

# Effects of buckwheat flour, gums and proteins on rheological properties of gluten-free batters and structure of cakes

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## RESEARCH ARTICLE

### Abstract

The main objective of the study is to determine the effects of buckwheat flour concentration and different gum types (xanthan, guar gum) or proteins (soy protein and whey protein) on rheological properties of gluten-free cake batters and physical properties of cakes (weight loss, porosity, specific volume, hardness and macro structure). Gum containing batters exhibited higher apparent viscosities. More uniform distribution of gas cells were observed in whey protein containing samples compared to other batters. Although increasing buckwheat flour concentration decreased weight loss, porosity and specific volume, it increased the hardness of the cakes. Cakes containing whey protein had the highest pore area fraction which was also correlated to porosity, specific volume and the texture results. Therefore, cakes containing 10% level buckwheat flour and whey protein can be recommended for coeliac patients as a high quality gluten-free cakes.

**Keywords:** baking, buckwheat flour, gluten-free cake, image analysis, rheology

### 1. Introduction

Coeliac disease is described as immune mediated disorder of intestinal mucosa that is triggered by protein; gluten (Mendoza, 2005). Storage proteins, such as gliadin, hordein, and secalin have roles not only in total protein content but also in quality of end product (Larrosa *et al.*, 2013; Shewry *et al.*, 1995). Although glutenin gives elasticity to dough, gliadin acts as a plasticizer that moderates bond strength which glutenin forms (Veraverbeke and Delcour, 2010). Although producing a gluten-free product has some difficulties, studies showed that products without gluten can maintain gas inside the structure in the presence of gluten mimicking material. Thus, hydrocolloids are used in gluten-free products for thickening, gelling, and texture improvement purposes (Mir *et al.*, 2015). In this study, xanthan and guar gums were selected as hydrocolloids in gluten-free cakes.

Whey protein is frequently preferred as an ingredient in bakery industry because of its functional attributes and high nutritional value. It contains essential amino acids, particularly lysine, leucine and methionine. In addition

to that, whey protein improves colour, flavour, and textural characteristics of the product (Marques *et al.*, 2016). Whey proteins are  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, immunoglobulins, and bovine serum albumin. They are mainly responsible for foaming, gelation, emulsification and hydration properties (Panaras *et al.*, 2011). The effect of replacement of egg protein by whey protein on wheat flour cakes was studied by Jyotsna *et al.* (2007) who showed that incorporation of whey protein to the batter increased the number of air cells in cake.

One of the most important plant source protein is soy bean. Soy protein, with its high nutritional value, has been regarded as an economical source of essential amino acids, especially lysine. Furthermore, soy proteins assist health promotion by reducing risk of cancer and cardiovascular diseases due to having large amounts of isoflavones (Majzoobi *et al.*, 2014). The effect of soy bean isolate, broad bean flour and chickpea flour on the sensorial and physicochemical attributes of biscuit has been studied and results indicated that soy protein fortification increased the hardness of biscuits (Rababah *et al.*, 2006).

Rice flour is generally regarded a main ingredient of gluten-free product formulations due to high level of readily digested carbohydrates, hypo-allergic features, colourless appearance and bland taste (Demirkesen *et al.*, 2010a). Moreover, chestnut flour (Demirkesen *et al.*, 2010b), soy flour (Menon *et al.*, 2014), sorghum and quinoa (Hager *et al.*, 2012), lupin, (Levent and Bilgiçli, 2011) are the other alternatives of gluten-free flours.

Buckwheat is one of the pseudo-cereals which has already known to have many health benefits. For example; it is very rich in terms of polyphenols and flavonoids (Torbica *et al.*, 2012). Because digestion of buckwheat carbohydrate monomers are slower; it is beneficial to gain glucose tolerance. Thanks to high mineral content, it has also cholesterol lowering abilities. Furthermore, it is a good source of dietary fibre (Mariotti *et al.*, 2013). A study conducted in 2014 mainly focused on the relationship between rheological properties and quality of gluten-free bread prepared with chickpea, millet, rice, corn, quinoa, and buckwheat (Burešová *et al.*, 2014). It was mentioned that there was a strong relation between rheological behaviour of bread dough and final loaf volume. Dough resistance, extensibility and extension area were the main parameters that influenced bread volume. A high correlation coefficient between these parameters has been the indicator of that hypothesis. As a result, final product quality or baking performance of dough mainly depends on dough resistance and stretching ability under uniaxial deformation.

Another research characterised sensorial and physicochemical properties of buckwheat biscuit with guar gum, gum acacia, xanthan gum, and gum tragacanth (Kaur *et al.*, 2015). It was recorded that addition of hydrocolloids to biscuit formulation led to higher moisture content, diameter thickness and weight of the samples. The effect of hydroxypropyl methylcellulose (HMPC) and buckwheat flour on bread quality was analysed in terms of specific volume, crumb texture, weight, height and colour (Mariotti *et al.*, 2013).

Research is still going on to improve both quality and nutritional aspects of gluten-free bakery products. Arslan *et al.* (2017) studied the effects of butter content (0, 10, 15, 20, 25 and 30 g/100 g flour) and different baking conditions (hot air baking, microwave baking and hot air microwave baking) on bread dough and bread characteristics in terms of moisture content, specific volume, hardness, colour, and starch digestibility and quality during storage. Furthermore, to improve the nutritional value and fibre content in gluten-free bread, guava pulp powder was added to gluten-free bread formulation at different levels 0, 2.5, 5, 7.5 and 10% (Srikanlaya *et al.*, 2017).

Although buckwheat flour has high amounts of polyphenols, flavonoids, fibre and minerals, there is not much research on

the use of buckwheat flour in gluten-free cakes. In addition, the studies in literature do not focus on the influence of buckwheat flour on physical properties and structure of cakes. Therefore, the main objective of the study was to determine the effects of buckwheat flour and addition of different gums/proteins (xanthan gum, guar gum, soy protein, and whey protein) on rheological properties of cake batter and physical properties of cakes. This study also gives information about the structure of gluten-free cakes, which is lacking in most of the gluten-free cake studies.

## 2. Materials and methods

### Materials

Rice flour and buckwheat flour were obtained from Başak Flour (Ankara, Turkey), and Yar (Antalya, Turkey), respectively. Salt (Billur Tuz, İzmir, Turkey), shortening (Sana, Unilever, Istanbul, Turkey), sugar (Bal Küpü, Aksaray, Turkey), and baking powder (Dr. Oetker, İzmir, Turkey) were purchased from local markets in Ankara. Egg white powder and emulsifier (monoglyceride and polyglycerol esters of fatty acid) were obtained from ETI Food Industry Co. Inc. (Eskişehir, Turkey). Xanthan gum and guar gum were bought from Sigma-Aldrich (St. Louis, MO, USA). Soy protein concentrate and whey protein concentrate with 80% protein were obtained from Smart Chemical Trading Co. Inc., İzmir, Turkey).

### Methods

#### *Preparation of cake batter*

A recipe of cake was adopted from the literature (Turabi *et al.*, 2008). Cake batter formulation contained 5% baking powder, 3% salt, 100% sugar, 9% egg white powder, 25% shortening, 3% emulsifier, and 90% water in terms of rice flour basis. Buckwheat flour at different levels (0, 10, 20 and 30%) was added to the formulation by replacing rice flour. Gum and protein addition levels were 0, 1% xanthan gum, 1% guar gum, 3% soy protein and 3% whey protein in terms of rice flour basis. Buckwheat flour, gum and protein free samples were considered as control. The first step of preparation of cake batter was mixing. Dry ingredients were mixed with a mixer (Kitchen Aid 5K45SS; Whirlpool Corporation, Benton Charter Township, MI, USA) for 2 min at 85 rpm. Gum was dispersed in water by high speed homogeniser at 7,200 rpm for 5 min (IKA T18Ultra-Turrax, Staufen, Germany). Melted shortening and gum suspension were added to the mixture and mixed further at 85 rpm for 5 min.

### Rheological properties of cake batter

Rheological behaviour of cake batter was examined using a parallel plate rheometer (Kinexus, Malvern, Worcestershire, UK). The gap between the plates was fixed to 1 mm. To understand the flow behaviour of batter, shear rate between 1-10/s was applied and the corresponding shear stress data was obtained. As a first step of dynamic oscillatory experiments, linear viscoelastic region of batter was detected as strains ranging between 0.01%-100% and at constant frequency of 1 Hz. After that, frequency sweep analysis was performed by changing frequency from 0.1 to 10 Hz with a constant strain rate of 0.1%. Finally, results were determined in terms of elastic modulus ( $G'$ ), and loss modulus ( $G''$ ).

### Physical properties of cake batter

Pycnometer was used in specific gravity measurement (Turabi *et al.*, 2008). To analyse the morphological characteristics of gas bubbles formed during mixing, batters were displayed under light microscope. Very thin layer of prepared batters was smeared on glass microscope slides. Then, it was placed under the microscope (Primo Vert; Zeiss, Jena, Germany). Images were obtained with the help of microscopic camera (Sony CCD Color Digital Video C-Mount Microscope Camera, Tokyo, Japan) and analysed using software TopView (SPECwise, West Palm Beach, FL, USA).

### Baking and analysis of cakes

For baking of cakes, an electrical oven (9411FT, Arçelik Inc. Co., Istanbul, Turkey) was used. Before starting the baking procedure, oven temperature was set to 175 °C. After oven was preheated for 10 min, four glass cups (diameter is 70 mm, and height is 95 mm) each containing 100 g batter were placed into the oven. Baking operation took 28 min. Then, weight loss, porosity, specific volume, texture and image analysis of cakes were carried out. Weight loss was determined using Equation 1:

$$\text{Weight loss} = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \times 100 \quad (1)$$

Where  $W_{\text{initial}}$  refers to weight of dough before baking,  $W_{\text{final}}$  represents the weight of cake after baking.

Porosity of cakes was measured with a compression method (Turabi *et al.*, 2008). To measure the specific volume, the rape seed displacement method was used (AACC, 1988). For texture analysis, after being cooled down for 1 h, two cakes were cut into cubic shape having dimensions of 3×3×3 cm. To measure the hardness value of cakes texture analyser with a cylindrical probe having diameter of 1 cm and load

cell of 50 kgf (TA Plus; Lloyd Instruments, Bognor Regis, UK) was used. Force required to compress the sample 25% of its initial height with a compression speed of 55 mm/min was measured.

For determination of pore properties, cakes were divided into two vertically cut parts. The cut side of one piece of cake was placed on the glass of a scanner (CanoScan, 3200F, Tokyo, Japan) and scanned with a scanning resolution 300 dpi. To analyse porosity of cakes and pore area, the software (ImageJ) was used (Turabi *et al.*, 2008). First, each scanned image was cropped to eliminate artifacts at the same cross section area. Then they were converted to grey scale (8 bit) and pixel values were converted to mm by using bars with known length. After that, binarise operation was done to differentiate two phases (solid part and pores) (Turabi *et al.*, 2008). Pore areas smaller than 0.5 mm<sup>2</sup> were not counted. Using the analyse option, pore area, size fraction and distribution values were obtained.

### Statistical analysis

Analysis of variance was carried out using MINITAB (Version 16; State College, PA, USA). If there was a significant difference, Tukey multiple comparison test was used for comparison ( $P \geq 0.05$ ). Baking was replicated twice for each cake formulation. The correlation coefficient between specific gravity, specific volume and hardness of cakes was expressed by Pearson correlation with 95% confidence level ( $\alpha=0.05$ ).

## 3. Results and discussion

### Rheological analysis of cake batter

Buckwheat containing cake batters showed shear thinning behaviour as seen from Figure 1. Apparent viscosity- shear rate relations between cake batter containing 20% and 30% buckwheat flour were very similar to 10% containing ones. Cake batters containing xanthan and guar gum always had higher apparent viscosity. On the other hand, control samples and samples with soy, and whey protein had almost identical consistency.

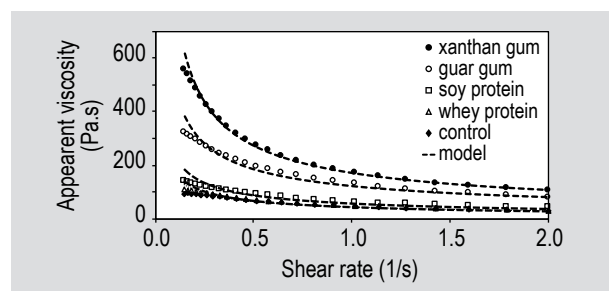


Figure 1. Apparent viscosity of 10% buckwheat flour containing batters with different gum/protein type.

Power law constants of gluten-free buckwheat added cake batters are shown in Table 1. Shear stress ( $\tau$ , Pa) versus shear rate ( $\dot{\gamma}$ , 1/s) data were well fitted to Power law model (Equation 2):

$$\tau = K(\dot{\gamma})^n \quad (2)$$

where K refers to consistency index ( $\text{Pa}\cdot\text{s}^n$ ), and n is flow behaviour index.

As all flow behaviour indices were lower than 1 (Table 1), all type of cake batter showed a shear thinning; in other words, a pseudo-plastic behaviour. With increasing shear rate, apparent viscosity of pseudo-plastic material started to decrease, due to disruption of aggregates and alignment of molecules in the direction of the flow (Moser *et al.*, 2013). Xanthan gum and guar gum added cakes had a higher consistency index that might be due to the complex aggregates developed by semi-rigid molecules and high water holding capacity of gum, which decreases available water that promotes movement of particles in cake batter (Demirkesen *et al.*, 2010b). Addition of soy protein to cake formulation also increased the consistency index which might be due to disulphide bonds. A study by Majzoobi *et al.* (2014) mentioned the effect of soy protein addition on wheat flour containing cake batter rheology. It was found that intermolecular interactions between proteins present in the cake batter created a medium with higher consistency. The responsible bonds were mainly electrostatic and disulphide bonds (Majzoobi *et al.*, 2014). Furthermore, a study by Gómez *et al.* (2011) on gluten containing dough found that mixing introduced a high number of newly formed

gluten entanglements due to S-S bonds. On the other hand, overmixing led to cleavage of these bonds and resulted in dough breakdown. Although the flours in this study do not contain gluten, the basic principle for S-S linkage is the same. In cake batter S-S bonds might occur between either soy proteins or soy protein and egg white proteins. However, addition of whey protein decreased the consistency index. This could be explained by the fact that more air incorporation during mixing had a decreasing effect on the consistency index.

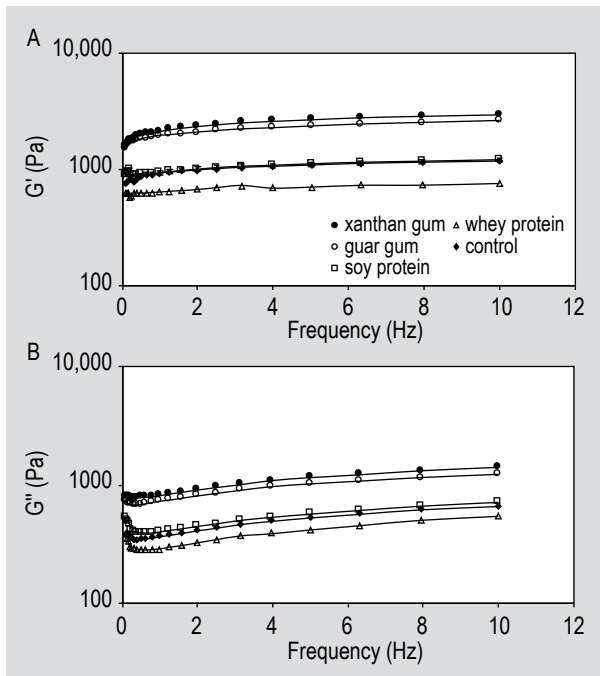
Increasing buckwheat concentration resulted in increasing consistency index (Table 1). This might be due to increasing fibre content. A study by Gularte *et al.* (2012) showed that addition of both soluble (inulin and guar gum) and insoluble (oat fibre) fibre in a gluten-free cake formulation resulted in increasing cake batter viscosity). Figure 2 shows the storage and loss modulus of batters containing 10% buckwheat flour, respectively. Batters with 20% and 30% buckwheat flour showed the same pattern as batter with 10% buckwheat flour. Increasing modulus with increasing frequency is evidence of shear dependent moduli (Figure 2). However, gentle slopes of storage modulus indicate a low dependency of the modulus to frequency change. Furthermore, the storage modulus of all samples was higher than the loss modulus. Moreover, as shown by Figure 2A, the addition of xanthan and guar gum had an enhancing effect on batter elasticity. Gum added samples had a higher storage modulus than the others. This could be explained by self-association of gum even at low concentrations (Peressini *et al.*, 2011). Another reason might be hydrocolloid-starch interactions; e.g. by attaching and enclosing of xanthan to starch granules (Peressini *et al.*, 2011).

**Table 1. Power law constants of buckwheat flour added cakes at 25 °C.<sup>1,2</sup>**

Buckwheat flour (%)	Gum/protein type	n	K (Pa.s <sup>n</sup> )	R <sup>2</sup>
10	control	0.44±0.00 <sup>a</sup>	44.63±6.25 <sup>i</sup>	0.99
10	xanthan gum	0.32±0.01 <sup>e</sup>	170.47±4.15 <sup>c</sup>	0.99
10	guar gum	0.39±0.00 <sup>abcd</sup>	121.35±1.15 <sup>e</sup>	0.99
10	soy protein	0.40±0.02 <sup>abc</sup>	59.00±2.67 <sup>hi</sup>	0.98
10	whey protein	0.42±0.00 <sup>ab</sup>	46.60±0.60 <sup>i</sup>	0.99
20	control	0.39±0.00 <sup>bcd</sup>	68.62±1.33 <sup>gh</sup>	0.99
20	xanthan gum	0.32±0.01 <sup>e</sup>	211.24±1.85 <sup>b</sup>	0.99
20	guar gum	0.40±0.01 <sup>abc</sup>	142.53±6.11 <sup>d</sup>	0.99
20	soy protein	0.41±0.00 <sup>ab</sup>	73.41±2.11 <sup>fg</sup>	0.99
20	whey protein	0.39±0.00 <sup>bcd</sup>	65.98±1.97 <sup>gh</sup>	0.99
30	control	0.40±0.00 <sup>abcd</sup>	86.17±1.60 <sup>f</sup>	0.99
30	xanthan gum	0.35±0.03 <sup>cde</sup>	230.88±8.98 <sup>a</sup>	0.99
30	guar gum	0.35±0.00 <sup>de</sup>	169.74±7.23 <sup>c</sup>	0.99
30	soy protein	0.38±0.00 <sup>bcd</sup>	107.061±4.78 <sup>e</sup>	0.98
30	whey protein	0.39±0.00 <sup>bcd</sup>	83.371±2.89 <sup>fg</sup>	0.99

<sup>1</sup> K = consistency index; n = flow behaviour index;

<sup>2</sup> Values in a row having different superscript letters are significantly different ( $P<0.05$ ).

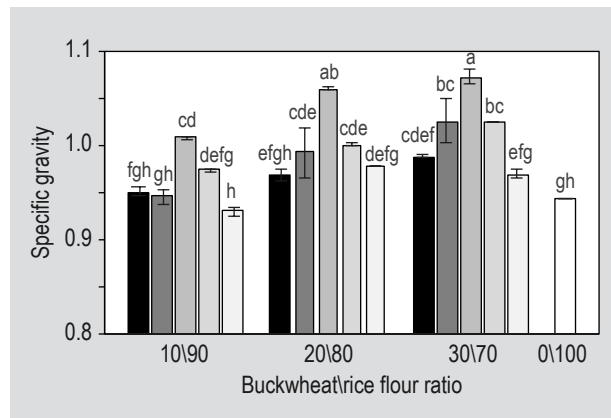


**Figure 2. (A) Storage modulus ( $G'$ ), and (B) Loss modulus ( $G''$ ) of batter samples containing 10% buckwheat flour.**

Tan  $\delta$ , which is the ratio of loss modulus to storage modulus at a constant frequency (1 Hz) (Herranz *et al.*, 2017; Peressini *et al.*, 2011) was calculated for all buckwheat added samples. Tan  $\delta$  values of all batters were less than 1, which implied that samples showed gel-like behaviour. In literature, similar results were found, confirming the solid like behaviour of gluten-free batters and doughs (Hadnadev Dapčević *et al.*, 2013; Sarabhai and Prabhasankar 2015). Although protein added samples, particularly whey protein, had a higher tan  $\delta$ , indicating a more liquid behaviour than gel-like, gum addition, especially of guar gum, decreased tan  $\delta$ , which showed that solid-like behaviour became more dominant. Tan  $\delta$  values of whey protein and guar gum added samples were between 0.379-0.601 and 0.337-0.406, respectively. Furthermore, when the elasticity of batters increases excessively, it becomes difficult to incorporate air into batter during mixing, leading to a lower quality (Peressini *et al.*, 2011).

### Specific gravity of batters

Specific gravity is a measurement of how much air is incorporated into batter during mixing. Figure 3 represents specific gravity values of buckwheat added cake batters prepared with gums and proteins. Guar gum added samples reached the highest, while whey protein added ones had the lowest value. Similar results were recorded for whey protein added cakes. Jyotsna *et al.* (2007) mentioned that cake batters with whey protein were lighter than those without, which was related to good foaming ability of whey protein.



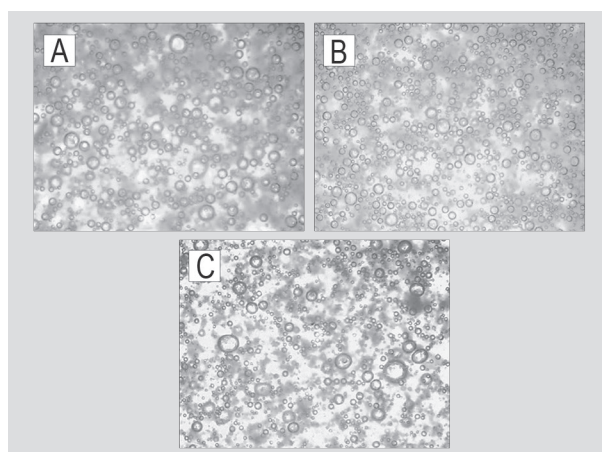
**Figure 3. Specific gravity of cakes prepared with different buckwheat flour ratio and gum/protein type. Bars having different letters are significantly different ( $P \leq 0.05$ ).**

Addition of xanthan gum and guar gum to 20 and 30% buckwheat containing samples resulted in higher specific gravity of the cake batter. This might be explained by the higher apparent viscosity of batter which made incorporation of air more difficult. Specific gravity of only rice flour containing cakes was not significantly different from cake batter containing 10% buckwheat, while those containing whey protein had the lowest specific gravity. This might be due to the positive effect of emulsifiers on aeration capacity (Khalil, 1998). Moreover, increasing buckwheat flour content in cake batter had a negative influence on specific gravity. Higher specific gravity values were recorded at higher buckwheat content, as increasing fibre content might obstruct mixing efficiency and aeration of gas bubbles.

### Morphological analysis of cake batters

Optical images of batters containing 10% buckwheat flour were obtained using light microscopy. Figure 4 shows that addition of protein and gum created differences in batter morphology. Cake batter with whey protein contained more gas bubbles than the control and guar gum containing batter.

More uniform distribution of gas cells was observed in whey protein containing samples compared to other batters. The size of gas cells present in whey protein added batter could be estimated as medium and small. On the other hand, cake batters prepared with guar gum had less gas bubbles compared to others. Moreover, these bubbles were either big or small in size and unevenly distributed. It has been known that addition of different hydrocolloids to cake formulation leads to different size and distribution of gas bubbles. Small size air bubbles are more desirable to obtain cakes with favourable texture and volume (Jia *et al.*, 2014). Therefore, it is expected that cakes with whey protein might have higher quality than the guar gum containing ones.



**Figure 4. Optical images of cake batters with 10% buckwheat (magnification 4×). (A) Control; (B) whey protein; and (C) guar gum.**

Moreover, the image analyses is supported by both rheology and specific gravity results. As discussed before, cake batters containing whey protein had the lowest specific gravity, which means that more air has been incorporated into the batter during mixing, due to the good emulsification and air aeration ability of whey proteins. Therefore, the high amount of air bubbles in whey added batters result in high quality of gluten-free cake. On the other hand, as seen in rheology results, batter formulated with guar gum showed the most solid like behaviour which obstructed aeration of air to the batter leading to the lowest number of air cells.

As shown by Figure 4, guar gum added batters had the lowest number of gas bubbles with non-homogenous gas bubble size distribution.

### Weight loss of cakes

Weight loss of cakes containing different levels of buckwheat flour is shown in Table 2. Increasing the amount of buckwheat flour means an increase in dietary fibre and starch content resulting in a decrease in weight loss of cakes. It has been reported that the water binding capacity of buckwheat starches is higher than corn and wheat starch, since starch granules of buckwheat have a smaller size thereby creating a higher surface area (Qian *et al.*, 1998). During baking, the crystalline structure of starch granules starts to disrupt and absorb water, resulting in swelling of starch granules. This acts as a barrier and prevents moisture loss, and consequently less weight loss (Xue and Ngadi, 2006). Furthermore, gums could weaken the starch structure and lead to a more uniform water distribution and better water retention (Kohajdová and Karovičová, 2009). This could explain why gum added samples have lower weight loss. It is known that high water soluble milk proteins, such as whey proteins, are less efficient than insoluble ones (e.g. casein) in terms of moisture retention. Although water retention ability of proteins increases with denaturation, whey proteins are not good at retaining moisture in cake after baking (Okun *et al.*, 2004).

**Table 2. Weight loss, porosity, specific volume and hardness of cakes prepared with buckwheat flour at different ratios and containing different gum/ protein.**

Buckwheat flour (%)	Gum/protein type	Weight loss (%)	Porosity	Specific volume (ml/g)	Hardness (N)
10	control	4.89±0.18 <sup>a</sup>	0.41±0.00 <sup>cde</sup>	1.77±0.02 <sup>bcd</sup>	1.44±0.09 <sup>f</sup>
10	xanthan gum	4.01±0.16 <sup>c</sup>	0.45±0.01 <sup>bc</sup>	1.66±0.01 <sup>def</sup>	2.01±0.06 <sup>e</sup>
10	guar gum	3.33±0.05 <sup>ef</sup>	0.32±0.01 <sup>ghi</sup>	1.56±0.08 <sup>f</sup>	4.51±0.02 <sup>a</sup>
10	soy protein	4.46±0.05 <sup>b</sup>	0.36±0.00 <sup>efgh</sup>	1.71±0.00 <sup>de</sup>	2.54±0.09 <sup>d</sup>
10	whey protein	4.41±0.12 <sup>b</sup>	0.60±0.03 <sup>a</sup>	1.98±0.00 <sup>a</sup>	0.64±0.02 <sup>h</sup>
20	control	3.70±0.10 <sup>cde</sup>	0.39±0.01 <sup>def</sup>	1.68±0.02 <sup>def</sup>	1.43±0.02 <sup>f</sup>
20	xanthan gum	3.76±0.05 <sup>cd</sup>	0.41±0.00 <sup>cde</sup>	1.66±0.04 <sup>def</sup>	2.13±0.00 <sup>e</sup>
20	guar gum	3.21±0.01 <sup>f</sup>	0.24±0.01 <sup>j</sup>	1.41±0.03 <sup>g</sup>	4.23±0.06 <sup>b</sup>
20	soy protein	3.78±0.05 <sup>c</sup>	0.32±0.01 <sup>ghi</sup>	1.64±0.01 <sup>ef</sup>	2.46±0.05 <sup>d</sup>
20	whey protein	3.92±0.10 <sup>c</sup>	0.50±0.00 <sup>b</sup>	1.85±0.00 <sup>b</sup>	0.97±0.04 <sup>7g</sup>
30	control	3.68±0.01 <sup>cde</sup>	0.31±0.01 <sup>ghi</sup>	1.62±0.02 <sup>ef</sup>	2.03±0.08 <sup>e</sup>
30	xanthan gum	3.79±0.08 <sup>c</sup>	0.37±0.00 <sup>efg</sup>	1.70±0.02 <sup>de</sup>	2.47±0.00 <sup>d</sup>
30	guar gum	3.41±0.02 <sup>def</sup>	0.28±0.02 <sup>ij</sup>	1.42±0.00 <sup>g</sup>	4.10±0.04 <sup>b</sup>
30	soy protein	3.73±0.01 <sup>cd</sup>	0.30±0.02 <sup>hij</sup>	1.64±0.02 <sup>ef</sup>	2.76±0.00 <sup>c</sup>
30	whey protein	3.73±0.12 <sup>cd</sup>	0.44±0.00 <sup>bcd</sup>	1.86±0.00 <sup>b</sup>	1.12±0.03 <sup>g</sup>
Rice flour	–	4.96±0.00 <sup>a</sup>	0.42±0.00 <sup>cde</sup>	1.73±0.00 <sup>cde</sup>	1.41±0.03 <sup>f</sup>

<sup>1</sup> Values in a row having different superscript letters are significantly different ( $P<0.05$ ).

## Porosity

The porosity of cakes with different buckwheat levels is shown in Table 2. Increasing amounts of buckwheat flour had a negative effect on porosity. The highest porosity was observed in cakes containing 10% buckwheat flour. These results were in good agreement with the specific gravity results, with a correlation coefficient of  $-0.797$  ( $P=0.000$ ). Whey proteins are known to be good surface active agents with good emulsifying ability. Because of this property and good foaming ability, cakes containing whey protein have a lower specific gravity. Soy protein did not have similar impact on porosity as whey protein. The reason for that might be the low solubility of soy protein related to the foaming ability (Kinsella, 1979). In addition, guar gum containing cakes had the lowest porosity which might be explained by specific gravity. Specific gravity of guar gum containing batters were the highest, which means during mixing not enough air could be entrapped in batter. Moreover, created gas bubbles might be unstable and could not be kept in the system for a long time.

## Specific volume

Specific volume of cakes was positively correlated with porosity values,  $0.881$  ( $P=0.000$ ). As shown by Table 2, cake with only rice flour had a higher specific volume than most cakes containing buckwheat flour. This might be due to the effect of emulsifiers, as it helps the incorporation of air bubbles during mixing (Seyhun *et al.*, 2005). Cake prepared with 10% buckwheat flour had a significantly higher specific volume than those containing 20 and 30% buckwheat flour. Increasing buckwheat flour content in cake formulation might make incorporation of air in cake batter more difficult due to higher fibre content, resulting in decreasing specific volume. As supported by specific gravity and porosity results, cakes with whey protein had the highest specific volume.

From literature, it is known that whey proteins are one of the globular proteins with great thermal gelling ability. During baking process, these proteins start to denature; tertiary structure protein bonds are destroyed at temperatures higher than  $70\text{ }^{\circ}\text{C}$ . After proteins become unfolded, new protein-protein interactions and interactions with other ingredients present in cake batter begin to form. These newly constructed interactions might be the reason of the increasing specific volume (Nunes *et al.*, 2009). Soy protein added samples had a similar volume as the control. According to a study by Matos *et al.* (2014), muffin volume was significantly affected by protein type. They stated that volume of vegetal origin protein (soy protein isolate, pea protein isolate and vital wheat gluten) added samples were not significantly different from control (no protein added). On the other hand, proteins from animal sources improved muffin volume (Matos *et al.*, 2014). Moreover, cakes with

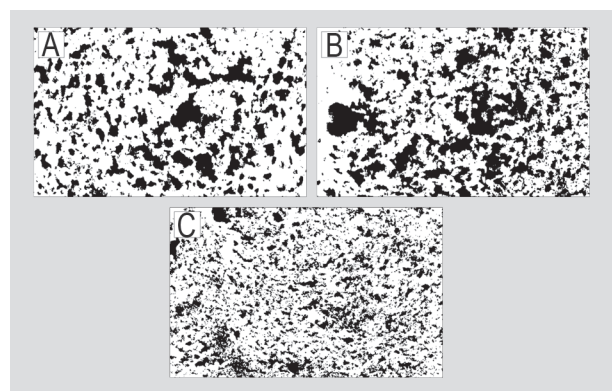
guar gum had the lowest volume, which was directly related to low specific gravity and more solid like behaviour. Higher apparent viscosity of batters can generally be interpreted as successful to prevent rising of gas bubbles. Besides, batters with guar gum did not have a high consistency index, like batters with xanthan gum. This meant that guar gum was not as sufficient as xanthan gum in gas retention.

## Hardness

Hardness results were found to be correlated with specific gravity and specific volume results. The correlation coefficient between specific volume and hardness was  $-0.879$  ( $P=0.000$ ). Besides, correlation coefficient between specific gravity and hardness was  $0.800$  ( $P=0.000$ ). The softest crumb was measured for buckwheat flour cakes with whey protein and the highest hardness was measured for buckwheat flour cakes with guar gum. This result is also supported by many other studies. It has been reported that guar gum added yellow layer cakes and rice cakes with guar gum had the hardest texture (Gómez *et al.*, 2007). Furthermore, addition of soy protein to the formulation did not show the desired influence on texture of gluten-free cakes. Similar results were obtained by Crockett (2009). She stated that disulphide linkages might reduce surface hydrophobicity and foam stability resulting in the loss of flexible film between water air interfaces. Therefore, lower incorporation of air into cake batters result in increasing hardness of cakes. Increasing buckwheat flour concentration from 10 to 20% did not create a significant difference. However, cakes with 30% added buckwheat had the highest hardness. This might be due to the thickening of cell walls by gas bubbles in the crumb (Gómez *et al.*, 2003).

## Macro structure of cake samples

Figure 5 represents binarised images of cakes containing 10% buckwheat flour. The difference in formulation of cakes created a significant difference in terms of pore area

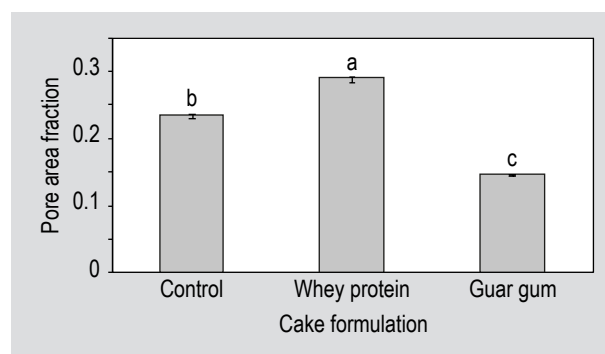


**Figure 5. Binarised images of cakes formulated with 10% buckwheat flour. (A) Control; (B) whey protein added; and (C) guar gum added.**

fraction (Figure 6). Whey added cakes reached the highest pore area fraction, whereas guar gum containing cakes had the lowest.

Rheology and specific gravity results supported such a distribution of area fraction. As mentioned before, whey added batter and control batter had the lowest consistency index value due to higher air incorporation into the batter. This is an indication of a more porous structure of baked cakes, which was also confirmed by specific gravity results. While the lowest specific gravity was measured in whey containing batters, guar gum added ones had the highest value. Batter morphology and pore area fraction results supported each other. Figure 4 showed that cake batter with whey protein had higher number of gas cells compared to control batter and guar gum added ones. Similarly, these cakes had the highest pore area fraction (Figure 6). Higher pore area fraction can be interpreted as more porous structure. Cakes containing whey protein had the highest area fraction which was also correlated to porosity, specific volume and the texture results. Due to good emulsification ability of whey protein, whey added samples always had a better quality. This was also supported by the study of Turabi *et al.* (2010). They implied that a higher pore area fraction meant higher volume and highly porous structure. Furthermore, this was related to rheological properties of batter. They stated that cakes with a xanthan-guar blend and only xanthan gum had the most porous structures, and also had higher volume and porosity. In addition, the easy entrapment of air bubbles during mixing led to these results (Turabi *et al.*, 2010).

Table 3 shows pore area distribution of cakes containing 10% buckwheat flour. Although guar gum had the highest number of pores, it had the lowest porosity. It means that size of pores and area distribution might be more important for cakes to have high porosity, rather than the number of pores. Half the pores from guar gum added cakes were of very small-size.



**Figure 6. Effect of formulation on pore area of cake with 10% buckwheat flour.**

**Table 3. Pore area distributions of cakes containing 10% buckwheat flour prepared with different formulations.**

Range of pore area (mm <sup>2</sup> )	Number of pores		
	Control	Whey protein	Guar gum
0.5-1	40	29	51
1-2	21	21	23
2-3	9	8	11
3-4	9	7	2
4-5	6	6	1
5-10	2	5	3
10-15	1	3	1
15-20	2	1	–
>20	1	2	–
Total number of pores	91	82	92

Furthermore, also morphological analysis of batters supported these results (Figure 4). Guar gum containing batters had very small sized gas bubbles with uneven distribution. On the other hand, in whey protein added cakes, a more uniform area distribution of cakes could be observed, which might be the reason these cakes had higher porosity than the others. Although the control cake had more pores than cake with whey protein, a more uniform size distribution of pores was obtained for whey protein added cakes, which might be the reason of lower hardness and higher specific volume of the cakes.

#### 4. Conclusions

Both the addition rate of buckwheat flour and of other ingredients (gum and protein) had an importance influence on all rheological properties and quality parameters of cakes. Whey protein generally improved the quality of batter and cake, due to the high emulsifying ability of due to low solubility of soy protein did not show the same effect. Consistency index, viscoelastic property of batter and gas bubble distribution were the parameters in terms of batter that influenced cake quality. High correlation coefficients between parameters were the indication of how the cake properties influenced with each other. Finally, in the future, staling properties of cakes or effect of baking type on cake quality might be investigated.

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