



## Characterization of structure of gluten-free breads by using X-ray microtomography



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### ABSTRACT

The effects of different gums and gum blends addition on crumb structure of gluten-free breads were studied by X-ray microtomography. The gums used were xanthan, guar, locust bean (LBG), agar, methylcellulose (MC), carboxymethylcellulose (CMC) and hydroxypropylmethylcellulose (HPMC) and the gum blends were xanthan–guar and xanthan–LBG. Porosity, number, average size, aspect ratio and roundness of pores were used as parameters to describe the internal structure of the crumbs. Characteristics of the crumb structure were related to the hardness, cohesiveness and springiness of breads. Gluten-free breads prepared with the addition of MC, agar and no additives had the highest porosity values with the lowest number of pores, which would be indicating a non-uniform crumb structure with large pores. There was a negative correlation between porosity and number of pores ( $r = -0.90$ ) and a positive correlation between porosity and average size of pores ( $r = 0.80$ ). Lowest porosity, lowest average pores area and the highest number of pores were observed on gluten-free breads prepared with the addition of xanthan, CMC, xanthan–guar, xanthan–LBG and HPMC. The higher number of smaller pores is associated with a finer crumb structure. Data showed that the hardness values of breads were positively correlated with porosity ( $r = 0.87$ ) and average area of pores ( $r = 0.87$ ) and negatively correlated with the number of pores ( $r = -0.92$ ). On the other hand, cohesiveness and springiness values of breads were negatively correlated with porosity ( $r = -0.86$  and  $-0.88$ ), average area of pores ( $r = -0.81$  and  $-0.90$ ) and positively correlated with number of pores ( $r = 0.89$  and  $0.88$ ).

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### 1. Introduction

Celiac disease is a condition predominantly of the small bowel, caused by sensitivity to ingested gluten in certain grains such as wheat, rye, barley and possibly oat. Recent studies showed that the prevalence of celiac disease in the United States is estimated to be at least 1:133, while it is approximately present in 1:150–300 in Europe (Fasano et al., 2003; McLoughlin et al., 2003). Moreover, it still remains under-diagnosed in most countries. The only way to overcome this disease is following a strict gluten-free diet through the patients' lifetime. Among the components of bread dough, gluten is unique in exhibiting viscoelastic networks that are responsible for the elastic and extensible properties which assists to retain gas produced from yeast fermentation and oven rise

(Demirkesen, Sumnu, & Sahin, 2013). Thus, breads formulated without gluten are of poor quality with low volume, poor texture, and flavor and fast staling. Since these products are not enriched and fortified, they do not contain adequate amount of vitamins, minerals, and fiber to meet the nutritional needs of celiac sufferers. Thus, gluten replacement remains to be one of the most challenging tasks for cereal technologist and scientists (Demirkesen, Mert, Sumnu, & Sahin, 2010a). To ensure acceptability of gluten-free products, modifications in formulations by replacing wheat flour by alternative flours and by using ingredients such as hydrocolloids, emulsifiers, sugars, shortening, enzymes and fibers have long been established by the gluten-free baking industry (Demirkesen et al., 2010a; Demirkesen et al., 2013; Demirkesen, Mert, Sumnu, & Sahin, 2010b; Demirkesen, Sumnu, Sahin, & Uysal, 2011; Matos & Rosell, 2012; Moreira, Chenlo, & Torres, 2011; Purhagen, Sjöö, & Eliasson, 2012).

Hydrocolloids, commonly known as gums, have been widely used in the bakery industry since they can provide viscoelastic

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properties that resemble those of dough. Also they enhance moisture retention, texture and extend shelf life of bakery products. Emulsifiers have the ability to reduce the surface tension of dough resulting in the subdivision of entrapped air bubbles into more and smaller bubbles during mixing. They can also improve the quality of products by providing stability to the dough during baking (Demirkesen et al., 2013). It has been demonstrated that the benefit of hydrocolloids as dough stabilizers can be promoted in the presence of surfactants (Bollaín & Collar, 2004; Demirkesen et al., 2010a). Thus, in recent years, there has been a growing interest in the use of hydrocolloids and emulsifiers as gluten-substitutes in gluten-free bread formulations (Demirkesen et al., 2010a, 2010b, 2011, 2013; Nunes, Moore, Ryan, & Arendt, 2009; Onyango, Unbehend, & Lindhauer, 2009).

The physical, textural and sensory properties of the bakery products are largely related to their structure at several levels that go from molecular to macroscopic levels. The microstructure of bread crumbs provide an accurate quantitative description of features of bread crumbs in terms of cell wall thickness, cell shape, void fraction and crumb fineness. Therefore, characterization of microstructure has an essential role in developing of products with desired quality. In order to evaluate microstructure of bread crumb, image analysis is a convenient and useful key to qualify and quantify the characteristics of crumb structure (Demirkesen et al., 2013). Up to date, image analysis techniques such as light microscopy (LM), confocal scanning laser microscopy (CSLM), electron microscopy (EM), atomic force microscopy (AFM), magnetic resonance imaging (MRI) have been applied to evaluate the relationship between microstructure and physical properties of bread. The use of X-ray microtomography (X-ray  $\mu$ CT), which is usually used in medical applications, introduces some advantages over other image analysis techniques. This new imaging technique creates a 3-D representation of the inside structure of bread from 2-D image slices allowing a set of projection measurements recorded from a certain number of points of view in non-destructive and non-invasive way. The visualization of the final image results can be recorded as by 3-D representations originated from the 2-D slices or their projections following arbitrary directions. It has the ability to create the contrast-enhanced imaging without any sample preparation that helps to overcome typical artifacts in the visualization of structures. In very recent studies, X-ray  $\mu$ CT has been used for quantitative characterization of bread crumbs by creating 3-D representation of the inside structure of bread from 2-D image slices (Besbes, Jury, Monteau, & Le Bail, 2012; Falcone et al., 2004, 2005; Lape, Jensen, Jeor, & Lendon, 2008; Primo-Martín et al., 2010; Wang, Austin, & Bell, 2011). Thus, the objective of the present study was to point out microscopic changes of gluten-free breads by using X-ray  $\mu$ CT and to relate crumb microstructure with textural properties of breads. Another objective of this research was to understand the influence of different gums (xanthan, guar, locust bean (LBG), agar, methylcellulose (MC), carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC)) or gum blends (xanthan–guar and xanthan–LBG) and emulsifier DATEM addition on crumb porous structure of gluten-free breads.

## 2. Materials and methods

### 2.1. Materials

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. Emulsifier DATEM (diacetyltartaric acid esters of

monoglycerides) was obtained from Danisco Co., (Copenhagen, Denmark). Xanthan, guar, LBG, agar, MC, CMC and HPMC were obtained from Sigma–Aldrich (St. Louis, MO, USA).

### 2.2. Bread making procedure

Basic dough recipe on 100 g rice flour basis consisted of 8% sugar, 8% shortening, 2% instant yeast, and 2% salt was used in the experiments. The amount of water (30 °C) added to the rice dough was 143% on flour basis. Gums (xanthan, guar, LBG, agar, MC, CMC, HPMC) or gum blends (xanthan–guar and xanthan–LBG gum) and emulsifier (DATEM) were added in the formulation at concentrations of 0.5% that were determined by using bread texture and volume results in our previous study (Demirkesen et al., 2010b).

The blends of xanthan–guar and xanthan–LBG were prepared by mixing equal amount of each gum. Before adding the gums or gum blends into dough mixture, each gum (0.5%) was dispersed in half of the water to be used in the dough formulation using a high speed homogenizer (Brinkmann Instruments, Westbury, NY, USA). During preparation of the bread formulations first, dry ingredients (rice flour, instant yeast, sugar, salt and emulsifier) were mixed thoroughly, and then the melted shortening was added. Finally 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 (Kitchen Aid, 5K45SS, ELKGROVE Village, USA). After complete mixing, fermentation was done at 35 °C and 85% RH in a proofing cabinet (Inter Metro Industries Co., Wilkes-Barre, PA) for 40 min. Gluten-free bread prepared without any additives (gums and emulsifiers) was used as control.

### 2.3. Baking

Gluten-free bread formulations was baked in a rotary electric oven (National Mfg. Co., Lincoln, NE) and breads samples (200 g each) were baked at 200 °C for 30 min.

### 2.4. Bread analysis

#### 2.4.1. Hardness

The TPA analysis was conducted on bread loaves which were allowed to cool for 1 h by using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Samples in cubic shapes having dimensions of 25 × 25 × 25 mm were taken from the center of bread. Samples were compressed to 25% of their initial height with a cylindrical probe (diameter a 25.4 mm) and the time between the compressions was 0.5 s. Trigger force was 5 g. A pre-test speed of 3 mm/s, test speed of 2 mm/s, and post-test speed of 2 mm/s were used. Hardness (N), cohesiveness, springiness (%), chewiness (N) and adhesiveness (Nm) values of the bread samples were determined by using the definitions given below. Hardness is the peak force during the first compression cycle (Sahin & Sumnu, 2006). Cohesiveness is the ratio of the second positive area to the first positive area. Adhesiveness is the negative force area for the first bite. Springiness is defined as the distance of the height of the product on the second compression divided by the original compression distance. Chewiness is defined as the product of hardness × cohesiveness × springiness values.

#### 2.4.2. X-ray micro computed tomography (X-ray $\mu$ CT) analysis

MicroCT 40 (Scano Medical Inc., PA) was used to study porous structure of gluten-free bread crumbs. The parameters of  $\mu$ CT were selected for foods to be most favorable at 45-kVp and 177  $\mu$ A intensity (Kelkar, Stella, Boushey, & Okos, 2011). The largest sample cell having 35.6 mm diameter and medium resolution was selected for scanning the crumbs. Each of the bread samples was cut to fit

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