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ORIGINAL PAPER

# Image Analysis of Gluten-free Breads Prepared with Chestnut and Rice Flour and Baked in Different Ovens

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**Abstract** In this study, the effects of chestnut flour and xanthan–guar gum blend–emulsifier DATEM mixture addition on macro- and microstructure of rice breads baked in conventional and infrared–microwave combination ovens were investigated by using the images obtained by a scanner and scanning electron microscopy (SEM). Pore area fraction, pore size distribution, and roundness values of pores were determined. The highest pore area fraction values were obtained in breads prepared by replacement of 46 % of rice flour with chestnut flour containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. On the other hand, rice breads containing no additives or chestnut flour had the lowest pore area fraction values. Infrared–microwave combination baking increased both pore area fraction values and total number of pores. Infrared–microwave combination baking caused approximately 23–28 % increase in number of the small pores (0–5 $mm^2$) in rice breads and 71 % increase in number of the large pores (>10 $mm^2$) in chestnut–rice breads. The fiber content and larger starch granules of chestnut flour contributed towards the stabilization of gas bubbles resulting in better crumb structure. More homogenous pore distributions were observed when additives and an infrared–microwave combination oven were used. When microstructure of gluten-free breads was investigated, it was seen that starch granules in chestnut–rice breads baked in an infrared–microwave combination oven did not disintegrate completely.

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**Keywords** Chestnut flour · Gluten-free bread · Infrared–microwave combination baking · Rice flour · Scanning electron microscopy

## Introduction

Gluten forms viscoelastic networks that are responsible for retaining gas produced from yeast fermentation and oven rise, so high volume and soft texture can be obtained in the wheat-based products. In fact, most gluten-free products have low volume, poor texture, and flavor and stales faster in the absence of a continuous protein network. In addition, gluten-free products do not meet the nutritional needs of celiac sufferers, since they do not contain adequate amount of vitamins, minerals, and fiber (Demirkesen et al. 2010a). For these reasons, gluten replacement is one of the most challenging tasks for cereal technologist and scientists. Recently, gluten-free breads have been produced by replacing wheat flour by alternative flours such as rice, corn, soy, sour, buckwheat, potato, and sorghum flour and using a thickening viscoelastic agent and emulsifier to provide bread with gas holding ability and stabilizing mechanisms (Van Riemsdijk et al. 2011).

High quality proteins with essential amino acids (4–7 %); relatively high amount of sugar (20–32 %), starch (50–60 %), dietary fiber (4–10 %); and low amount of fat (2–4 %) makes chestnut flour one of the most suitable flour for preparing gluten-free products. In addition, chestnut flour contains some important vitamins and minerals such as vitamins E, B group vitamins, potassium, phosphorous, and magnesium. Demirkesen et al. (2010a) showed that addition of chestnut flour improved functional and technological properties of dough as well as color and flavor properties of breads.

Although microwave heating has a number of advantages such as energy efficiency, faster heating, space saving, precise process control, selective heating, and food with high nutritional quality, microwave-baked products do not meet with consumer acceptance due to their unacceptable quality (Sumnu 2001). The advantages of infrared–microwave combination heating over the microwave heating have been realized over the past few years (Sumnu et al. 2007). This combination technology has also been found as a good alternative to conventional heating to produce bakery products (Demirekler et al. 2004; Keskin et al. 2004; Ozkoc et al. 2009). In a recent study by Demirkesen et al. (2011), it was shown that gluten-free breads baked in an infrared–microwave combination oven had comparable volume, color, and texture with conventionally baked ones.

Quality of a baked product depends on appearance, texture, loaf volume, and sensory properties (Zghal et al. 1999). These properties are significantly affected by structure of foods varying from the molecular to macroscopic levels. Thus, knowledge of macro- and microstructure is essential. However, examining food microstructure is difficult, since food materials are complex and the majority of structural elements are below the 100-μm range (Aguilera 2005). Several microscopy, scanning, and spectrometric techniques that allow visualization of changes in structure at different levels without intrusion has been proposed as useful tools for image acquisition (Falcone et al. 2006).

In recent years, image analysis based on a large variety of microscopic techniques has been applied for characterization of bread crumbs. Size, distribution, wall thickness, and number of cells were determined in these studies (Datta et al. 2007; Farrera-Rebollo et al. 2012; Ozkoc et al. 2009; Polaki et al. 2010; Rosell and Santos 2010; Rouille et al. 2005; Sanchez-Pardo et al. 2008; Sapirstein et al. 1994; Zayas 1993; Zghal et al. 2002). Scanning electron microscopy (SEM) is one of the most important image analysis technique, since it provides the combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation. SEM studies have been assessed to determine the changes that occur during baking qualitatively (Ozkoc et al. 2009; Polaki et al. 2010; Rosell and Santos 2010; Sanchez-Pardo et al. 2008). In a recent study by Turabi et al. (2010), SEM has been used to obtain quantitative information on macro- and microstructure of gluten-free rice cakes.

In the present study, it was aimed to obtain both quantitative and qualitative information on macro- and microstructure of gluten-free breads baked in different ovens. Another objective of this research was to understand the influence of replacement of rice flour with chestnut flour and the effects of xanthan–guar gum blend–DATEM mixture addition on structure of gluten-free breads.

## Materials and Methods

### Materials

Chestnut flour containing 10.79 % moisture, 47.80 % starch, 21.51 % sugar, 9.50 % fiber, 4.61 % protein, 3.80 % crude fat, and 1.99 % ash was supplied by Kafkas Pasta Şekerleme San. & Tic. A.Ş. (Karacabey, Bursa). Rice flour (Knorr-Çapamarka, Istanbul, Turkey) having 10 % moisture, 79.9 % starch, 0.12 % sugar, 1.28 % fiber, 6.0 % protein, 2.1 % crude fat, and 0.6 % ash was obtained from a local market. Sugar (sucrose), salt, instant yeast containing natural dough yeast (*Saccharomyces cerevisiae*) and emulsifier blend (citric acid esters of mono- and diglycerides) (Dr. Oetker, Turkey), and shortening (Becel, Unilever, İstanbul, Turkey) were also purchased from local markets. Emulsifier DATEM (diacetyltartaric acid esters of monoglycerides) was obtained from Ankara Halk Ekmek Fabrikası (Ankara, Turkey). Xanthan gum and guar gum were obtained from Sigma-Aldrich (Steinheim, Germany).

### Methods

#### *Bread-Making Procedure*

In order to prepare gluten-free breads, the optimum formulations determined in our previous studies were used (Demirkesen et al. 2010a, 2011). Basic dough recipe on 100-g flour basis contained 8 % sugar, 8 % shortening, 1 % instant yeast, and 2 % salt. Bread formulations were prepared from only rice flour and by replacement of rice flour with chestnut flour. In conventionally baked breads, 30 % of rice flour was replaced with chestnut flour, while in breads baked in an infrared–microwave combination oven, 46%. These formulations were optimized in preliminary experiments. The effect of xanthan–guar gum blend–DATEM mixture addition was also studied in bread formulations. The gum blend (xanthan–guar gum) was added as 0.5 % (w/w) of flour amount. Emulsifier DATEM used in gluten-free bread formulations to be baked in conventional and infrared–microwave combination ovens were determined as 0.50 and 0.62 % (in flour basis), respectively, by the study of Demirkesen et al. (2010b) and Demirkesen et al. (2011). On flour basis, the amount of added water in different formulations was varied between 150 % and 186 %. Water content used for each bread formulation was determined by conducting experiments based on the quality tests of breads in terms of specific volume and hardness (Demirkesen et al. 2010a and 2011).

The gum blend was prepared by mixing equal amounts of each gum. Before adding the gum blend into the dough mixture, the gum blend was dispersed in half of the water to be used in the dough formulation using a high-speed homogenizer (IKA T18 Ultra-Turrax, Staufen, Germany). During dough preparation, mixing of dry ingredients (chestnut flour, rice flour, instant yeast, sugar, salt, and emulsifier) was followed by the addition of melted shortening. Then gum blend suspension and water were added slowly and mixed for 3 min at 85 rpm and then 2 min at 140 rpm using a mixer (Kitchen Aid, 5K45SS, Elkgrove Village, USA). After complete mixing, dough was fermented in an incubator (Nüve EN 400, Ankara, Turkey) at 30 °C for 40 min. Following fermentation, gluten-free bread samples were baked either in a conventional or infrared–microwave combination oven. Rice breads prepared without xanthan–guar gum blend–DATEM mixture addition were used as controls.

*Baking*

Infrared–microwave baking was performed in a combination oven (Advantium oven, General Electric Company, Louisville, KY, USA). The power of the oven was determined as 682 W by using IMPI 2-L test (Buffler 1993). The oven has three halogen lamps, each having 1,500 W. To maintain the humidity in the oven, four beakers each containing 400 ml water, were placed in the corners of the oven during baking. Four dough samples (100 g each) were placed at the center of turn table and baked using 40 % upper and 70 % lower infrared power, and 30 % microwave power for 9 min which was the optimum formulation and baking condition determined by experiments (Demirkesen et al. 2011).

For conventional baking, four dough samples (100 g each) were baked in a conventional oven (Arçelik A. Ş., İstanbul, Turkey) at 200 °C for 25 min.

*Scanning of Bread*

Gluten-free breads were cut into halves vertically by an electric knife (Arzum AR 156 Colte, Ankara, Turkey). The cut side of one of the halves was placed over the glass of a scanner (CanoScan 3200F, Tokyo, Japan). Scanning was performed with a resolution of 300 dpi.

*Scanning Electron Microscopy (SEM) Analysis*

For SEM analysis, bread crumbs which were cut in cubes were frozen in liquid nitrogen and freeze dried. Freeze-dried samples were sputter coated with gold-palladium to render them electrically conductive by using a HUMMLE VII Sputter Coating Device (Anatech Electronics, Garfield, NJ, USA). Samples were then examined and images were recorded with a scanning electron microscope (JSM-6400, JEOL, Tokyo, Japan) at an accelerating voltage of 20 kV. Samples were observed at magnification levels of 20× and 1,000×. In the case of 1,000× magnification level, both outside and inside of bread crumbs were examined.

*Image Analysis*

Crumb cell characteristics of the scanned images and SEM micrographs at magnification of 20× were analyzed using the software Image J (http://rsb.info.nih.gov/ij/). In the software, the contrast between two phases (pores and solid part) for each images were used. In the case of scanned images, each color image was first converted to gray scale (8 bit). Values of scanned images were obtained in pixels and converted into centimeter by using bars of known lengths. Segmentation was carried out using Image J software by applying the manual thresholding tool. The largest possible cross-section of the images (5×5 cm) was selected for each image. However, the whole area was analyzed without any cropping in the case of SEM micrographs. To determine the pore distribution in bread crumbs, the method and software used in the study of Impoco et al. (2007) was used. This software is in the form of a plug-in for Image J. The plug-in encompasses two commands: Binarise SEM and Compute Stats.

Binarise SEM segments the input image into "holes" and "structure." The command Compute Stats is used for the output of the previous application Binarise SEM to obtain image statistics about the distribution of pores. Crumb structure of bread samples were analyzed by calculating pore area fraction, pore size distribution, and mean roundness values by this software. Roundness was calculated using the following formula:

$$\text{Roundness} = \frac{4A}{\pi D_{max}^2} \tag{1}$$

where $A$ is net area and $D_{\max}$ is the maximum diameter. An $R$ value of 1 indicates a perfect circle.

*Statistical Analysis*

Two-way ANOVA was used to determine whether gluten-free bread formulations and oven type affected structure of bread formulations significantly ($p \leq 0.05$). If a significant difference was found, means were compared by using Tukey multiple comparison test ($p \leq 0.05$) by using Minitab (Version 15) software.

## Results and Discussion

### Image Analysis

Figure 1 shows the scanned images of gluten-free bread samples prepared with different formulations and baked in different ovens. The scanned images of bread samples prepared without additives and baked in different ovens are shown in Fig. 1a, c, e and g. Breads formulated with rice flour and containing no gum and DATEM had nonuniform and larger pores (Fig. 1a). This might be due to the fact that the viscosity and viscoelastic properties of rice dough were not sufficient to allow bubble capture during the fermentation and baking processes (Demirkesen et al. 2010a). The

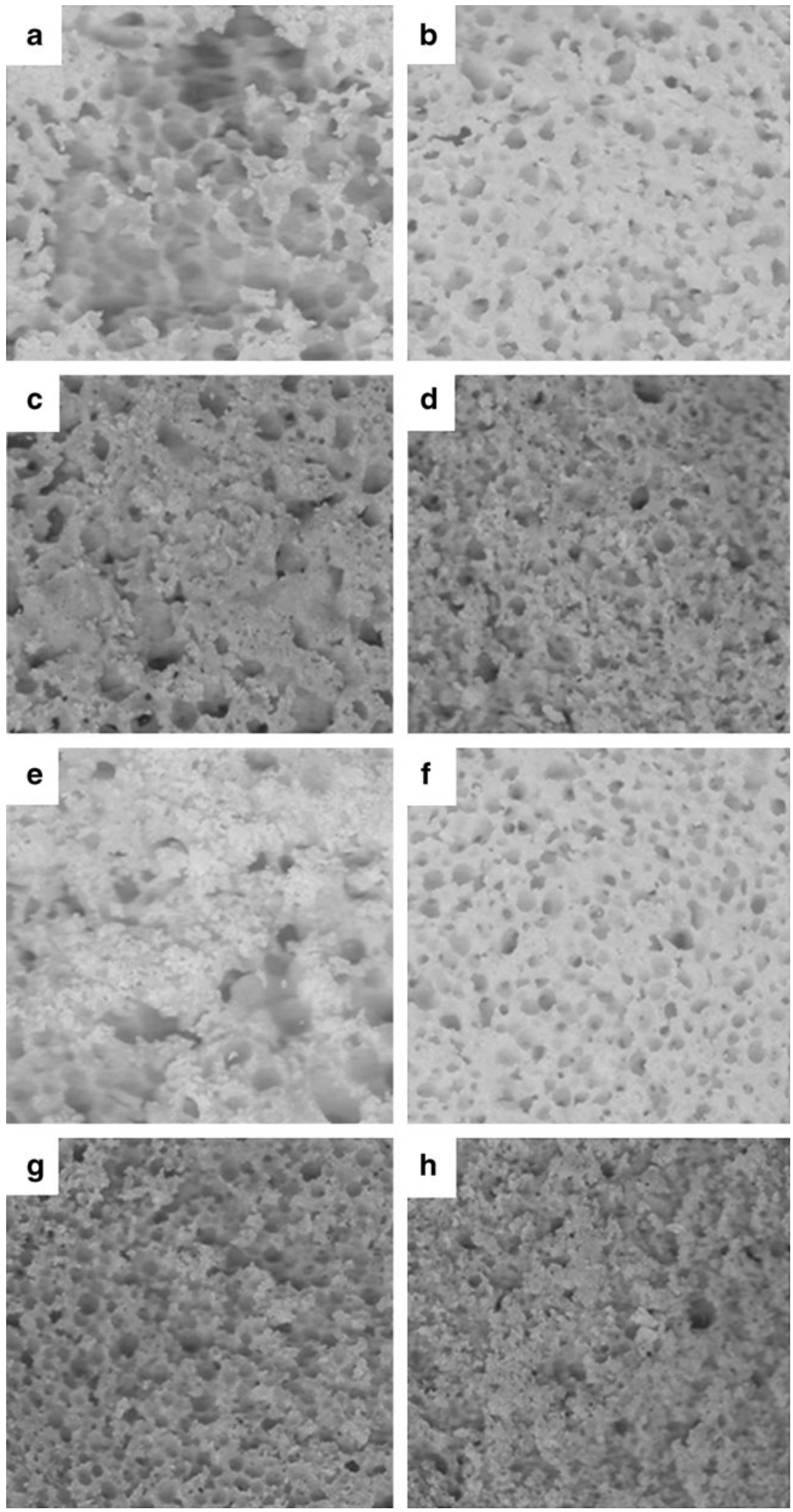


**Fig. 1** Scanned images of different gluten-free bread formulations baked in different ovens. **a** Rice bread baked in a conventional oven. **b** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **c** Chestnut–rice breads baked in a conventional oven. **d** Chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **e** Rice bread baked in an infrared–microwave combination oven. **f** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in infrared–microwave combination oven. **g** Chestnut–rice breads baked in an infrared–microwave combination oven. **h** Chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven

puffing effect of infrared–microwave combination baking was not effective on the size and distribution of pores in rice bread crumb either (Fig. 1e). Therefore, control rice breads had heterogeneous and coarser crumb with very large pores (Fig. 1a and e). On the other hand, in the presence of chestnut flour, even in the absence of additives, large pores in bread crumb were prevented (Fig. 1c and g). This might be explained by the higher fiber content of chestnut flour that enhanced the viscoelastic properties and resulted in entrapment of more air bubbles (Demirkesen et al. 2010a). Moreover, using different baking ovens resulted in noticeable differences in size and distribution of pores of chestnut–rice bread crumbs (Fig. 1c and g). During infrared–microwave combination heating, higher internal pressure and faster vaporization occurred inside chestnut–rice breads that created a puffing effect (Demirkesen et al. 2011). Therefore, among all gluten-free breads prepared without additives, the most uniform structure with small pores was obtained from breads prepared using chestnut and rice flour and baked in an infrared–microwave combination oven (Fig. 1g).

It has been shown that the benefit of hydrocolloids as dough stabilizers can be promoted in the presence of surfactants (Bollaín and Collar 2004). This may be due to the fact that the interaction between hydrocolloids and emulsifiers assists the entrapment of air bubbles during the mixing and fermentation processes. While hydrocolloids improve bread quality by increasing water absorption and viscoelastic properties of dough (Kohajdova and Karovicova 2009), emulsifiers lower the surface tension of dough leading to the subdivision of the entrapped air bubbles into more and smaller bubbles during mixing (Kokelaar et al. 1995; Ribotta et al. 2004). Consequently, in the presence of xanthan–guar gum blend–DATEM mixture, the pores of gluten-free breads were smaller and more uniform in size (Fig. 1b, d, f and h). Among all gluten-free breads, the most homogenous structure was obtained in the presence of chestnut flour, xanthan–guar gum blend–DATEM mixture addition, and infrared–microwave combination baking (Fig. 1).

Figure 2 shows the pore area fractions of gluten-free breads prepared with different formulations and baked in different ovens based on scanned images. According to two-way ANOVA, both gluten-free bread formulations and oven type were found to be significantly effective on the pore area fractions of crumb structure ($p \leq 0.05$). The lowest pore area fraction values were obtained from rice breads without any additives. In the absence of xanthan–guar gum blend–DATEM mixture, rice dough had very low viscosity and viscoelastic moduli values, which prevented entrapment of air bubbles resulting in low specific volume values (Demirkesen et al. 2010b). However, fiber content of chestnut flour provided higher apparent viscosity and viscoelastic properties to dough. This property helped the entrapment of more air bubbles into chestnut flour containing dough and caused higher specific volume values (Demirkesen et al. 2010a). Thus, chestnut flour containing breads had higher pore area fractions as compared to rice breads.

As mentioned before, gums and emulsifiers have the ability to improve volume and texture of breads by increasing water absorption and gas retention ability of dough during the mixing and fermentation processes and by providing stability to the dough during baking. Recently, it has also been demonstrated that addition of emulsifiers together with hydrocolloids into gluten-free formulations is critical, since the complex formed by hydrocolloid, emulsifier, and dough components have an important role in the enhancement of dough handling ability and bread quality (Demirkesen et al. 2010b, 2011; Nunes et al. 2009). Therefore, in the presence of xanthan–

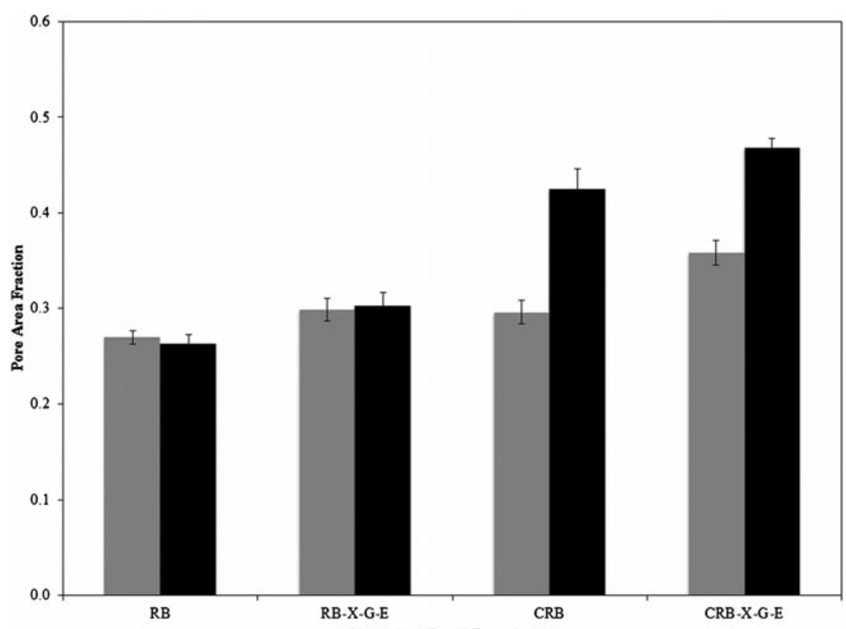


**Fig. 2** Based on scanned images, pore area fractions of different gluten-free bread formulations baked in conventional (*gray*) and infrared–microwave combination ovens (*black*). (*RB* rice bread, *RB-X-G-E* rice bread containing xanthan–guar gum blend–DATEM mixture, *CRB* chestnut–rice bread, *CRB-X-G-E* chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture)

**Table 1** Pore area distribution of gluten-free breads prepared with different formulations and baked in different ovens

| Number of pores<br>Range of pore area (mm$^2$) | Oven type | B-X-G-E | RB | B-X-G-E | CRB |
|---|---|---|---|---|---|
| 0–5 | Conventional | 161 | 98 | 278 | 339 |
| 5–10 | | 62 | 23 | 54 | 32 |
| 10–20 | | 24 | 18 | 28 | 24 |
| >20 | | 3 | 11 | 1 | 5 |
| Total number of pores | | 250 | 150 | 361 | 400 |
| 0–5 | Infrared–microwave combination | 259 | 271 | 291 | 292 |
| 5–10 | | 54 | 25 | 27 | 26 |
| 10–20 | | 16 | 23 | 26 | 25 |
| >20 | | 0 | 5 | 17 | 19 |
| Total number of pores | | 329 | 324 | 361 | 362 |

*RB* rice bread, *RB-X-G-E* rice bread containing xanthan–guar gum blend–DATEM mixture, *CRB* chestnut–rice breads, *CRB-X-G-E* chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture

guar gum–DATEM mixture, higher pore area fraction values were obtained in rice and rice–chestnut breads.

As shown in Fig. 2, there were significant differences in the pore area fraction values of gluten-free breads baked in different ovens ($p \leq 0.05$). Pore area fraction values of breads baked in infrared–microwave combination oven were significantly higher than that of conventionally baked ones ($p \leq 0.05$). The high internal heat generation in infrared–microwave combination baking produces higher internal pressure, which creates a puffing effect (Demirkesen et al. 2012). This puffing effect might be the reason of looser and more porous structure in gluten-free breads baked in an infrared–microwave combination oven. The increased effect of an infrared–microwave combination oven on pore area fraction values has also been recognized for wheat breads (Ozkoc et al. 2009).

Among all gluten-free breads, the highest pore area fraction values were obtained from breads prepared with chestnut flour with xanthan–guar gum blend–DATEM mixture addition and baked in an infrared–microwave combination oven (Fig. 2). This result is in good agreement with a previous study in which volume of breads containing chestnut flour and baked in an infrared–microwave oven were found to be significantly higher than that of a conventionally baked one ($p \leq 0.05$) (Demirkesen et al. 2011).

Pore area distributions of different gluten-free bread formulations and baked in conventional and infrared–microwave combination ovens are presented in Table 1. According to two-way ANOVA, both bread formulations and oven types affected pore area distributions of breads significantly ($p \leq 0.05$). Among all gluten-free breads, the lowest total number of pores was obtained from conventionally baked rice breads prepared without additives. As discussed before, the reason for this is the lack of incorporation of sufficient air bubbles. The addition of xanthan–guar gum blend–DATEM mixture caused entrapment of more air into dough resulting in an increase in total number of pores in conventionally baked

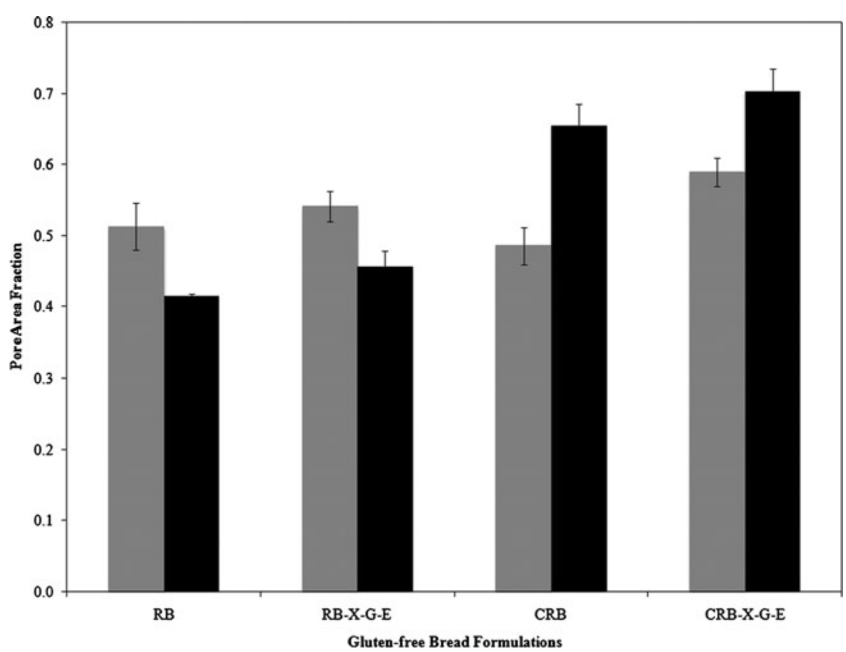


**Fig. 3** Based on SEM, pore area fractions of different gluten-free bread formulations baked in conventional (*gray*) and infrared–microwave combination ovens (*black*). (*RB* rice bread, *RB-X-G-E* rice bread containing xanthan–guar gum blend–DATEM mixture, *CRB* chestnut–rice bread, *CRB-X-G-E* chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture). Magnification: 20×

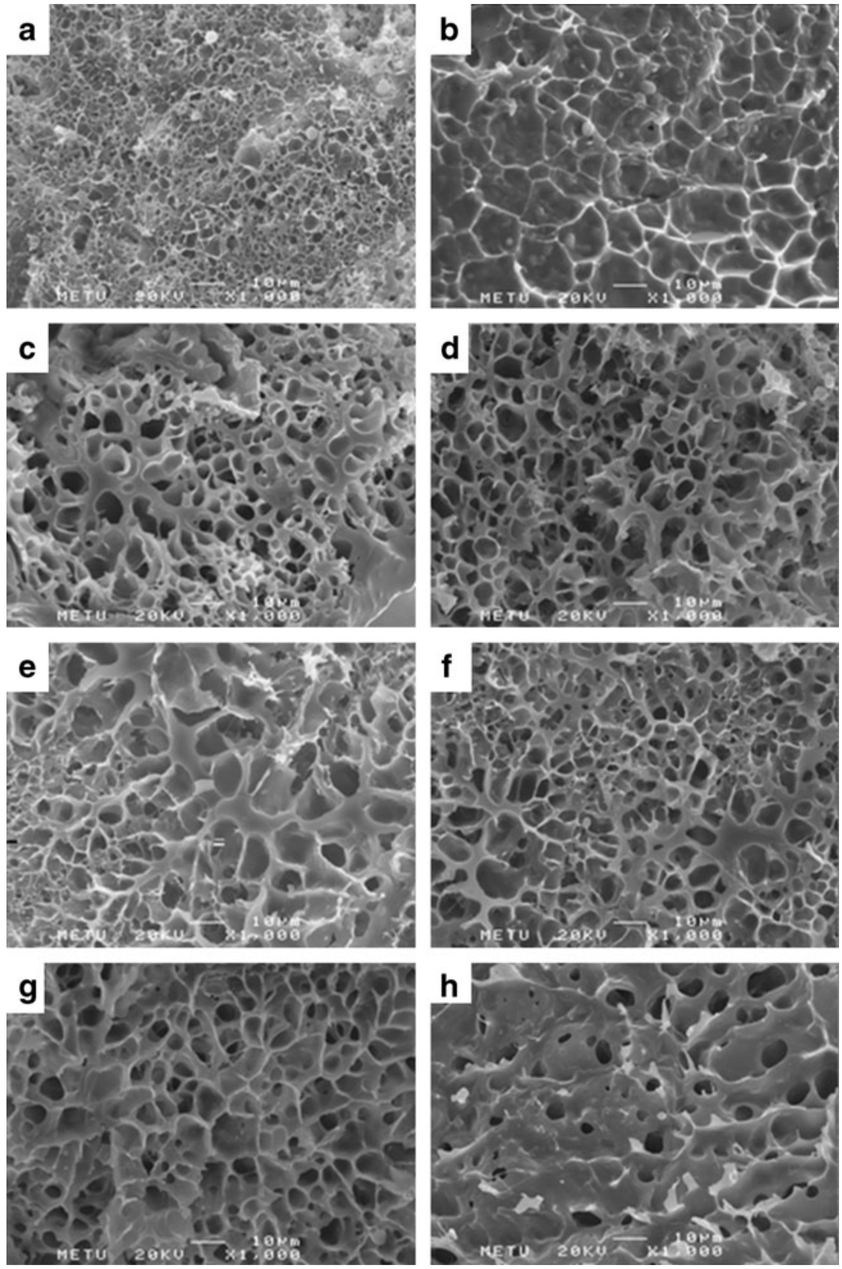


**Fig. 4** SEM micrographs of outside of gluten-free bread crumb samples baked in different ovens. **a** Rice bread baked in a conventional oven. **b** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **c** Chestnut–rice breads baked in a conventional oven. **d** Chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **e** Rice bread baked in an infrared–microwave combination oven. **f** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. **g** Chestnut–rice breads baked in an infrared–microwave combination oven. **h** Chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. Magnification: 1,000×

rice breads (67 %). However, addition of xanthan–guar gum blend–DATEM mixture did not cause such a noticeable increase in the total number of pores of rice breads baked in an infrared–microwave combination oven. This may be due to the fact that high pressure gradient occurring inside the breads during infrared–microwave combination was found to be more effective on the total number of pores of these breads as compared to additives. In general, it can be said that baking type resulted in noticeable change in the total number of pores and distribution of pores of rice breads, and when rice bread formulations were baked in infrared–microwave baking oven, approximately 23–28 % increase in small size of pores (0–5 $mm^2$) occurred (Table 1).

As opposed to rice dough, chestnut flour containing dough could incorporate sufficient amount of air bubbles during the mixing and fermentation processes. Both the presence of additives and baking type did not result in noticeable change in the total number of pores of chestnut–rice breads. However, despite the slight differences in total number of pores of chestnut–rice breads, high pressure during infrared–microwave baking changed pore area distribution of chestnut–rice breads resulting in approximately a 71 % increase in the number of large pores (>10 $mm^2$).

Pore roundness of the gluten-free bread samples were also determined by analyzing the scanned images of crumbs and no significant difference was obtained in roundness

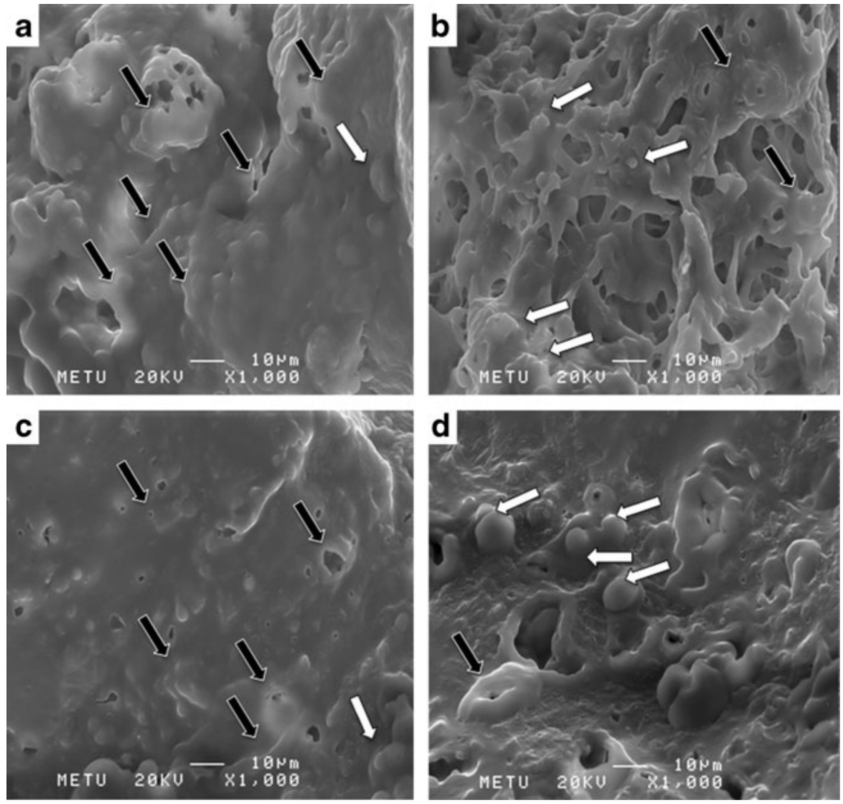


**Fig. 5** SEM micrographs of inside of gluten-free bread crumb samples baked in different ovens. **a** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **b** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. **c** Chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **d** Chestnut–rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. (*White arrows* represent starch granules residues and *black arrows* represent deformed starch granules). Magnification: 1,000×

values of gluten-free breads ($p \leq 0.05$). The roundness values of pores were in between 0.61 and 0.67 showing that the pores in breads were not circular in shape.

## SEM Analysis

The image analysis method was also used for obtaining quantitative information on bread samples examined with SEM at magnification of 20×. Like scanned images, pore area fraction values of breads showed that breads formulated with chestnut flour with xanthan–guar gum blend–DATEM mixture addition and baked in an infrared–microwave combination oven were found to be the highest (Fig. 3). Although fiber had a critical role in the enhancement of rheological properties of dough and bread quality, it was not sufficient to stabilize gas cell in gluten-free bread formulations alone (Demirkesen et al. 2010a). As mentioned above, addition of xanthan–guar gum blend–DATEM mixture and using of infrared–microwave combination baking were found to be necessary to obtain higher pore area fraction values from breads.

SEM results of the outside and inside of bread crumbs prepared with different formulations and baked in different ovens can be seen in Figs. 4 and 5, respectively. In gluten-free breads, the viscosity of the batters prior to starch gelatinization is critical to prevent the settling of the flour particles and escaping of gas cells prior to starch gelatinization and hence, provide a homogenous system during fermentation and baking until starch gelatinization (Alvarez-Jubete et al. 2010). In the absence of additives, rice breads especially conventionally baked ones had less developed pores, since sufficient amount of air bubbles could not be entrapped into the dough (Table 1 and Fig. 4a and e). The replacement of rice flour with chestnut flour resulted in higher amount pores (Table 1 and Fig. 4c and g), since fiber content of chestnut flour provided higher viscosity values to gluten-free dough. In addition, the presence of fiber may decrease starch–protein binding resulting in a more homogenous structure as compared to rice breads (Figs. 2 and 4a and c) (Sabanis et al. 2009). The difference in the distribution of starch granule size may also have implications on appearance of crumb structures. The rice starch granules were smaller in size ranging between 3 and 7 μm in diameter, while chestnut starches had larger granules with a diameter of around 8–12 μm. In the study of Park et al. (2004), the significant relationship between starch granule size and gas retention was found to be responsible for final crumb appearance. Flours that have larger starch granules tended to release more amylose during baking, since they contained more amylose as compared to small granules. As a result, a film-like structure was formed by the interaction between that amylose and protein which might coalesce less during baking (Park et al. 2004; Alvarez-Jubete et al. 2010). This is in agreement with the increase in crumb fineness with the replacement of chestnut flour observed in our study. However, the pores of conventionally baked chestnut–rice breads were not evenly distributed as much as those of baked in an infrared–microwave combination oven. In addition, some swollen and evenly dispersed starch granules created a continuous sheet on some part of chestnut bread crumb (Fig. 4c). Similar to scanned images of breads, SEM observation showed that among all breads prepared without any additives, the most homogenous structure was obviously obtained from breads formulated with chestnut flour and baked in an infrared–microwave combination oven (Fig. 4g).

The addition of xanthan–guar gum blend–DATEM mixture improved bread structures, since more homogenous pore distributions were obtained in gluten-free breads (Fig. 4b, d, f and h). As can be seen in Fig. 4h, in the presence of high internal pressure, fiber, and additive, the surface of the starch granules were stretched and rolled up into fibrils and formed a veil-like structure in gluten-free chestnut–rice breads.

According to inside bread crumb images obtained at 1,000× magnifications, breads baked in conventional and infrared–microwave combination ovens had granular and deformed starch together (Fig. 5). However, breads prepared with both rice and chestnut flour and baked in an infrared–microwave combination oven had more granular residues (Fig. 5d). Furthermore, the starch granules in these breads did not lose their identity and did not disintegrate completely. Incomplete disintegration of starch granules may be due to shorter processing time that affects swelling and gelatinization. In the study of Demirkesen et al. (2012), gelatinization degrees in breads baked in an infrared–microwave combination oven (84–88 %) were found to be lower than those in conventionally baked gluten-free breads (92–94 %). Higher fiber and sugar content of flour are also effective in incomplete disintegration of starch granules. It was also found that higher fiber content and sugar content in chestnut flour increased gelatinization temperatures resulting in hindering of starch gelatinization during baking (Demirkesen et al. 2012). Thus, in the presence of both chestnut flour and infrared–microwave combination baking, more granular residues were obtained.

## Conclusion

Different formulations and oven types were found to be effective on pore area fractions and pore area distributions of bread crumbs. Breads prepared with chestnut flour and xanthan–guar gum blend–DATEM mixture had a more porous structure. In addition, pore area fraction values of breads increased when the infrared–microwave combination baking method was used. Based on scanned and SEM images, the highest pore area fractions were obtained from gluten-free breads containing chestnut flour and xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. Generally, the usage of infrared–microwave combination baking increased the number of small pores in rice breads and large pores in chestnut–rice breads. The replacement of rice flour with chestnut flour resulted in a more uniform structure. The presence of additives and infrared–microwave combination oven increased the uniformity of microstructure of rice and rice–chestnut breads. SEM observation showed that breads prepared with chestnut flour and baked in an infrared–microwave combination oven had more starch granules, which did not lose their identity and did not disintegrate completely.

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