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PARTIAL WHEAT FLOUR REPLACEMENT WITH GLUTEN-FREE FLOURS IN BREAD - QUALITY, TEXTURE AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Consumers are increasingly looking for products in which gluten is not present or its content has been reduced by the conversion of conventional raw materials to gluten-free. The influence of the partial wheat flour replacement (from 10% to 40%) with gluten-free flours from amaranth, quinoa and millet on bread properties, including texture and crumb structure of the final product, as well as antioxidant potential and consumer acceptance were evaluated. The effect of replacement on pasting properties of flours mix was determined as well. It was found that the increased addition of millet and quinoa flours resulted in an increased maximum viscosity, whereas adding amaranth flour exerted an opposite effect. The wheat flour replacement influenced the colour of the product reducing its lightness and shifting colour balances as well. Due to a lower content of gluten proteins in the bread formulation the firmness of the crumb increased. Wholemeal flours are good source of antioxidants, antioxidant activity increased with a higher proportion of gluten-free flours.

Keywords: texture, crumb structure, bread quality, gluten-free flours addition, antioxidant properties

INTRODUCTION

Bread is often a staple of the diet and it is consumed almost every day around the world. The bread satisfies about 10% of the daily requirement of such ingredients as proteins, niacin, folic acid, thiamine, iron, copper, zinc and magnesium. In addition, it satisfies the demand for fiber and calcium in 20% **(Grafenauer and Curtain 2018; Lamacchia *et al*. 2018; O'Connor 2012)**. Due to the universal nature, bread can be enriched with many nutrients, which can contribute to the elimination of nutrient deficiencies. The use of unconventional ingredients also allows to vary the sensory characteristics. Among such ingredients, whole-wheat gluten-free flours are particularly noteworthy, in particular from amaranth, millet and quinoa. It is worth noting, however, that gluten is a fundamental structure in bread production and is responsible not only for the appearance or structure, but also the consumers acceptance of bakery products **(Rybicka *et al. 2019)***. The high nutritional value and pro-health properties must be accompanied by sensory attractiveness **(Sun-Waterhouse and Wadhwa 2013).**

Amaranth, more and more often referred to as the crop of the 21th century, is a plant indigenous to South America. Common on all continents, it is known under different names and used for various purposes. The nutritional value of amaranth seeds is much higher than that of seeds of other crops. Amaranth flour contains significant amounts of protein, fiber and valuable fatty acids necessary for the proper functioning of the body. It is also a source of mineral compounds (calcium, phosphorus, iron), as well as vitamins (B1, B2, B3, B9, A, C, PP) **(Venskutonis and Kraujalis 2013)**. The proportion of protein, one of the most vital elements of the seed, is about 16% - 20%. The biological value of the protein is high considering the nutritional benefits that it can provide. The nutritional value of amaranth exceeds the value of soy protein as it is rich in all essential amino acids. Amaranth protein has an even higher biological value than milk protein **(Escudero *et al.* 2004)**. An important advantage of amaranth flour is the high content of the limiting amino acid in most cereal products - lysine **(Ayo 2001; García Salcedo *et al.*, 2018)**. Amaranth seeds additionally contain numerous mono- and polyunsaturated fatty acids, including GLA (γ-Linolenic acid), a health-promoting acid especially valuable for its hypotensive effect **(Mondal *et al.* 2016)**. Millet seeds contain a high proportion of carbohydrates, amounting to 60-80%, and about 11% of protein. The exceptional nutritional value of millet protein can be attributed to the essential amino acids such as leucine, isoleucine and methionine, as well as to high content of lecithin. Millet is a valuable source of mineral salts, including potassium, iron and magnesium. Vitamins, including B_1, B_2, B_6, PP, biotin, pantothenic acid and folacin are also found in relatively high proportions **(Kalinova and Moudry 2006)**. Quinoa will be popular because of its nutritional value (high content of protein and minerals, including phosphorus, iron and calcium), as well as undemanding growing conditions (resistance to changing weather conditions, alkaline soil pH or low rainfall) **(García Salcedo *et al.* 2018)**. The seeds are rich in vitamin B_2 (riboflavin), vitamin E, and contain more calcium, magnesium and phosphorus than other cereals. Phytoestrogens in quinoa are known as substances preventing atherosclerosis, breast cancer and osteoporosis **(Comai *et al.* 2007)**. With the protein content of 14-18% it is more valuable in this respect than wheat, rice, oats and maize.

Recently, scientific research has been focusing on the effects of free radicals, particularly the reactive oxygen species (ROS), on the human body. Oxidative stress, understood as the imbalance between the ROS activity and antioxidants, may lead to many chronic diseases, as radicals react with proteins, fats and the DNA. Permanent damage as a consequence of the reactions may result in cardiovascular diseases (especially atherosclerosis), macular degeneration, cataract, Parkinson's and Alzheimer's disease, and cancers **(Brewer 2007; Cadet *et al.* 2005; De Flora and Izzotti 2007; Perry *et al.* 2002; Valko *et al.* 2006).** Therefore, scientists are urgently searching for bioactive compounds to counteract the negative effects of free radicals in humans. Using wholemeal flour in bakery products is likely to increase the proportion of phenolic compounds and antioxidant potential of bread as well. Available literature data suggest that not only whole quinoa seeds but also decorticated and milled fractions of seeds may serve as functional ingredients of gluten-free foods thanks to phenolic acids (vanillic and ferulic acid) and flavonoids (rutin, quercetin). Millet seeds are also rich in phenolic acids like gallic and syringic acid. The content of phenolic compounds in seeds is, however, dependent on the crop conditions and variety **(Hemalatha *et al.* 2016; Seifried *et al.* 2007**).

The present study aimed at evaluating the effect of various proportions of different types of gluten-free flours (amaranth, millet and quinoa flours) on the quality, crumb texture, and antioxidant properties of wheat bread.

MATERIAL AND METHODS

Materials

Wheat flours (WF) was obtained from mill Komplexmłyn sp. z o.o. (Poland). Wholemeal millet flour (WMF), wholemeal quinoa flour (WQF) and wholemeal amaranth flour (WAF) were purchased from Grano Mill (Poland). The characteristics of the flours used are shown in Table 1, according to the producers' data. Salt was bought from Kłodawa S.A. (Poland), compressed baker's yeasts from Lesaffre Polska S.A. (Poland).

Table 1 Basic characteristics of the flours

Parameter	WF	WMF	WQF	WAF
Carbohydrates [%]	70.8	70.1	70.0	61.5
Fat [%]	1.3	3.2	5.8	7.7
Fiber [%]	2.2	1.9	6.0	12.8
Protein [%]	10.6	11.0	5.8	15.1

Dough preparation and baking

Wheat bread dough (control sample C) was prepared using the following ingredients: 500 g of plain wheat flour 7.5 g of salt, 15 g of yeasts and 300 g of tap water. Other variants of bread for analysis contained 10%, 20%, 30% and 40% of wheat flour substitutes: wholemeal millet flour (M10, M20, M30 and M40, respectively), wholemeal quinoa flour (Q10, Q20, Q30, Q40), and wholemeal amaranth flour (A10, A20, A30 and A40). The dough was mixed for 120 seconds using a KitchenAid mixer at the speed of 70 rpm, then left to ferment for 60 minutes (fermenter conditions: temperature 30°C, relative humidity 75%). After 30 minutes of fermentation the dough was kneaded. On completion of fermentation, two equal loaves of 450 g were formed, put on baking forms and kept in the fermenter for another 15 minutes. Finally, the dough was baked at 220°C for 30 minutes in an oven MIWE condo (MIWE Michael Wenz GmbH, Germany) **(Kowalczewski *et al.* 2019)**.

Measurements of gelatinisation of starch by RVA

Changes in the properties of starch gelatinisation were evaluated using the Rapid Visco Analyser Tec Master (Perten Instruments, Sweden) **(Makowska *et al.* 2014)**. Each analysis evaluated a wheat and gluten-free flours mix of 3.5 g and 25 g of distilled water (taking into account any corrective measures to attain humidity of 14%). The proportions of wheat flour and gluten-free flours for every bread variant were in accordance with the dough recipes and were the basis for evaluating the effect of wheat flour substitution. The suspension was stabilised for 1 minute at 50 °C, then the sample was heated at 6 °C per minute until it reached 95 °C and kept at this temperature for 5 minutes. After thermostating the sample was cooled to 50 °C by lowering the temperature by 6 °C per minute. During the process the gelatinisation temperature and viscosity of the paste were measured.

Evaluation of the quality of bread

Measurement of the volume of bread was performed according to AACC 10-05 International Approved Methods **(AACC 2009a)**, moisture content measurement was performed according to AACCI 44-19.01 **(AACC 2009b)**. Additionally, bake loss and yield were calculated **(Leuschner *et al.* 1997)**.

Analysis of the colour of bread crumb

The colour characteristics were evaluated using the Chroma Meter CR-410 by Konica Minolta Sensing Inc. (Japan) in the CIE L*a*b* colour space **(Pauter *et al.* 2018)**. Lightness of the sample (L*) and colour saturation were measured: a* red (+) / green (-), and b* - yellow (+) / blue (-). The Total Colour Difference (TCD) was calculated on the basis of the measurements, according to the following formula:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Evaluation of the texture of bread crumb

The texture was determined with a TA.XTplus texture analyser (Stable Micro Systems, UK) **(AACC 2009c)**. For each analysis, a 2.5 cm crumb sample was taken from the middle of the loaf. The test parameters were: pre-test speed: 1 mm/s; test speed: 5 mm/s; post-test speed: 10 mm/s. To evaluate the firmness of bread, the force required to compress the bread crumb by 25% was determined.

Evaluation of the structure of bread crumb

Photographs taken with a GO-3 (QImaging, Canada) camera served as the basis for bread crumb structure analysis. The photographs were compared in the Image-Pro Plus software (Media Cybernetics Company).

Extraction of bioactive compounds

The extraction of phenolic and antioxidant compounds from freeze-dried bread was performed using 80% (v/v) methanol. The volume to solvent ratio was 1:5. The extraction process took 45 minutes using a S50 shaker (CAT, Germany). After 45 minutes of extraction the samples were centrifuged at 7800g, and the obtained supernatant was decanted from precipitate.

Measurements of antioxidant properties and total phenolics

Antioxidant properties were measured using the ABTS radical cation **(Re *et al.* 1999)**. Antioxidant activity per 1 gram of dry mass was expressed as the Trolox equivalent antioxidant capacity (TEAC). A spectrophotometric determination of total phenolics was performed with the Folin – Ciocalteu reagent **(Fang *et al.* 2006)**. Total phenolic compounds per 1 gram of dry mass were expressed as the ferulic acid equivalent (FAE).

Statistical analysis of the results

The measurements were subjected to the one-way analysis of variance (ANOVA) using Statistica 10 (StatSoft, Poland) at the 0.05 significance level. To indicate homogenous groups, the post-hoc Tukey HSD test was used.

RESULTS AND DISCUSSION

Partial substitution of wheat flour with gluten-free flours resulted in changes in starch gelatinisation analysed with RVA (Table 2). In most cases, temperature of starch gelatinisation increased, from 60.7 ± 0.54 °C for the control sample, to even 67.2 ± 0.49 °C for bread with 40% of millet flour. Similarly to gelatinisation temperature, the value of viscosity throughout the analysis depends on several factors, such as botanical origin, starch concentration in the mixture, solvent volume, pH level of the mixture, and presence of other components, for example proteins or hydrocolloids **(Hossen *et al.* 2011; Makowska *et al.* 2015)**. Maximum viscosity was found to increase with an increased proportion of millet and quinoa flours added to wheat flour. On the contrary, adding amaranth flour led to a gradual decrease of viscosity.

Table 2 RVA test results

Sample	Peak viscosity [cP]	Trough [cP]	Breakdown [cP]	Final Viscosity [cP]	Seatback [cP]	Peak time [s]	Pasting Temperature [°C]
C	$2156^{b} \pm 32$	$1348^{b} \pm 33$	$807^{c} \pm 3$	$2542^{e} \pm 42$	$1194^{e} \pm 9$	$6.0^{a} \pm 0.1$	$60.7^{d} \pm 0.5$
M10	$2174^{b} \pm 25$	$1290^{c} \pm 14$	$884^{b} \pm 11$	$2733^{d} \pm 25$	$1443^{d} \pm 11$	$5.8^{c} \pm 0.1$	$61.2^{cd} \pm 0.5$
M20	$2259^{a} \pm 25$	$1306^{bc} \pm 40$	$952^{a} \pm 18$	$2932^{c} \pm 4$	$1625^{c} \pm 41$	$5.9^{b} \pm 0.1$	$62.0^{c} \pm 0.5$
M30	$2270^{a} \pm 72$	$1219^{cd} \pm 50$	$951^{a} \pm 23$	$3060^{b} \pm 75$	$1841^{b} \pm 24$	$5.8^{c} \pm 0.0$	$64.3^{b} \pm 0.8$
M40	$2282^{a} \pm 5$	$1211^{d} \pm 9$	$970^{a} \pm 10$	$3352^{a} \pm 17$	$2138^{a} \pm 18$	$5.7^{d} \pm 0.0$	$67.2^{a} \pm 0.5$
A10	$1984^{c} \pm 15$	$1310^{bc} \pm 10$	$674^{e} \pm 37$	$2486^{f} \pm 17$	$1176^{e} \pm 67$	$6.0^{a} \pm 0.1$	$61.2^{c} \pm 0.5$
A20	$1864^{c} \pm 17$	$1264^{c} \pm 11$	$600^{e} \pm 63$	$2292^{g} \pm 17$	$1028^{f} \pm 72$	$6.0^{a} \pm 0.1$	$61.7^{c} \pm 0.8$
A30	$1662^{d} \pm 16$	$1101^{e} \pm 90$	$521^{f} \pm 71$	$1975^{h} \pm 14$	$874^{g} \pm 54$	$5.8^{c} \pm 0.1$	$63.2^{b} \pm 1.0$
A40	$1624^{d} \pm 11$	$1141^{e} \pm 68$	$522^{f} \pm 45$	$1941^{h} \pm 97$	$800^{g} \pm 30$	$5.7^{d} \pm 0.1$	$63.8^{b} \pm 0.4$
Q10	$2116^{b} \pm 14$	$1360^{b} \pm 10$	$756^{d} \pm 4$	$2596^{e} \pm 13$	$1236^{e} \pm 13$	$6.0^{a} \pm 0.1$	$60.6^{d} \pm 0.5$
Q20	$2125^{b} \pm 32$	$1321^{b} \pm 20$	$603^{e} \pm 12$	$2461^{f} \pm 32$	$1139^{e} \pm 14$	$6.0^{a} \pm 0.1$	$61.1^{c} \pm 1.0$
Q30	$2149^{b} \pm 26$	$1237^{cd} \pm 16$	$511^{f} \pm 10$	$2286^{g} \pm 32$	$1049^{f} \pm 16$	$6.0^{a} \pm 0.2$	$61.2^{cd} \pm 0.6$
Q40	$2222^{a} \pm 51$	$1446^{a} \pm 24$	$576^{f} \pm 26$	$2614^{e} \pm 47$	$1168^{e} \pm 23$	$5.9^{b} \pm 0.0$	$61.9^{c} \pm 0.9$

Legend: Table shows mean values and standard deviations; mean values in columns denoted by different letters differ significantly ($p < 0.05$).

Although gluten-free flour may increase the nutritional value of bread, it may also lead to undesirable structure changes. Bread volume decreased when gluten-free flours were added (Table 3), however, a 10% proportion of millet flour did not result in a significant decrease in volume, compared to the control sample. A smaller content of gluten proteins makes bread smaller and less spongy. Since whole gluten-free seeds are usually milled, the obtained flour has a high fibre content, which inhibits fermentation of the dough and, as a consequence, reduces the volume of a loaf after baking **(Sullivan *et al.* 2011)**. Substitution of wheat flour with quinoa flour reduced bake loss by about 2% compared to both the control sample and other mixes of wheat flour with gluten-free flours. Additionally, bread moisture content was lost proportionally to an increased content of gluten-free flours.

Table 3 Quality parameters of analyzed breads

Sample	Volume of 100 g loaf [mL]	Bread yield [%]	Bake loss [%]	Moisture of bread crumb [%]
C	$312.4^{a} \pm 10.2$	$136.7^{a} \pm 6.4$	$7.1^{b} \pm 0.2$	$44.35^{a} \pm 0.64$
M10	$318.2^{a} \pm 9.8$	$138.1^{a} \pm 5.3$	$7.2^{b} \pm 0.4$	$43.72^{b} \pm 0.11$
M20	$280.9^{b} \pm 4.6$	$131.7^{b} \pm 2.9$	$7.9^{a} \pm 0.3$	$42.64^{c} \pm 0.04$
M30	$228.1^{c} \pm 4.7$	$138.5^{a} \pm 6.1$	$6.7^{c} \pm 0.6$	$42.45^{c} \pm 0.03$
M40	$216.0^{c} \pm 3.2$	$135.0^{a} \pm 3.3$	$7.2^{b} \pm 0.3$	$42.06^{c} \pm 0.06$
A10	$279.8^{b} \pm 2.6$	$134.5^{a} \pm 3.1$	$7.7^{ab} \pm 0.6$	$44.45^{a} \pm 0.29$
A20	$213.8^{c} \pm 4.1$	$138.0^{a} \pm 4.7$	$7.4^{b} \pm 0.3$	$43.82^{b} \pm 0.40$
A30	$200.9^{d} \pm 2.2$	$130.7^{b} \pm 4.2$	$8.0^{a} \pm 0.5$	$43.53^{b} \pm 1.05$
A40	$140.8^{e} \pm 6.4$	$136.7^{a} \pm 5.8$	$7.4^{b} \pm 0.3$	$42.49^{c} \pm 0.07$
Q10	$280.4^{b} \pm 3.5$	$137.7^{a} \pm 5.5$	$5.3^{d} \pm 0.3$	$45.38^{a} \pm 1.02$
Q20	$225.4^{c} \pm 7.1$	$128.7^{c} \pm 3.9$	$5.0^{d} \pm 0.3$	$43.37^{b} \pm 1.04$
Q30	$190.1^{d} \pm 4.1$	$131.5^{b} \pm 3.7$	$5.5^{d} \pm 0.4$	$42.24^{c} \pm 0.09$
Q40	$197.1^{d} \pm 3.3$	$114.1^{d} \pm 4.4$	$5.5^{d} \pm 0.6$	$42.20^{c} \pm 0.11$

Legend: Table shows mean values and standard deviations; mean values in columns denoted by different letters differ significantly ($p < 0.05$).

Different proportions of wholemeal gluten-free flours added to wheat flour resulted in a change of the colour of bread crumb (Table 4). Lightness L* of each variant was found to decrease on substituting wheat flour with gluten-free flour. Moreover, within particular groups of variants an increased proportion of gluten-free flour increased lightness loss. The breads with gluten-free flours were darker, thus their color resembled commercially available wholemeal breads **(Kurek *et al.* 2017; Mialon *et al.* 2002)**. Dark bread is much more often chosen by consumers due to the growing awareness of the presence of health-promoting compounds in whole flour, and consequently in the bread made from it **(Bakke & Vickers 2007; Heiniö *et al.* 2016).** The intensity of the red colour (a*) also changed; it increased when quinoa and amaranth flours were added. Millet flour contributed to a slight increase of the intensity of green in the bread crumb. The analysis of balance between yellow and blue (b*) revealed that adding quinoa flour slightly shifted the colours towards blue, whereas the other flours shifted the colours towards yellow. On the basis of the obtained colour values the total colour difference ΔE was determined. Available literature data suggest that consumers, even without expert knowledge, perceive the colour difference ΔE ranging from 2 to 3.5 **(Mokrzycki and Tatol 2011)**. Total colour difference, in reference to traditional bakery products, ranged from 2.96 for A10 to as much as 16.78 for Q40, which proves that changes in bread colour are clearly visible.

Table 4 Color determinants and total color difference (ΔE) of crumb bread

Sample	L*	a*	b*	ΔE
C	$75.62^{a} \pm 1.21$	$0.55^{d} \pm 0.04$	$18.05^{b} \pm 2.71$	-
M10	$73.16^{b} \pm 2.95$	$0.55^{d} \pm 0.03$	$19.70^{b} \pm 1.17$	2.96
M20	$71.01^{b} \pm 1.92$	$0.48^{d} \pm 0.09$	$22.38^{a} \pm 1.94$	6.32
M30	$71.71^{b} \pm 1.27$	$0.35^{d} \pm 0.07$	$22.95^{a} \pm 2.04$	6.27
M40	$70.34^{b} \pm 1.13$	$0.16^{e} \pm 0.07$	$24.62^{a} \pm 1.82$	8.44
A10	$70.86^{b} \pm 1.17$	$1.92^{b} \pm 0.24$	$20.08^{ab} \pm 1.93$	5.35
A20	$66.05^{c} \pm 2.71$	$3.12^{a} \pm 0.47$	$21.13^{a} \pm 2.06$	10.38
A30	$63.50^{d} \pm 1.92$	$4.02^{a} \pm 0.97$	$21.43^{a} \pm 1.76$	13.39
A40	$62.07^{e} \pm 2.08$	$4.65^{a} \pm 0.91$	$21.74^{a} \pm 2.31$	14.97
Q10	$68.01^{c} \pm 2.01$	$1.14^{c} \pm 0.14$	$18.48^{b} \pm 2.24$	7.64
Q20	$65.25^{d} \pm 1.33$	$1.34^{c} \pm 0.11$	$17.66^{bc} \pm 1.61$	10.41
Q30	$61.94^{e} \pm 2.47$	$1.72^{b} \pm 0.27$	$17.61^{c} \pm 1.11$	13.87
Q40	$58.96^{f} \pm 2.28$	$2.09^{b} \pm 0.31$	$16.77^{c} \pm 1.08$	16.78

Legend: Table shows mean values and standard deviations; mean values in columns denoted by different letters differ significantly ($p < 0.05$).

Gluten proteins in wheat bread are responsible for its proper structure **(Demichelis *et al.* 2019)** and reducing its quantity can significantly affect the characteristics of bread. Smaller bread volume entailed changes in the structure of bread crumb. The analysis of photographs of the bread crumb (Fig. 1) revealed considerable differences in porosity and pore distribution. Pore walls became thicker and the structure more compacted when the proportion of gluten-free flours increased. The differences may stem from the fact that gluten-free flours have a different chemical composition and, consequently, alter the content of the protein responsible for dough leavening during fermentation. The addition of hydrocolloids or milk protein seems to be an effective method of counteracting volume loss **(Gallagher *et al.* 2003; Lazaridou et al. 2007)**.

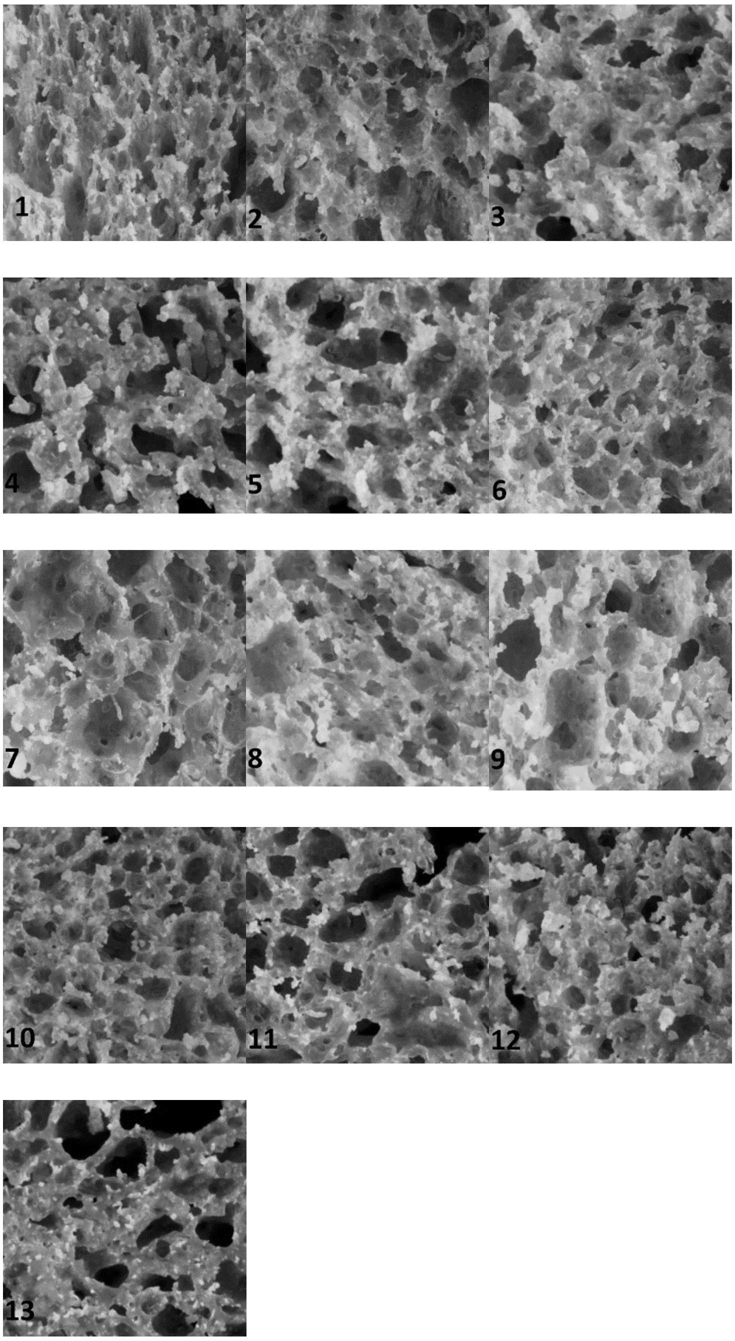

Figure 1 Photos of crumb porosity 1-13 respectively: C, A10, A20, A30, A40, M10, M20, M30, M40, Q10, Q20, Q30, Q40

Different proportions of gluten-free flours were found to affect texture of the crumb of baked bread (Table 5). Appropriate porosity and elasticity of the crumb is guaranteed by high content of starch in the dough and low content of dietary fibre **(Deora *et al.* 2015)**. Including gluten-free flours in the recipes apparently increased firmness of the bread crumb. Due to high fibre content of quinoa seeds (twice higher than that of wheat grain), wheat-quinoa breads were noticeably firmer than other variants of bread.

Table 5 Texture of bread analyzed

Sample	Springiness [%]	Hardness [N]
C	$72.45^{e} \pm 1.56$	$10.69^{g} \pm 0.75$
M10	$96.58^{a} \pm 0.30$	$10.96^{g} \pm 1.68$
M20	$95.97^{a} \pm 1.14$	$12.47^{f} \pm 0.99$
M30	$92.36^{c} \pm 1.07$	$19.42^{cd} \pm 3.49$
M40	$82.01^{d} \pm 1.48$	$22.97^{c} \pm 3.15$
A10	$94.56^{b} \pm 0.98$	$11.03^{g} \pm 0.96$
A20	$92.36^{c} \pm 0.67$	$18.15^{d} \pm 2.02$
A30	$72.11^{e} \pm 1.06$	$23.07^{c} \pm 1.90$
A40	$71.94^{e} \pm 0.98$	$26.06^{b} \pm 2.23$
Q10	$98.16^{a} \pm 0.06$	$13.53^{f} \pm 1.25$
Q20	$98.10^{a} \pm 0.33$	$16.73^{e} \pm 1.97$
Q30	$97.05^{a} \pm 0.09$	$23.83^{c} \pm 3.84$
Q40	$96.42^{a} \pm 0.35$	$35.75^{a} \pm 3.33$

Legend: Table shows mean values and standard deviations; mean values in columns denoted by different letters differ significantly ($p < 0.05$).

Evaluation of total antioxidant activity becomes more and more important as it provides useful information on health-related and functional properties of raw materials without analysis of individual antioxidant compounds **(Scalfi *et al.* 2000)**. Antioxidant activity was more potent in case of all gluten-free flours (Table 6), and it increased with higher content of gluten-free flours. Phenolic compounds, especially phenolic acids, are found mainly in the seed coating **(Tang and Tsao 2017)**. In the present study, whole-grain gluten-free flours were used, and the expected result would be a significant increase in the content of polyphenolic compounds in the breads obtained. However, only wholemeal quinoa flour appeared to be the only type of flour which significantly increased polyphenolic content of baked breads, as compared to wheat bread. Published research on quinoa seeds led to the identification of 23 phenolic compounds in free or conjugated forms, most of which were phenolic acids consisting of vanillic acid, ferulic acid and their derivatives, as well as flavonoids, quercetin, kaempferol and their glycosides **(Tang *et al.* 2015; 2016)**. Also the highest antioxidant activity was exhibited by bread with 40% quinoa content. Polyphenolic compounds are sensitive to heat, therefore they are subject to degradation upon baking. The total antioxidant activity is changed even at mixing and kneading of the dough, probably due to enzymatic activity increasing when water was added to flour **(Holtekjølen *et al.* 2008; Leenhardt *et al.* 2006)**.

Table 6 Total phenolic content and antioxidant activity of bread analyzed

Sample	Total phenolic content [µg/g DM]	Antioxidant properties [µmol Trolox/g DM]
C	$2.9^{e} \pm 0.03$	$2.82^{g} \pm 0.14$
M10	$2.1^{j} \pm 0.01$	$2.98^{f} \pm 0.02$
M20	$2.4^{h} \pm 0.01$	$3.54^{c} \pm 0.05$
M30	$2.5^{g} \pm 0.01$	$3.98^{b} \pm 0.07$
M40	$2.7^{f} \pm 0.01$	$4.02^{b} \pm 0.03$
A10	$2.3^{i} \pm 0.02$	$2.87^{g} \pm 0.09$
A20	$2.4^{h} \pm 0.04$	$3.01^{f} \pm 0.06$
A30	$2.4^{h} \pm 0.01$	$3.03^{f} \pm 0.07$
A40	$2.5^{g} \pm 0.02$	$3.33^{d} \pm 0.06$
Q10	$3.6^{d} \pm 0.03$	$3.21^{e} \pm 0.02$
Q20	$5.8^{c} \pm 0.01$	$3.65^{c} \pm 0.08$
Q30	$6.1^{b} \pm 0.06$	$3.97^{b} \pm 0.11$
Q40	$7.3^{a} \pm 0.03$	$4.21^{a} \pm 0.09$

Legend: Table shows mean values and standard deviations; mean values in columns denoted by different letters differ significantly ($p < 0.05$).

CONCLUSION

Partial replacement of wheat flour with gluten-free flours altered the process of starch gelatinisation of the bread blends. The temperature of gelatinisation were higher in doughs with gluten-free flour addition compared to the control sample. It was also shown that viscosity significantly decreased due to the use of amaranth flour. Presumably, this is related to the high protein and fat content of this raw material. The colour of baked bread differed from the control bread; the bread crumb was darker (value of parameter L*), and the balance of colours was shifted (value of parameters a* and b*). The bread obtained as a result of the replacement of wheat flour with gluten-free flours resembled wholemeal bread, widely recognized as healthier. Changes were observed in the texture of baked bread as crumb firmness increased with higher content of gluten-free flours. Adding quinoa and amaranth flours increased elasticity of bread crumb. The reduction of the gluten protein content resulted in impeding the creation of the proper structure and obtaining a crumb with a smaller porosity. Whole-wheat gluten-free flours are a source of antioxidant compounds, which is why a significant increase in the antioxidant activity of breads with these flours was observed. The increase was proportional to the content of gluten-free flour.

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