



Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads

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ABSTRACT

The aim of the study was to investigate the effect of adding (in two different doses 15% and 30%) pseudocereal (buckwheat, amaranth and quinoa) flour on the antioxidant properties and sensory value of breads. Buckwheat flour had the highest phenolic content (7.25 ± 0.23 mg/g dw). The content of total flavonoids in flours was about 2–4 fold higher when compared to breads. The addition of buckwheat flour to wheat bread, particularly in higher dose, was more effective in enhancing antioxidant activity, as evaluated by means of FRAP and DPPH, which increased by 2.36 fold, and 3.64 fold respectively, in comparison with other pseudocereal flours (amaranth, quinoa), which caused, in higher doses, the changes of above parameters within the ranges 1.20–1.79 fold, and 0.60–1.71 fold. Analysis of sensory results of breads showed that addition of buckwheat flour to the dough might improve subjective properties of bread and increase acceptable quality attributes such as taste, colour or odour. All these observations suggest that addition of buckwheat flour into bread can improve antioxidant as well as sensory properties of bread. Bread fortified with pseudocereal flours, and especially with buckwheat flour, may be placed on the market as a functional food.

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1. Introduction

Whole pseudocereal grains such as buckwheat, amaranth and quinoa are rich in a wide range of compounds e.g. flavonoids, phenolic acids, trace elements, fatty acids and vitamins with known effects on human health (Gorinstein et al., 2008; Kalinova & Dadakova, 2009; Li & Zhang, 2001; Tomotake et al., 2007). Whole grain products consumption have been associated with reduced incidence of diseases such as cancer (Chan, Wang, & Holly, 2007; Slavin, 2004) cardiovascular disease (Jacobs & Gallaher, 2004; Mellen, Walsh, & Herrington, 2008), high blood pressure (Behall, Scholfield, & Hallfrisch, 2006; Flint et al., 2009) and diabetes (Lutsey et al., 2007; Qi & Hu, 2007; Rave, Roggen, Dellweg, Heise, & tom Dieck, 2007). Therefore, increased consumption of grain products has been recommended, and cereals products should be the main part of the daily menu (Richardson, 2003). The pseudocereals are not often used in technology of bread but they can be useful in dietotherapy of celiac disease (Thompson, 2001). Home-made breads containing pseudocereals could be an important part

of traditional type of food, i.e. “slow food”, which is now becoming more popular. Increasing consumption of this kind of bread in our daily menu can improve antioxidant potential of our diet because it is known that the pseudocereals' grains are rich in different antioxidant compounds (Lin, Liu, Yu, Lin, & Mau, 2009; Paško, Sajewicz, Gorinstein, & Zachwieja, 2008). Pseudocereals can provide beneficial health effects (Christa & Sorel-Śmietana, 2008; Martirosyan, Miroshnichenko, Kulakova, Pogojeva, & Zolodov, 2007), therefore bread with addition of buckwheat, amaranth or quinoa flour, as a staple product could diversify ordinary (daily) model of nutrition. That is why, the sensory value of bread is very important, because taste, smell, and flavour of bread significantly influence consumer preferences of cereal products.

The sensory characteristics, chemical, rheological and storage properties of breads from various species of wheat flour or breads from different portions of other cereal flours, have been thoroughly investigated. It was observed, that the additions of some flours having valuable nutrient profile to poor quality common soft wheat improved the breadmaking properties, extended the shelf life of the corresponding product, made crumb more softer, and lowered firming rate. The nutritive properties of breads (e.g. the protein quality and quantity, dietary fibre content, and unsaturated fats profile) were also enhanced (Raffo et al., 2003). However, little is

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known about antioxidant effect (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2009) and sensory characteristics of pseudocereal breads, especially about breads with addition of amaranth and quinoa flour (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2009; Nikolić, Sakac, & Mastilovic, 2011; Schoenlechner, Drausinger, Ottenschlaeger, Jurackova, & Berghofer, 2010). Therefore, the aim of our study was to investigate the effect of addition of pseudocereal flour on the antioxidant properties and sensory value of breads.

2. Materials and methods

2.1. Materials

The flour used in the formula of control bread was wheat flour (Królowa Mąk Tortowych, PPHU Młynpol Sp. j. Gromadka, Krzyżowa, Poland), type 400. The rest of breads were baked with addition of buckwheat flour (Futuro c.m., Kraków, Poland), amaranth flour (PHU Szariat s.c., Łomża, Poland) or quinoa flour (Futuro c.m., Kraków, Poland). The pseudocereal flours were added in two different doses 15 g/100 g and 30 g/100 g, respectively. All of used flours were purchased from a local shop with “slow food” in Krakow, Poland. The other ingredients used in the formula of dough were: sugar (Kryształ, Krajowa Spółka Cywilna S.A., Siennica Nadolna, Poland), iodinated salt (Tesco/Poland/Sp. ZO.O., Presov, Slovak Republic), yeast (Dr Oetker Poland Sp. ZO.O., Gdańsk, Poland) and distilled water. Distilled water was used to eliminate possible influence of some metals, e.g. iron and copper, on the evaluation of antioxidant activity, as it is well known that active compounds in produced breads such as flavonoids and other polyphenols are vulnerable to oxidation in the presence of transient metals and ambient oxygen.

De-ionized water for chemical analyses 18 Mohm cm was obtained from Milli Ro & Q water purification system (Millipore, Warsaw, Poland); methanol, acetone, hydrochloric acid 36 g/100 g, ferric chloride (FeCl₃), aluminium chloride hexahydrate, sodium nitrite, sodium hydroxide, sodium acetate and acetic acid were purchased from Chempur, Piekary Śląskie, Poland. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, catechin and gallic acid were purchased from Sigma Poznań, Poland. All reagents were of analytical grade.

2.2. Bread making

The materials for dough-making were mixed according to the formula proportions listed in Table 1. In this experiment, standard baking procedures were not used because the aim of this work was to present results of home-made breads baked using popular home bread oven. All types of baked breads were tin breads. The breads were baked in the oven Alaska BM 2600 (METRO Group, Düsseldorf, Germany) in temperature 180 °C. Cycle of bread making composed

of eight stages, i.e.: 15 min mixing, 15 min growing without raising temperature, 10 min mixing, 15 min growing without raising temperature, 45 min growing in low temperature (35 °C), 10 min mixing, 15 min growing in medium temperature (45 °C), 50 min baking. In case of buckwheat breads, time of baking had to be prolonged because in shorter time slack-baked bread was obtained. Detailed data about time of baking and weight of bread after baking were also collected in Table 1.

2.3. Extracts preparation

After baking, breads were allowed to cool down to room temperature for 3 h. Subsequently, the breads were sliced (slices about 1.5 cm thick), and kept frozen (−20 °C) until analysis. After thawing, the slices were dried and then manually crumbed, grounded in traditional stone mortar and sieved through at 2 mm sieve to obtain bread powder. Powdered samples of breads (2 g) were extracted for 2 h with 40 mL of solvent consisting of methanol, 0.16 mol/L hydrochloric acid and water, mixed in proportion 8:1:1, respectively. The extracts were separated by decantation and the residues were extracted again with 40 mL of 70 g/100 g acetone for 2 h. The initial methanol extracts were added to prepare mixture, which was subsequently decanted, centrifuged and stored in darkness in a freezer in temperature of −20 °C.

2.4. Determination of total phenols

Total phenols (TP) were determined colorimetrically using Folin–Ciocalteu reagent, as described previously (Paško et al., 2009). Total phenols assay was conducted by mixing 2.7 mL of de-ionized water, 0.3 mL of extracts, 0.3 mL 7 g/100 g Na₂CO₃ and 0.15 mL Folin–Ciocalteu reagent. Absorbance of mixture was measured at 725 nm using the spectrophotometer Jasco UV-530 (Medson, Paczkowo, Poland). A standard curve was prepared with gallic acid. Final results were given as gallic acid equivalents (GAE).

2.5. Determination of total flavonoids

Total flavonoid content was determined by a colorimetric method as described previously (Gorinstein et al., 2007). Briefly, 0.25 mL of the 80 g/100 g methanolic extract was diluted with 1.25 mL of distilled water. Then 75 µL of 5 g/100 g NaNO₂ solution was added to the mixture, and after 6 min 150 µL of 10 g/100 g AlCl₃·6H₂O solution was added. The mixture was allowed to stand for 5 min and next 0.5 mL of 1 mol/L NaOH was added and the total was made up to 2.5 mL with distilled water. The solution was mixed well and the absorbance was measured immediately against the blank at 510 nm using a spectrophotometer Jasco UV-530. The results were expressed as mg of catechin equivalents.

Table 1
The formulations of dough.

Type of bread	Wheat flour [g]	Pseudocereal flour [g]	Salt [g]	Sugar [g]	Yeast [g]	Water [mL]	Time of procedure [h min]	Weight of bread after baking [g]
Control bread	500	—	10	10	5	350	2.55	780
Amaranth bread 15 g/100 g	425	75	10	10	5	350	2.55	780
Amaranth bread 30 g/100 g	350	150	10	10	5	350	2.55	787
Buckwheat bread 15 g/100 g	425	75	10	10	5	350	3.25	734
Buckwheat bread 30 g/100 g	350	150	10	10	5	350	3.25	748
Quinoa bread 15 g/100 g	425	75	10	10	5	350	2.55	781
Quinoa bread 30 g/100 g	350	150	10	10	5	350	2.55	778

2.6. Determination of FRAP activity

FRAP (Ferric Reducing Ability of Plasma) assay was carried out according to Benzie and Strain (1996), and modified to 48-well plates and automatic reader (Synergy-2, BioTek/USA) with syringe rapid dispensers. Briefly, the oxidant in the FRAP assay (reagent mixture) consisted of ferric chloride solution (20 mmol/L), TPTZ solution (10 mmol/L TPTZ in 40 mmol/L HCl) and acetate buffer (pH = 3.6) in a proportion of 5:5:10, respectively, and was freshly prepared. To each plate, 0.4 mL of acetate buffer (pH 3.6) was dispensed, followed by 50 µL of sample, standard or blank. The plate was conditioned at the temperature of 37 °C for 2 min, and then 0.2 mL of reagent mixture was added and shaken for 30 s; afterwards, absorbance at 593 nm was measured with kinetic mode for 15 min. The final results were expressed as mg Trolox/100 g DW (dry weight).

2.7. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Yen and Chen (1995) with modification (Paško et al., 2009). For measurement of sample scavenging activity 0.4 mL of methanolic acetate buffer was added to the cuvettes containing the increasing volumes of sample (e.g. 0, 0.1, 0.2, 0.3, 0.45, 0.6 mL) with adequate volumes of methanol to make total volume of 1 mL. Acetate buffer was made from 0.2 mol/L solutions of sodium acetate and acetic acid in methanol mixed at the volume ratio 7.9:2.1. The pH of the buffer was 5.2. 1 mL of DPPH stock solution (12 mg DPPH was dissolved in 100 mL of methanol; absorbance 1.3) was added to each cuvette, then absorbance was measured after 24 h. The absorbance of the resultant solution was determined using Jasco UV-530 spectrometer (Japan) at 514 nm. The total antioxidant capacities (TAA) were estimated as Trolox equivalents (TEAA) by interpolation to 50% inhibition (TEAA50).

2.8. Sensory evaluation

The sensory evaluation was carried out on the breads 12 h after baking. The 31 consumers (20 women; 11 men) aged 19–24 years old completed the questionnaire. They were recruited among students of Faculty of Pharmacy in Krakow. The samples were sliced into equal sizes (2 cm × 2 cm) before serving to the respondents on coloured coded plates. The consumers made hedonic evaluation of the samples. The scorecard was developed with 10-point category scale (disliked = 0; extremely liked = 10) (Wronkowska, Troszyńska, Sorel-Śmietana, & Wotejszo, 2008), each testers was

asked to assess the breads for overall quality, based on the colour, odour and consistency. The students were also asked about taste of bread. They could choose: interesting, tasty, natural, strange taste, vapid, not to eat, bad taste, gummy, crusty, little pronounced, sweet, salty, tart, difficult to determine, delicate, milky.

2.9. Statistical analysis

Results of biochemical analyses are given as means ± SD based on four measurements for each sample of flour and bread. Where appropriate, the data were tested by one-way ANOVA, followed by Tukey post hoc test. For sensory parameters the median values and confidence intervals of medians were calculated (Bland, 2000). The possible differences between various breads, in respect to sensory parameters, were analysed using Kruskal–Wallis test. Dunn's post-test was used to reveal the differences between the paired brands of breads. Differences with $p < 0.05$ were considered to be statistically significant. Pearson correlation coefficients were calculated for pairs of biochemical parameters. Statistical calculations were carried out using the commercially available packages Statistica v.5.1 (StatSoft, Inc., Tulsa, USA), and GraphPad Prism v. 3.02 (GraphPad Software, San Diego, USA).

3. Results and discussion

3.1. Phenolic contents of flour and breads

The phenolic contents of the four kinds of flour and seven breads were expressed as mg gallic acid per gram of dry weight (Table 2). Buckwheat flour had the highest phenolic content (7.25 ± 0.23 mg/g dw) and the next one was wheat (6.96 ± 0.11 mg/g dw). Amaranth and quinoa flour had the lowest phenolic content (2.71 ± 0.1 mg/g dw and 2.8 ± 0.1 mg/g dw, respectively) and the differences between them and the former two were statistically significant. Our results for phenolic content in buckwheat flour were in between those given by Sensoy, Rosen, Ho, and Karwe (2006). They observed lower content of total phenolics in buckwheat white, raw flour, but higher content of total phenolics of buckwheat dark, raw flour in comparison with phenolic content in our buckwheat flour.

Consistently with the above results, the content of phenols in breads was highest in breads baked with 30 g/100 g addition of buckwheat flour (2.65 ± 0.10 mg/g dw). However, the second highest result was, surprisingly, for the same dose of amaranth flour (2.61 ± 0.04 mg/g dw), followed by bread with 30 g/100 g addition of quinoa flour (2.54 ± 0.11 mg/g dw) and with 15 g/100 g addition of buckwheat flour (2.1 ± 0.08 mg/g dw). Remaining

Table 2
Mean values (±SD, $n = 4$) of total phenolic content, total flavonoids content, FRAP and DPPH for flours and breads under study (results marked with the same letter in upper index within each column differ significantly).

Material tested	Total phenolic content [mg/g dw]	Total flavonoids content [µg/g dw]	TEAC FRAP [mg Trolox/100 g dw]	TEAC DPPH [mmol Trolox/kg dw]
Wheat flour	$6.96 \pm 0.1^{a,b,p,l}$	$70 \pm 11^{a,b,f}$	$158.3 \pm 3.0^{a,b,c,z}$	$3.95 \pm 0.11^{b,h,k,\gamma}$
Buckwheat flour	$7.25 \pm 0.2^{p,c,d,m,n}$	$153 \pm 12^{a,c,d,g,h}$	$214.9 \pm 3.5^{a,e,f,u,w}$	$8.80 \pm 0.52^{c,h,i,\delta,\epsilon}$
Amaranth flour	$2.71 \pm 0.1^{a,c,o}$	$65 \pm 8^{c,e,i,j}$	$38.6 \pm 1.2^{b,e,h,x,y}$	$3.60 \pm 0.34^{l,m,\phi}$
Quinoa flour	$2.8 \pm 0.1^{b,d,r}$	$92 \pm 9^{b,d,e,k,l}$	$58.7 \pm 1.5^{c,f,h,z,\beta}$	$6.22 \pm 0.2^{a,b,c,k,m,\gamma,\eta}$
Yeast	–	–	82.7 ± 2.8	14.50 ± 2.84
Control bread	$1.7 \pm 0.07^{h,i,j,k,l}$	20.3 ± 9^f	$63.8 \pm 2.2^{k,l,m,n,o,p,z}$	$2.07 \pm 0.17^{b,s,w,x,z,\alpha,\beta,\gamma}$
Buckwheat bread 15 g/100 g	$2.1 \pm 0.08^{e,h,m}$	33.4 ± 3.5^g	$111.0 \pm 1.8^{r,u,k}$	$5.42 \pm 0.17^{n,w,\delta}$
Buckwheat bread 30 g/100 g	$2.65 \pm 0.1^{e,i,n}$	32.9 ± 4^h	$150.8 \pm 2.2^{r,w,l}$	$7.53 \pm 0.18^{n,x,\epsilon}$
Amaranth bread 15 g/100 g	$1.73 \pm 0.09^{f,o}$	20.6 ± 8^i	$73.7 \pm 1.6^{s,m,x}$	$2.62 \pm 0.19^{o,p,t,z,\phi}$
Amaranth bread 30 g/100 g	$2.61 \pm 0.04^{f,j}$	34.9 ± 10^j	$114.1 \pm 3.0^{s,n,y}$	$3.55 \pm 0.22^{o,r,u}$
Quinoa bread 15 g/100 g	$1.88 \pm 0.07^{g,r}$	27.5 ± 8.5^k	$71.0 \pm 3.1^{o,z}$	$1.10 \pm 0.15^{a,t,\alpha,\gamma}$
Quinoa bread 30 g/100 g	$2.54 \pm 0.1^{g,k}$	28.7 ± 7^l	$76.5 \pm 1.3^{p,\beta}$	$1.25 \pm 0.11^{b,p,r,s,u,\beta,\eta}$

– not analysed.

breads – bread with addition of 15 g/100 g quinoa flour, 15 g/100 g amaranth flour and control bread made up of wheat flour had the similar low total phenolic content: 1.88 ± 0.07 mg/g dw, 1.73 ± 0.09 mg/g dw, 1.70 ± 0.07 mg/g dw, respectively. Why control bread had so low phenolic content is not clear for us and needs further investigation.

There were significant differences in respect to phenolic content between all kinds of breads prepared either with addition of 15 g/100 g or 30 g/100 g of pseudocereals flour. The significant differences in all cases between control bread and pseudocereals breads also were observed, except for breads baked with 15 g/100 g addition of amaranth and quinoa flour, which had similar phenols content as wheat bread. The contents of phenols in breads were in all cases lower than in respective flour. Based on works of Holtekjølen, Baevre, Rødbotten, Berg, and Knutsen (2008) and Leenhardt et al. (2006) it is known that antioxidant active compounds presented in flours might be damaged or degraded as a consequence of the heat/thermal process during baking. However, losses of antioxidants during dough mixing and kneading were also observed. Antioxidant activity of breads could be modified by active oxidative enzymes presented in ingredients of compounds used in breads production, or oxidized by ambient oxygen. The addition of water will initiate enzyme activities, while a substantial incorporation of oxygen occurs during the initial dough mixing and the remoulding into smaller pieces. Second obvious reason for lower antioxidant activity in breads as compared with pseudocereal flours in current study was the fact that pseudocereal flours accounted for only 15–30 g for 100 g of all breads ingredients.

The phenols in control wheat bread were on similar level as shown by Holtekjølen et al. (2008). Additionally, in accordance with our results, Alvarez-Jubete, Auty et al. (2009), Alvarez-Jubete, Wijngaard et al. (2009) who investigated bread baked with 50 g/100 g addition of buckwheat, amaranth and quinoa flour, also observed the highest content of phenols in buckwheat bread. However, in our investigation the level of phenolic compounds in breads with amaranth flour ranged 1.73–2.61 mg/g and overlapped with phenolic compounds content in quinoa breads (1.88–2.54 mg/g), while Alvarez-Jubete, Auty et al. (2009), Alvarez-Jubete, Wijngaard et al. (2009) observed significantly lower phenolic concentration in amaranth bread than in quinoa bread.

3.2. Flavonoids contents of flour and breads

The content of total flavonoids in flour was about 2–4 fold higher when compared to breads in all cases. All these differences were statistically significant. Buckwheat flour was a better source of flavonoid compounds than quinoa, wheat or amaranth flour (Table 2). Our results concerning total flavonoids in breads made with addition of pseudocereals' flour did not show such differences, i.e. they did not depend on dosage of pseudocereals' flour. The lowest flavonoid level was presented in control bread.

According to the presented results the buckwheat breads showed a much higher loss of flavonoids than amaranth or quinoa. Similarly, Zhang, Chen, Li, Pei, and Liang (2010) observed that total flavonoids in buckwheat flour were decreased significantly after thermal processing such as roasting pressured steam-heating and microwave heating, while total phenolics were not influenced considerable. Sensoy et al. (2006) indicated that roasting (200 °C for 10 min) slightly reduced antioxidant activity of buckwheat flour. Dietrych-Szostak and Oleszek (1999) noticed that even short temperature treatment of buckwheat grain (heating for 10 min to 150 °C) caused a significant decrease (20% of total) in flavonoid concentration. Prolonged temperature treatment (heating process prolonged to either 1 h and 10 min or 2 h and 10 min) resulted in

drastic reductions of flavonoid concentrations (about 40%) in comparison with groats manually dehulled. In the most severe temperature treatment (the grains treated with steam (pressure 0.35 MPa, 164 °C) for 20 min, followed by 50 min of treatment with steam (0.4 MPa, 150 °C) and final drying), the reduction of flavonoid content was 75%. It is not well understood which processes are responsible for the observed flavonoid losses. Dietrych-Szostak and Oleszek (1999) concluded that such changes might be due to flavonoid breakdown during heating and/or extraction of glycosides by the steam. In conclusion, the different thermal processing could result in the decrease of flavonoids content, causing a drop in antioxidative activities, particularly in buckwheat, but also, to less extent, in amaranth and quinoa. Therefore in order to obtain health-promoting pseudocereals products, the processing condition should be optimised to keep the loss of constituents as low as possible. Further evaluations in this field are required.

3.3. Total antioxidant activities of flour and breads

Two methods were used to test the antioxidant activity of flours and breads. First one (FRAP method) is based on determination of ferric-tripyridyltriazine complex reducing capacity of the studied extracts. Among polyphenols the greatest antioxidant efficacies in this test were shown for quercetin, tannic acid, caffeic acid and gallic acid, while catechin and resveratrol had the lowest ones (Pulido, Bravo, & Saura-Calixto, 2000). Because of metal ion involvement in analytical reaction, FRAP method is fast, sensitive and spans relatively wide range of antioxidant substrates. However, the results obtained using FRAP method expressed the corresponding concentrations of electron-donating antioxidants, and the compounds that act by radical quenching, e.g. thiol antioxidants (such as glutathione) and carotenoids cannot be determined by this assay. On the other hand, DPPH method is based on the evaluation of the reducing ability of antioxidants toward DPPH', which is stable nitrogen radical, possessing an odd electron. The colour of its solution fades rapidly when it encounters radical scavengers. In this case, steric accessibility is a major determinant of the analytical reaction. Thus, this assay is adequate mainly for reactive small molecules that have good access to the radical site, and is less sensitive for bigger molecules. The most effective antioxidants scavenging DPPH' are gallic acid, tannic acid, ascorbic acid, and quercetin.

These two methods (FRAP and DPPH) are complementary to some degree. Both methods are recommended as easy, speedy, reproducible, and inexpensive for measuring the antioxidant activity in food extracts.

3.3.1. FRAP

The total antioxidant activities (TAA) determined by FRAP of flour and breads were expressed as mg of Trolox per 100 g dry weight (Table 2). TAA of flour extracts, which were used to bake breads, was highest in case of buckwheat flour. TAA of wheat flour was on the level of 158.3 ± 3.0 mg Trolox/100 g dw. The antioxidant activity of quinoa and amaranth flours were the lowest two. All differences in antioxidant activities of evaluated flours were significant.

The antioxidant activity in breads was highest for bread baked with 30 g/100 g addition of buckwheat flour, followed by the same dose of amaranth flour, and then bread with 15 g/100 g addition of buckwheat flour. Remaining breads with amaranth and quinoa flour had the similar lower antioxidant activity. The control bread had the lowest TAA. There were significant differences between total antioxidant activity of all kinds of breads prepared with addition of either 15 g/100 g or 30 g/100 g pseudocereals flour, as well as between control bread and pseudocereals breads in almost

all cases. TAA of wheat and buckwheat flours were significantly higher than in breads made from these flours. On the contrary, opposed effects were found in TAA of flour and breads made with addition of amaranth and quinoa seeds. In comparison with literature data (Alvarez-Jubete, Auty et al. 2009; Alvarez-Jubete, Wijngaard et al. 2009) about antioxidant activity of pseudocereals flour, our results obtained by FRAP method were lower for amaranth, quinoa and buckwheat flour. Compared to the few data on FRAP in bread made with addition of buckwheat flour and quinoa flour, available in the literature (Alvarez-Jubete, Auty et al. 2009; Alvarez-Jubete, Wijngaard et al. 2009), our FRAP activity are in essential agreement.

3.3.2. DPPH

The total antioxidant activities (TAA) determined by DPPH of flours and breads were expressed as mmol of Trolox per 1 kg dry weight (Table 2). Total antioxidant activity (DPPH) was highest in buckwheat flour, followed by quinoa flour and then wheat flour. Amaranth flour had the lowest total antioxidant activity. The differences between buckwheat and quinoa flours and wheat and amaranth flours were statistically significant.

For breads, the total antioxidant activity in bread with addition of 30 g/100 g buckwheat flour was higher than that in bread with addition of 15 g/100 g buckwheat flour. Similar results were obtained in buckwheat bread by Alvarez-Jubete, Auty et al. (2009), Alvarez-Jubete, Wijngaard et al. (2009). Breads with the addition of amaranth flour (independently on dose of flour) and control bread had higher total antioxidant activity than bread with addition of 30 g/100 g quinoa flour. Bread with addition of 15 g/100 g quinoa flour had the lowest antioxidant activity. There were significant differences between total antioxidant activities of all kinds of breads depending on percentage of flour, except breads made up of quinoa flour. The significant differences for total antioxidant activity (DPPH) between control bread and investigated pseudocereals breads were also observed in all cases. The DPPH scavenging activity of wheat bread was similar to data of Martinez-Villaluenga et al. (2009).

TAA (DPPH) of all kinds of flours was higher than in breads made up of these flours (significant differences were in case of wheat and quinoa flours and breads).

The decreasing levels of TP, FRAP, DPPH in breads in comparison to the flours used as an additional ingredient were revealed in all cases. These results suggest that the antioxidants, which were present in cereals, can be modified during thermal processes. Other authors made similar observations (Holtekjølén et al., 2008; Martinez-Villaluenga et al. 2009; Moore, Luther, Cheng, & Yu, 2009).

There were positive and significant correlations between total phenolic compounds and FRAP of breads ($r = 0.710$; $p < 0.0001$), total phenolic compounds and DPPH of breads ($r = 0.455$; $p < 0.01$), FRAP and DPPH of breads ($r = 0.924$; $p < 0.001$). However, there was no correlation between FRAP and DPPH of flours. All these results suggest, that: (1) there was different trend in antioxidant capacity between FRAP and DPPH in flours

(i.e. between ferric-reducing capacity and nitrogen radical scavenging), but similar one in breads; (2) despite substantial loss of phenolics content during baking processes, still significant contribution to antioxidant power and scavenging ability in breads comprised phenolic compounds; (3) baking processes reduced the variability and quantity of antioxidants in flour, but those remaining in breads are equally well determined by both methods (FRAP and DPPH); therefore it would be interesting to evaluate more closely how much in common do these species bear in respect to such features like their solubility and phase of localization, reaction kinetics, and also physiologic aspects of their varying reactivity.

3.4. Sensory evaluation

The results of hedonic scale analysis of colour, odour and consistency were shown in Table 3. Organoleptic evaluation (colour, odour, consistency) of breads revealed that in a ten-point hedonic scale, all sensory results were in the range of 4–8 indicating that these breads were moderately acceptable. The best colour of bread was observed in case of bread with 30 g/100 g addition of buckwheat flour, in contrast to bread with 15 g/100 g addition of quinoa flour, which had the worst colour of all examined breads. The similar observation about buckwheat was presented by Lin et al. (2009), who suggested that buckwheat improved colour of wheat breads because it contains more phenolic compounds which could inhibit the browning processes during baking.

Bread with 30 g/100 g addition of buckwheat flour had the best odour of all breads, and bread with addition of 15 g/100 g of amaranth flour had the worst one. The results suggested that substituting 15 g/100 g of amaranth flour in the bread formula would not be advantages for bread acceptability. The best influence of odour on evaluated breads had a buckwheat flour, and this effect was previously observed by Lin et al. (2009).

The consistency of bread was the best in case of bread with the addition of 15 g/100 g of buckwheat. Consistency of control bread and amaranth bread were of the same quality. The worst consistency was observed in bread with 15 g/100 g addition of quinoa flour.

The statistically significant difference in colour was found only between bread with 30 g/100 g addition of buckwheat flour and bread with 15 g/100 g addition of quinoa flour ($p < 0.01$). In case of odour the significant difference was only between bread with 15 g/100 g addition of amaranth flour and bread with 30 g/100 g addition of buckwheat flour ($p < 0.01$). No statistically significant differences were found in consistency of examined breads, but in respect to colour and consistency breads with quinoa flour had the worse scores of organoleptic analysis. Breads with buckwheat and amaranth flour had a good valuation of their features, and these results suggest that these kinds of breads were pretty acceptable for consumers.

The effects of the addition of pseudocereals flour into wheat flour on the taste of breads was shown in Figs. 1–3.

Table 3
The ratings of control bread and breads with addition of pseudocereals flour (median values and 95% confidence intervals for medians; the scorecard was developed with 10-point category scale (disliked = 0; extremely liked = 10), number of testers $n = 31$; results marked with the same letter in upper index within each row differ significantly, $p < 0.01$).

Attributes	Bread							
	Control bread	Buckwheat bread 15 g/100 g	Buckwheat bread 30 g/100 g	Amaranth bread 15 g/100 g	Amaranth bread 30 g/100 g	Quinoa bread 15 g/100 g	Quinoa bread 30 g/100 g	
Colour	8 (7–9)	7 (5–7)	8 (7–8) ^a	6 (5–7)	7 (6–8)	5 (4–6) ^a	6 (6–7)	
Odour	7 (6–9)	6 (4–7)	8 (7–8) ^b	5 (4–7) ^b	7.5 (5–7)	7.5 (5–7)	7 (6–8)	
Consistency	7 (6–9)	7.5 (5–7)	7 (6–8)	7 (5–7)	7 (6–7)	4 (2–6)	7 (6–8)	

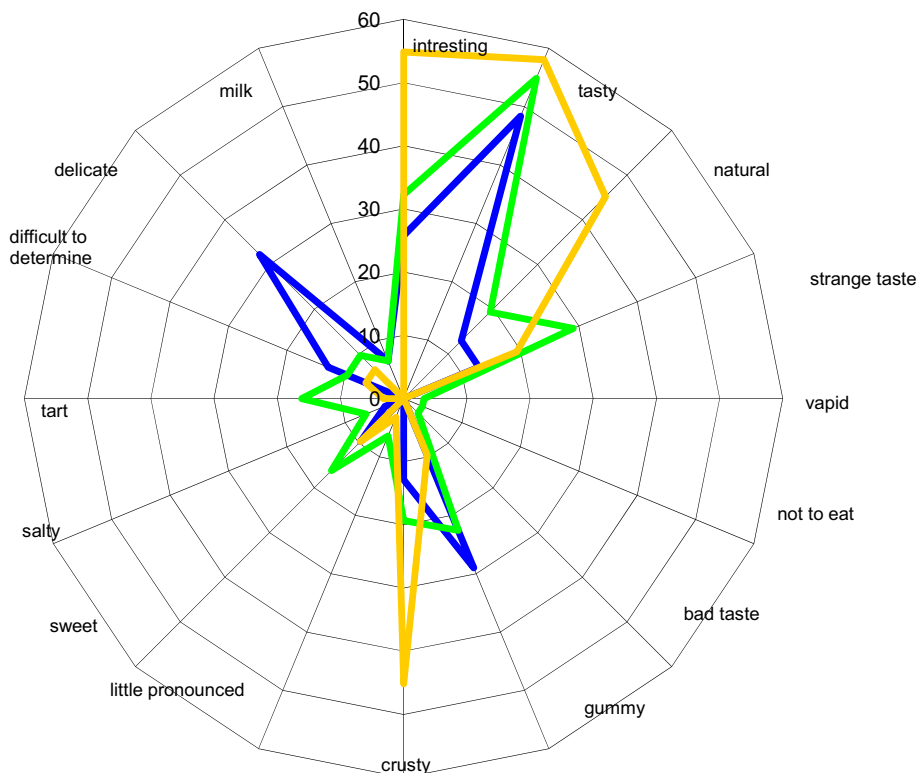


Fig. 1. Spider diagram of the sensory evaluation [%] of the different buckwheat breads and the comparison to the control bread. *Breads were tested 12 h after backing. % values in the diagram were calculated as % of testers ($n = 31$) who approved respective feature. Blue line – % of positive answers given by testers to control bread; green line – % of positive answers given by testers to bread with addition of 15% buckwheat flour; yellow line – % of positive answers given by testers to bread with addition of 30% buckwheat flour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

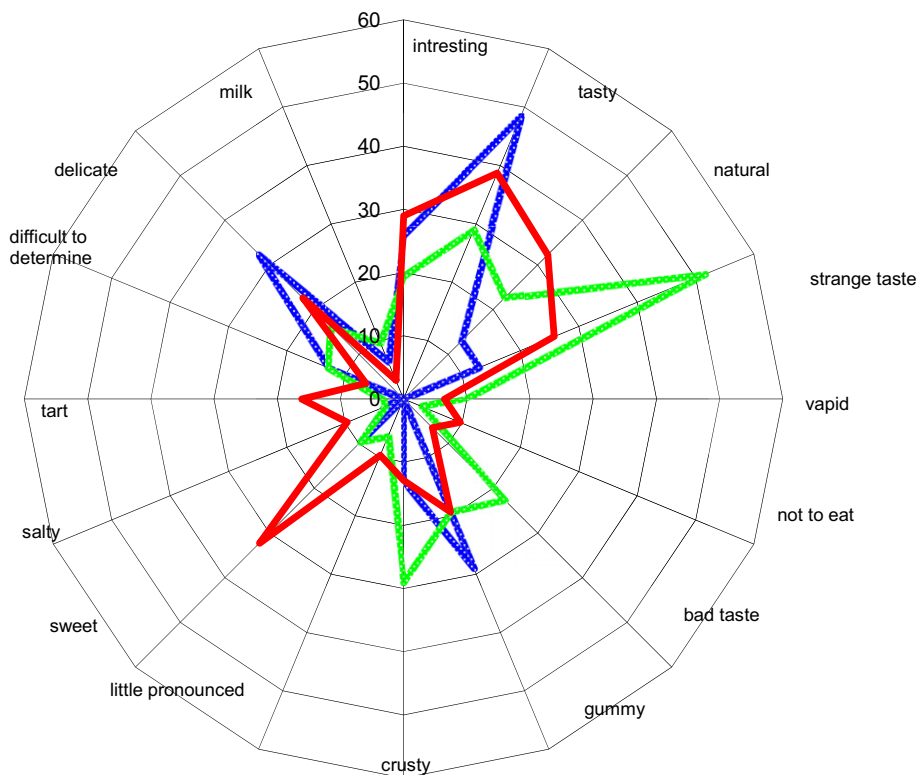


Fig. 2. Spider diagram of the sensory evaluation [%] of the different amaranth breads and the comparison to the control bread. *Breads were tested 12 h after backing. % values in the diagram were calculated as % of testers ($n = 31$) who approved respective feature. Blue line – % of positive answers given by testers to control bread; green line – % of positive answers given by testers to bread with addition of 15% amaranth flour; red line – % of positive answers given by testers to bread with addition of 30% amaranth flour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

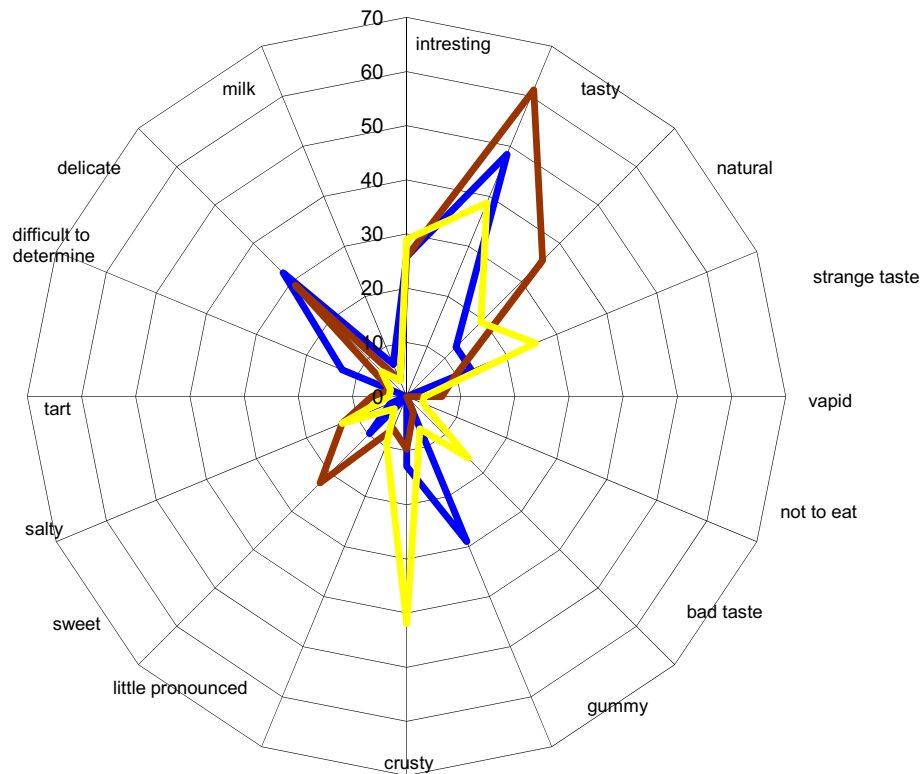


Fig. 3. Spider diagram of the sensory evaluation [%] of the different quinoa breads and the comparison to the control bread. *Breads were tested 12 h after backing. % values in the diagram were calculated as % of testers ($n = 31$) who approved respective feature Blue line – % of positive answers given by testers to control bread; brown line – % of positive answers given by testers to bread with addition of 15% quinoa flour; yellow line – % of positive answers given by testers to bread with addition of 30% quinoa flour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The influence of buckwheat flour is shown in Fig. 1. The characteristics of buckwheat bread taste indicate that bread with addition of 30 g/100 g of buckwheat flour had an interesting and natural taste and was crusty as more than 40% of testers declared such opinion. Control bread and bread with 15 g/100 g of buckwheat flour were not crusty and testers declared gummy taste. Results suggest that buckwheat flour (30 g/100 g) improved the sensory value of bread and reduced attributes (i.e. gummy), which could decrease value of bread. Bread with 30 g/100 g buckwheat flour had a highest scores of sensory profile such as in buckwheat bread examination by Wronkowska et al. (2008).

The influence of amaranth flour is shown in Fig. 2. In case of amaranth bread it was noticed that it had some negative opinions (about 10–30% testers) stating that this bread was “not to eat”, gummy, and had a bad, strange or difficult to determine taste. Only 30% testers declared an interesting taste of amaranth bread. Therefore, the addition of amaranth flour to wheat bread does not seem to be a favourable modification. The significant negative observations of changes of sensory attributes associated with quantity of different flour (rice, soy or maize germ flour) were previously described by Sabanis and Tzia (2009) or Siddiq et al. (2009).

The influence of quinoa flour is shown in Fig. 3. In opposite to amaranth bread, quinoa breads were really appreciated by testers. More than 30% of them suggested an interesting, delicate and crusty taste. Only 15% testers declared that bread with addition of quinoa flour (30%) had a bad taste.

Analysis of sensory quality results of breads shown that addition of pseudocereals flour (especially buckwheat and quinoa flour) to wheat flour may increase acceptable quality attributes such as taste, colour and odour. The buckwheat breads had the best opinion about their colour and odour. These observations suggest that

addition of buckwheat flour into bread can improve not only antioxidant but also sensory properties of bread.

4. Conclusions

The addition of buckwheat flour to wheat flour improved more effectively antioxidant status of bread than other studied pseudocereals, amaranth and quinoa. Buckwheat bread had a highest content of phenolic compounds.

The sensory profile results suggest that the buckwheat bread has more positive sensory value than amaranth and quinoa breads. Results showed also that addition of buckwheat flour up to 15 g/100 g or 30 g/100 g levels to wheat flour improved satisfactory bread properties and attributes such as colour, odour and taste. These results indicate that consumers may willingly choose buckwheat breads.

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