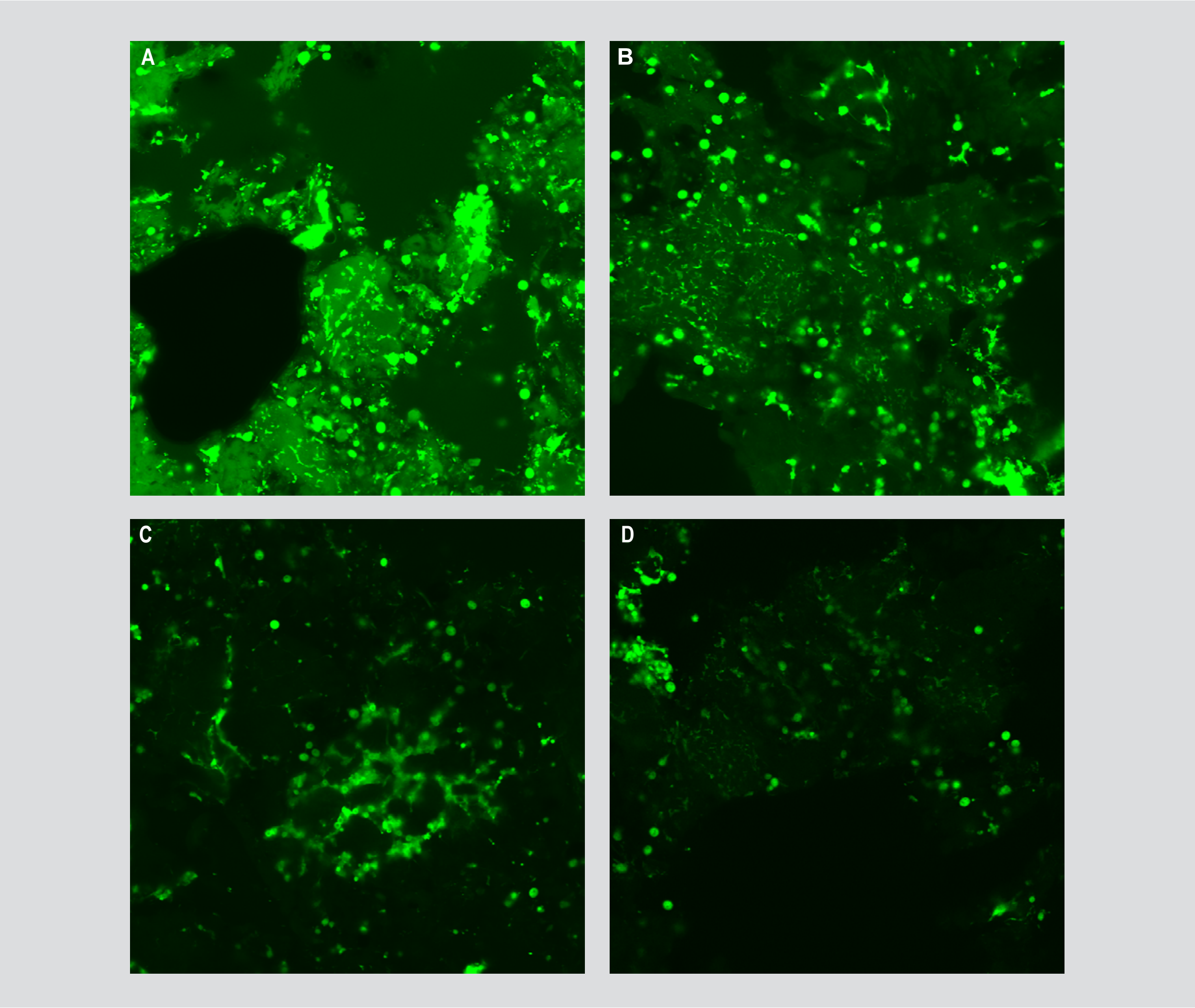

Figure 5. Confocal laser scanning microscopy images of gluten-free control chestnut-rice breads and breads prepared with the addition of 20% of sourdough and stored at 0 and 96 h. (A) Control chestnut-rice bread stored at 0 h; (B) control chestnut-rice bread stored at 96 h; (C) bread prepared with addition of 20% sourdough stored at 0 h; (D) bread prepared with addition of 20% sourdough stored at 96 h.

4. Conclusions

Gluten-free breads formulated with the addition of different amounts of sourdough were evaluated using rheological, baking and microstructural methods. Rheological measurements showed that the addition of sourdough led to decreases in the complex modulus (G^*) of the dough samples, thus promoting their softening. From baking tests, it was found that breads prepared with the addition of 20% sourdough had the best quality parameters. However, elevated levels of sourdough led to some deterioration in quality parameters. X-ray microtomography results indicated that addition of sourdough resulted in more opened structures, reductions in the number of pores and enlargements of pore areas of gluten-free bread crumbs. The most homogenous structure with high number of smaller pores was obtained with the addition of 20% sourdough. Proteins in sourdough containing breads were smaller as compared to control bread showing the degradation of proteins into smaller peptides by the sourdough fermentation. Due to less interface of protein with the starch gel upon heating process, sourdough incorporation enhanced bread quality. Therefore, the results of the present study showed that the quality of gluten-free chestnut-rice breads can be successfully improved by the addition of 20% sourdough.

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