DOI 10.1002/mnfr.200500258 Mol. Nutr. Food Res. 2006, *50*, 824 – 832

Antioxidants in thermally treated buckwheat groats

Henryk Zieliński, Anna Michalska, Mariusz K. Piskuła and Halina Kozłowska

Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

The seeds of buckwheat (*Fagopyrum esculentum* Moench L.) were dehulled and then, following milling, extruded on a counter rotating, twin-screw extruder with the different barrel temperature profiles: 120, 160, and 200°C. After extrusion cooking process, the following compounds were analyzed: free and conjugated phenolic acids, total polyphenols (TPC), tocopherols (T) and tocotrienols (T3), inositol phosphates (IP), reduced glutathione (GSH), and melatonin (MLT). The antioxidant capacity and superoxide dismutase-like activity (SOD-like activity) were determined in the groats and extrudates. Extrusion caused a significant decrease in all the compounds tested, except for phenolic acids. The content of IP decreased by 13%, that of GSH by 42%, and that of T + T3 by 62%. A three-fold lower level of MLT and TPC was noted whereas the SOD-like activity disappeared when compared to the nonextruded material. A two-fold higher content of phenolic acids (free and released from ester bonds) was observed. In spite of the clear decrease in the investigated antioxidants, the extruded dehulled buckwheat seeds contained still significant content of bioactive compounds, which resulted in as little as an average 10% decrease of the antioxidant capacity.

Keywords: Bioactive compounds / Buckwheat groats / Extrusion / Health benefits / SOD-like activity

Received: December 9, 2005; revised: April 10, 2006; accepted: May 9, 2006

1 Introduction

In search of food with a high nutritive value and rich in bioactive compounds with antioxidant properties, special attention is paid to common buckwheat (*Fagopyrum esculentum* Moench L.), a crop adapted to cool, moist climate, short growing season (70–90 days), and negligible crop protection. Buckwheat belongs to the *Polygonaceae* family, unlike major cereals such as wheat, barley, oat, rice, and corn. However, it is usually grouped along with cereals due to its ways of cultivation and utilization. The buckwheat seed is actually a fruit, an achene, which, when dehulled (the pericarp removed), is referred to as groats.

Buckwheat is primarily used for human consumption and is at present considered as a food component of high nutritional value because of high level of vitamin B_1 and B_2, lysine, and balanced amino acid composition [1]. Moreover, buckwheat contains many biologically active compounds with beneficial action on human body. Among them are flavonoids such as catechins [1], rutin, quercetin, orientin, isoorientin, vitexin, isovitexin [2], phytosterols composed mainly of β-sitosterol, campesterol and traces of stigmasterol [3], unique fagopirins such as fagopyritol A1 and fagopyritol B1 [4, 5], and thiamin-binding proteins [6]. It should be also mentioned that buckwheat seeds contain some compounds which can exert negative action on human body. Among them are allergenic proteins, fagopirins, and trypsin inhibitors which are responsible for allergenic reaction type I, photosensitization in animals but not in humans, and low utilization rate of buckwheat food, respectively [7].

Most buckwheat in human diet is used as processed flour or in pancake mixes. Buckwheat flour can be light in color if hulls (a quarter of the grain in weight) are completely removed before grinding groats; but often, some hull fractions remain in the ground material, giving the flour a dark color. Japanese use buckwheat flour largely for noodles and curds; in addition its pericarp is used for pillow stuffing materials. In Eastern Europe, buckwheat roasted groats called "kasha" is cooked and served like rice. Groats are sometimes used in the US as a breakfast cereal. Both kasha and groats can be baked, steamed, or boiled for nutritious alternatives to potatoes and rice [8].

Recently, researchers have focused on the development of buckwheat as a potential functional food material. Buckwheat can be used to produce extruded cereal and snack

Correspondence: Dr. Henryk Zieliński, Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland
E-mail: haziel@pan.olsztyn.pl
Fax: +48-89-5240124

Abbreviations: GSH, reduced glutathione; **IP,** inositol phosphates; **MLT,** melatonin; **OPT,** *o*-phthalaldehyde; **SOD,** superoxide dismutase; **T,** tocopherols; **T3,** tocotrienols; **TEAC,** Trolox equivalent antioxidant capacity; **TPC,** total polyphenols

products. Extruded buckwheat products are of very high nutritional quality when compared with products extruded from maize, wheat, or barley alone [8]. Buckwheat appears to be a suitable component of food products from the point of view of its antioxidant activity. Several authors studied its antioxidative properties [1, 9, 10]. However, the data compiled on the effect of thermal treatments on the bioactive compounds and antioxidant capacity of buckwheat groats are limited. Therefore, changes in bioactive compound levels and antioxidant properties after extrusion cooking of buckwheat groats were addressed in this study. Antioxidant activity of raw and thermally treated material was related to the total polyphenols (TPC), free and conjugated phenolic acids, tocopherols (T) and tocotrienols (T3), reduced glutathione (GSH), inositol phosphates (IP), and melatonin (MLT). The contribution of these compounds to the Trolox equivalent antioxidant capacity (TEAC) of raw and extruded buckwheat groats was calculated. The next objective of this study was to characterize the antioxidant properties of extruded buckwheat groats in comparison to the extruded main cereal grains.

2 Materials and methods

2.1 Reagents

Glutathione (γ-glutamyl-cysteinyl-glycine; GSH), *o*-phthalaldehyde (OPT), sodium phytate, and *N*-acetyl-5-methoxytryptamine (cold MLT) were obtained from Sigma (Sigma Chemical, St. Louis, MO). Phenolic acid standards and tocopherols (α-T, β-T, γ-T, δ-T), and tocotrienols (α-T3, β-T3, γ-T3) standards were obtained from Merck and Sigma. (*O*-methyl3H)-MLT (specific activity 87 Ci/mM) was purchased from Du Pont NEN; and antiserum G/S/704-6483 from Stockgrand, University of Surry, Guilford, UK. The superoxide dismutase (SOD) kit (RANSOD, Cat. No. SD 125) was from Randox Laboratories (Crumlin, Antrim, UK). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

2.2 Samples

Buckwheat (*F. esculentum*, variety Kora) was provided by the local plant breeding station in Northeast Poland. Dehulling process was performed in the local groats plant by crushing the seeds without thermal treatment following which hulls and groats were separated by sieving through a set of sieves.

2.3 Thermal treatment

The experiment was carried out on a counter-rotating, twin-screw extruder (type 2S-9/5 Metalchem, Poland) with a die bore diameter of 6 mm and constant screw configuration. The extruder barrel configuration consisted of five head sections with an extruder profile for expanded breakfast cereals and snacks. The initial head section of the profile consisted of 90 mm diameter conveying and kneading screws. The final head element was conical-shaped and had flights cut for more effective mechanical energy development. The process was carried out on disintegrated buckwheat groats. The following barrel temperature profiles were used: 80–100–120–120–120°C, 100–130–160–160–120°C, and 120–160–200–200–120°C. Temperatures were recorded for all head sections by thermocouples that were mounted into the extruder head sections and were flushed with the interior of the extruder barrel. Water injected downstream from the solid feed port provided a moisture content of 20% (wet basis). The extruder parameters were kept constant at 500 rpm screw speed and 225 g/min mass flow rate. The extruded material was cooled to room temperature and next moisture content was determined using the method of the AOAC (Association of Official Analytical Chemists) [11]. The moisture content of the raw buckwheat material was 12.2%, whereas those of the extruded samples at 120, 160, and 200°C were 11.8, 11.0, and 10.5%, respectively. Approximately 0.5 kg of the buckwheat extrudate and raw material used for extrusion cooking were ground in a laboratory mill type WZ-1 (Factory of Machines and Mechanisms for the Food Industry, Znin, Poland). Ground samples were stored at −30°C until chemical analysis. Samples from two replications of each process were chosen for analysis.

2.4 Analytical methods

2.4.1 Preparation of PBS and methanolic extracts

The buckwheat groats were ground and extracted in triplicate with pH 7.4 PBS (15 mL/1 g of sample) or with 80% aqueous methanol (1/10 w/v) for 2 h of shaking at 37°C. The samples were then centrifuged at 12 000 × *g* for 15 min in a Beckman GS-15 R centrifuge (Beckman Instruments, Palo Alto, CA, USA). The fresh PBS extracts were used to determine their ability to scavenge superoxide anion radicals and the content of soluble proteins, whereas 80% methanolic extracts were used to determine their ability to scavenge $ABTS^{\bullet+}$ radicals and the content of TPC.

2.4.2 Determination of phenolic acids

Five-gram samples of disintegrated raw and extruded buckwheat groats were extracted for 2 h with 50 mL of 80% v/v aqueous methanol at 35°C. The extracts were centrifuged at 4000 × *g* for 10 min (Centrifuge MPW-360) and the supernatants were evaporated to dryness under nitrogen. The residue was dissolved in water adjusted to pH 2 (6 N HCl)

and then extracted with diethyl ether for 24 h. The ether extracts were dehydrated with anhydrous sodium sulfate, filtered and evaporated to dryness under vacuum at room temperature and the dry residue was used for the analysis of free phenolic acids according to the method described by Zadernowski [12]. The water solution left after extraction of ether was neutralized and then evaporated to dryness. The dry residue was dissolved in 50 mL of 2 M NaOH and hydrolyzed for 4 h in a nitrogen atmosphere at room temperature. Phenolic acids released from soluble esters were extracted from the hydrolysate by liquid-liquid extraction with diethyl ether after acidification to pH 2. The free and esterified phenolic acids were separated by HPLC on LiChrospher RP-18 5 μm particle size, 4 × 250 mm column. Twenty microliters of each sample was injected into the column. The HPLC systems consisted of a Shimadzu model LC pump series 10 AD, and a Shimadzu UV detector. The mobile phase was water : ACN : acetic acid (88 : 10 : 2 v/v/v) and the flow rate was 1 mL/min. The peaks were detected using a wavelength of 290 nm and they were quantitated against their corresponding phenolic acid standards.

2.4.3 Determination of TPC

The content of TPC was determined according to Shahidi and Naczk [13]. Exactly 0.25 mL aliquot of the 80% methanolic extract was mixed with 0.25 mL Folin-Ciocalteu reagent (previously diluted with water, 1 : 1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution, and 4 mL of water. The mixture was allowed to stand at room temperature for 25 min and then it was centrifuged at 2000 × *g* for 10 min. Absorbance of the supernatant was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The results were expressed as rutin equivalents.

2.4.4 Determination of tocopherols and tocotrienols by HPLC

Tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) were extracted with 80% methanol (0.5 g of sample/7 mL of solvent) and evaporated extracts were redissolved in *n*-hexane. The tocols were separated by HPLC on a LiChrospher Si 60 5 μm particle size, 4 × 250 mm column, according to the method described by Peterson and Qureshi [14]. Twenty microliters of each sample was injected into the column. The HPLC systems consisted of a Shimadzu model LC pump series 10 AD, and a Shimadzu RF-535 fluorescence spectrometer. The mobile phase was 0.5% isopropanol in hexane. The flow rate was 1 mL/min, and the peaks were detected using an excitation wavelength of 295 nm and emission wavelength of 330 nm. The tocols contents were calculated from the peak areas using standard curves of tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3). Vitamin E content, expressed in micrograms of α-T-equivalents (α-TE), was calculated according to Mc Laughlin and Weihrauch [15] using biological activities of 1.0 for α-T, 0.3 for α-T3, 0.4 for β-T, 0.05 for β-T3, 0.1 for γ-T, 0.01 for γ-T3, and 0.01 for δ-T.

2.4.5 Determination of IPs (IP6 – IP3) by HPLC

Ground groats and extrudates were extracted with 20 mL of HCl (0.5 M) for 5 h using a BM1 magnetic stirrer. The extract was centrifuged at 3500 × *g* for 40 min (Centrifuge MPW-360) and the supernatant was decanted, frozen overnight (–18°C), thawed at room temperature, and recentrifuged at 3500 × *g* for 40 min. The supernatant (15 mL) was vacuum evaporated to dryness at 40°C and dissolved in 15 mL of 0.025 M HCl. The samples were then transferred to the mini-columns filled with Dowex AG 1-X8 resin, from which the IPs were eluted using 2 M HCl (5 × 4 mL). After the solvent had been removed by evaporation, the dry residue was dissolved in a mixture of methanol, 0.05 M formic acid, and 1.5 mL/100 mL TBA-OH and analyzed by HPLC according to Sandberg and Ahderinne [16] and Sandberg *et al.* [17] using a Shimadzu chromatograph (LC-10 AD pump, refractometric detector RID-6A, CTO 6A column oven) and a Nova-Pak C_{18} column. The mobile phase was a mixture of methanol, 0.05 mol/L formic acid (51/49 v/v), and 1.5 mL/100 mL TBA-OH. The flow rate was 0.4 mL/min. Sodium phytate was the external standard and injections were made with a 20 μL loop.

2.4.6 Determination of GSH

Extraction and assay of GSH were conducted according to Smith *et al.* [18] and Hissin and Hilf [19], respectively. Raw and extruded buckwheat groats (3 g) were ground in an electric coffee mill. The flour was transferred to a centrifuge tube and mixed with phosphate buffer (15 mL, 0.2 M with EDTA, 1 mM, pH 7.5) and potassium chloride (KCl, 0.33 g). The mixture was homogenized for 30 s using a Polytron homogeniser at full speed. Polyvinylpolypyrrolidone (PVPP; 0.25 g) was added and after thorough mixing the mixture was centrifuged (2000 × *g*, 10 min, 4°C). After recentrifugation, the supernatant was kept on ice and assayed for the GSH content as follows: exactly 60 μL of the extract was mixed with 1.84 mL of 0.1 M sodium phosphate – 0.005 M EDTA buffer (pH 8.0), and 100 μL of the OPT solution, containing 100 μg of OPT in reagent-grade absolute methanol. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette. Fluorescence was determined at 420 nm with excitation at 350 nm. A series of GSH standards were prepared in phosphate-EDTA buffer, pH 8.0, ranging from 0.033 to 6.51 nmol/60 μL. The assays were performed using a Perkin-Elmer LS 50 B Luminescence Spectrometer.

2.4.7 Determination of MLT

Raw and extruded groats samples were ground in a laboratory mill and then extracted in duplicate with 0.01 M PBS pH 7.4 (5 mL/1 g of sample) during 2 h of shaking at 37°C. They were next centrifuged at 12 000 × *g* (Beckman centrifuge GS-15R) and the fresh supernatant was assayed for MLT. The MLT immunoreactivity was assayed by a slightly modified direct method of Fraser *et al.* [20]. Briefly, 200 µL of antiserum G/S/704-6483, diluted 1 : 6000 in assay buffer (tricine 0.1 M, sodium chloride 9 g/L, gelatine 1 g/L) was added to 500 µL of sample or standard (0 – 500 pg/mL prepared in PBS), and the mixture was incubated at room temperature for 30 min. Then, 100 µL of 3H-MLT ((*O*-methyl3H)-MLT, specific activity 87 Ci/mM), diluted in the assay buffer to approximately 1 µCi/10 mL, was added. Following overnight incubation at 4°C, antibody-bound MLT was separated from the free fraction by incubation with 500 µL dextran-coated charcoal (0.9 g Norit A and 60 mg dextran in 100 mL of the assay buffer) for 15 min at 4°C. After centrifugation, the radioactivity of 700 µL of supernatant was measured using liquid scintillation method. Samples were assayed in duplicates. For each sample, the nonspecific binding was determined (in duplicates) by adding tricine buffer instead of antibody solution. The assay was validated by running of the samples containing different amounts of exogenous MLT. The MLT concentration was calculated using four-parameter logistic curve (Immuno Fit EIA/RIA ver. 3. 0a, Beckman). The mean standard curve of the direct assay employing antiserum G/S/704-6483 was generated during 6-assay series. The mean specific binding was 39.5% of the total radioactivity, the mean unspecific binding in PBS was 1.4% of the total radioactivity, the unspecific binding of samples varied from 4 to 12% of the total radioactivity. The sensitivity of this assay was below 5 pg/mL. The intra-assay coefficients of variation ($n = 6$) for the samples containing 28.50 and 57.82 pg/mL of MLT were 6.3 and 5.6%, respectively.

2.4.8 Determination of TEAC

This test was based on the reduction of the $ABTS^{\bullet+}$ radical cation by antioxidants present in 80% methanolic extracts. The $ABTS^{\bullet+}$ radical cation was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture had to remain for 12 – 24 h until the reaction was complete and the absorbance was stable. TEAC was determined following a procedure described by Re *et al.* [21] with a minor modification described below. For measurements, the $ABTS^{\bullet+}$ solution was diluted with 80% methanol to the absorbance of 0.700 ± 0.020 at 734 nm. For the photometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 µL of the extracts or Trolox standards were mixed and measured immediately after 6 min at 734 nm, at 30°C using a spectrophotometer (UV-160 1PC, Shimadzu). Appropriate solvent blanks were run in each assay. The TEAC of 80% methanolic extracts was calculated, using Trolox standard curve, on the basis of percentage inhibition of absorbance at 734 nm.

2.4.9 Determination of SOD-like activity

The SOD-like activity of the PBS extracts was measured according to SOD kit (RANSOD). The assays were performed using a thermostated recording spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu) adjusted to 37°C inside the cuvettes. The test required 50 µL of sample, with a read time of 3 min. The results were finally calculated into milligrams of soluble protein assayed according to the bicinchonic acid (BCA) protein micro assay [22]. The SOD with the activity of 5.3 U/mL was used as a standard and was supplied as a part of the reagent kit.

2.4.10 Statistics

All measurements were replicated three times for each treatment and their means are reported. The means were tested for significance using one-sided *t*-test. Differences were considered significant when $P \leq 0.5$.

3 Results and discussion

It is well known that thermal processing causes chemical changes in food products. Since buckwheat groats may undergo considerable changes in composition during thermal treatment, the antioxidant properties and the content of selected bioactive compounds before and after extrusion cooking, used in this experiment as a model of hydrothermal processing, were addressed [23].

In this study, small quantities of the following free and ester bound phenolic acids were identified in buckwheat groats: vanillic, syringic, ferulic, and coumaric (Table 1). They were found mainly as ester bound phenolic acids and the highest level was noted for syringic and coumaric acid. The content of free and ester bound phenolic acids was significantly lower when compared to cereal grains. For example, it was twice lower compared to barley, four times lower compared to wheat, and from 10 to 20 times lower than in rye and oat [24]. They formed only 0.1% of total phenolic compounds in buckwheat, which confirmed that flavonoids and flavonols, including proanthocyanidins, are the main phenolics in buckwheat groats [1, 2, 25]. The applied hydrothermal process caused significant changes in the phenolic acids content in the extruded buckwheat groats (Table 1). An increase in all the analyzed free and ester bound phenolic acids was found, except for vanillic acid. The latter was not found in the hydrothermal processed groats. This finding may be explained on the ground of previous evidence that the increase in phenolic acid content is

Table 1. Content of free and conjugated phenolic acids (μg/g dry mass) and TPC (mg/g dry mass) in buckwheat groat before and after extrusion cooking[a)]

Phenolic acids	Vanillic	Syringic	Ferulic	Coumaric	Total phenolic acids	TPC
Free phenolic acids						
Groat:	tr.	tr.	tr.	0.13 ± 0.01	0.13 ± 0.02[a]	4.08 ± 0.20[a]
Extrusion 120°C	tr.	1.42 ± 0.07	0.37 ± 0.02	0.74 ± 0.04	2.53 ± 0.13[b]	1.17 ± 0.06[b]
Extrusion 160°C	tr.	1.49 ± 0.10	0.37 ± 0.03	0.75 ± 0.05	2.61 ± 0.18[b]	0.83 ± 0.05[bc]
Extrusion 200°C	tr.	1.75 ± 0.09	0.30 ± 0.02	0.72 ± 0.04	2.77 ± 0.14[bc]	1.41 ± 0.07[bcd]
Conjugated phenolic acids						
Groat:	0.15 ± 0.01	0.97 ± 0.08	0.18 ± 0.01	1.22 ± 0.09	2.52 ± 0.20[a]	
Extrusion 120°C	tr.	4.96 ± 0.25	1.49 ± 0.07	2.23 ± 0.11	8.68 ± 0.43[b]	
Extrusion 160°C	tr.	5.18 ± 0.36	0.93 ± 0.07	2.13 ± 0.15	8.24 ± 0.58[b]	
Extrusion 200°C	tr.	5.39 ± 0.27	1.37 ± 0.07	2.27 ± 0.11	9.03 ± 0.30[b]	

a) Mean value ± SD of three determinations. Different superscripts in the column for total free and conjugated phenolic acids and TPC imply significant difference between the raw and extruded groat ($P \leq 0.05$).

primarily due to the increased release of phytochemicals from the matrix to make it more accessible in the extraction, similarly to the lycopene increase after thermal processing of tomatoes [26]. As a result, the liberated phenolic acids may contribute to the antioxidant capacity of buckwheat extrudates. This small increase of phenolic acids after extrusion cooking was statistically significant ($P \leq 0.05$). Following extrusion cooking of the groats at 120, 160, and 200°C phenolic acids made 0.95, 1.31, and 0.84% of total phenolic compounds, respectively, and were masked by the decrease in total phenolic compounds from 4.08 mg/g dry mass (groats) to 1.17, 0.83, and 1.41 mg/g dry mass for extrudates at 120, 160, and 200°C, respectively. It was mainly due to the thermal degradation of the main buckwheat groats flavonoids – rutin and isovotexin – as it was previously described by Dietrych-Szostak and Oleszek when extensive heat treatment was applied during processing [2]. The significant decrease in rutin content was also found after cooking of buckwheat groats suggesting that the presence of rutin-degrading enzyme was also responsible for this effect [27]. In contrast, other authors did not find any statistically significant changes in total phenolic compound contents after roasting (200°C, 10 min) and extrusion (die temperature of 170°C) of dark buckwheat flour, perhaps due to short processing time at high temperature [28]. However, they noted qualitative changes in both polar and nonpolar compounds after roasting, and in polar compounds after extrusion of dark buckwheat flour, at least five times richer source of phenolic compounds than white buckwheat flour [28, 29]. The latter was similar to the material used in our study, since white flour was obtained from crushed whole buckwheat grain from which groats were hand separated and milled into flour.

In this study, γ-T was the main isomer found among the analyzed tocopherols (29.9 mg/kg dry mass); however, a small quantity of α-T was also detected (0.8 mg/kg dry mass). Among all tocotrienols, only trace amount of α-T3 was noted (Table 2). The tocopherols and tocotrienols profile of the buckwheat groats were quite different from these noted previously for the main cereal grains (wheat, oat, barley, and rye), which contained more tocotrienols than other food products [15, 30]. When γ-T, α-T, and α-T3 were expressed in terms of vitamin E activity and their biological activities were taken into account, buckwheat groats proved to contain a smaller amount of vitamin E when compared to wheat, barley, and oat, and a comparable level when compared to oat grain [24]. Extrusion caused a statistically significant decrease in vitamin E content, on average 63% for all the temperatures used, mainly due to γ-T thermal degradation (Table 2). The vitamin E data provided here may be used as a baseline of vitamin E activity which is left after hydrothermal processing of buckwheat, and may be used as an initial point before buckwheat-based products are vitamin E fortified.

The qualitative and quantitative analyses of IPs were carried out on dehulled buckwheat seeds. In this material, the inositol hexaphosphate (IP6) makes 98% of total IPs. Apart from IP6, trace amounts of inositol pentaphosphate (IP5) and inositol tetraphosphate (IP4) were detected (Table 3). These results confirm that buckwheat groats are a good source of inositol hexaphosphate, approximately about twice richer when compared to such cereal grains as wheat, rye, dehulled oat, and barley [31 – 33]. Extrusion cooking caused no statistically significant degradation of inositol hexaphosphates, only by 13%; no links were observed between the process temperature and degradation of IP6. However, only in the case of the highest barrel temperature profile of 200°C, about twice higher level of IP5 was detected (Table 3). This difference was statistically significant at $P \leq 0.5$. The degradation degree of inositol hexaphosphates indicates that this compound is resistant against hydrothermal treatments.

Buckwheat groats are a rich source of glutathione (γ-glutamyl-cysteinyl-glycine; GSH). From the technological point of view, this compound has been considered to play an

Table 2. Concentration of tocopherols and tocotrienols in buckwheat groat before and after extrusion cooking (μg/g dry mass)[a)]

Source	Tocopherols					Tocotrienols				Vitamin E (IU/kg)
	α-T	β-T	γ-T	δ-T	Total	α-T3	β-T3	γ-T3	Total	
Groat	0.78 ± 0.04	29.88 ± 1.49			30.66 ± 1.53	0.09 ± 0.01			0.09 ± 0.01	5.6 ± 0.28[a]
Extrusion 120°C	0.30 ± 0.02	10.62 ± 0.74			10.92 ± 0.76	0.08 ± 0.01			0.08 ± 0.01	2.1 ± 0.15[b]
Extrusion 160°C	0.29 ± 0.02	10.05 ± 0.51			10.34 ± 0.52	0.06 ± 0.01			0.06 ± 0.01	2.0 ± 0.14[b]
Extrusion 200°C	0.35 ± 0.03	10.99 ± 0.77			11.34 ± 0.79	0.07 ± 0.01			0.07 ± 0.01	2.2 ± 0.15[b]

a) Mean value ± SD of three determinations. Different superscripts in the column for vitamin E content imply significant difference between the raw and extruded groat ($P \leq 0.05$)

Table 3. Content of IPs in buckwheat groat before and after extrusion cooking (mg/g dry mass)[a)]

Buckwheat groat	IPs			
	IP4	IP5	IP6	Total
Before extrusion				
	0.1 ± 0.05[a]	0.2 ± 0.05[a]	17.3 ± 1.35[a]	17.6 ± 1.31[a]
After extrusion				
120°C	tr	0.2 ± 0.05[a]	15.1 ± 0.52[a]	15.3 ± 0.52[b]
160°C	tr	0.2 ± 0.05[a]	15.7 ± 0.37[a]	15.9 ± 0.37[b]
200°C	0.1 ± 0.05[a)]	0.4 ± 0.03[b]	15.4 ± 1.06[a]	15.9 ± 1.06[ab]

tr < 0.05 mg/g dry mass

a) Mean value ± SD of three determinations. The same different superscript in the column for IPs means significant difference between the raw and extruded groat ($P \leq 0.05$).

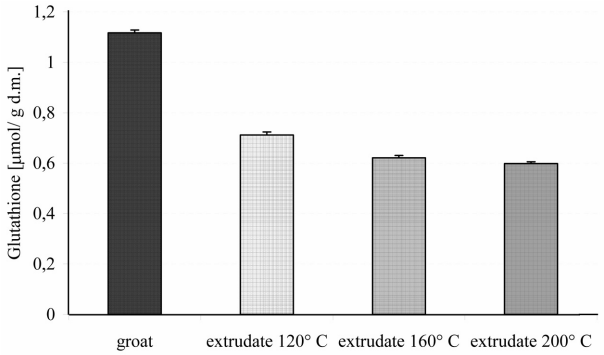

Figure 1. GSH content due to the extrusion cooking of buckwheat groats in different temperature profiles.

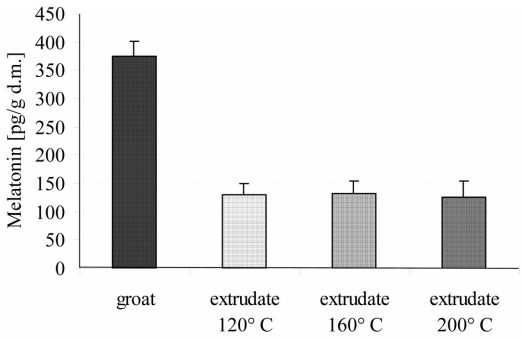

Figure 2. Content of the RIAable MLT in buckwheat groats before and after extrusion cooking.

important role in redox reactions in flour and baking technology [34]. Buckwheat groats contained twice higher amounts of GSH than wheat and rye grains, and almost three-fold higher amounts when compared to barley and oat grains [24]. This finding is in agreement with recent studies which showed that cereals and breads as well as dairy products were generally low in GSH. Moreover, different forms of food processing and preservation generally resulted in extensive loss of GSH with one exception made to freezing [35]. The extrusion cooking performed in this study resulted in a statistically significant decrease of GSH on average by 42%. In details, the extrusion performed at 120°C caused a significant decrease in GSH content when compared to raw material. The next higher temperature profiles of 160 and 200°C caused only a slight decrease of GSH in extrudates when compared to extrusion cooking at 120°C (Fig. 1). The differences between GSH content in extrudates were not statistically significant at $P \leq 0.5$.

The MLT level found in buckwheat groats (470 pg/g dry mass) was comparable to those of cereal grains (200–700 pg/g dry mass) [27]. The amount of MLT in extruded groats was statistically significantly decreased, on average by 63% (Fig. 2). The used temperature profiles from 120 to 200°C did not cause statistically significant differences between extrudates. The amount of MLT found in groats and extrudates was lower than that found previously in medicinal plants [36]; however, it was higher than that found in edible-plant products [37]. Moreover, considering the potent antioxidant activity of MLT and its synergistic positive interactions with antioxidants such as Trolox, glutathione, ascorbate in aqueous system [38] and with α-T in a lipid bilayer [39], it may be concluded that ingestion of cereal-based products selected for their MLT content may play a role in the protection against radical-mediated cellular damage as well as cancer progression and promotion *in vivo*.

The SOD-like activity of the PBS extract originated from buckwheat groats, which represents its ability to scavenge superoxide anion radicals, was almost three times lower (3.04 ± 0.12 U/mg proteins) when compared to previously published data for wheat and barley, and about twice lower compared to rye and oat grains [40]. Moreover, the ability to scavenge superoxide radicals by extracts prepared from the extrudates completely disappeared after thermal processing. The remaining SOD-like activity of extrudates made only about 1% of the initial activity noted for groats (data not included).

In this study, the antioxidant capacity of the raw and extruded buckwheat groats was determined. The term "antioxidant capacity" used here corresponds to the measure of moles of a given free radical scavenged by a test solution, independently of the antioxidant activity of any antioxidant present in the mixture [41]. Then, the measure of the TEAC of food extracts considers the cumulative action of all the antioxidants present in the extract, including their chain-breaking, scavenging, and chelating effects, and thus providing an integrated parameter rather than a simple sum of measurable antioxidants. As a result of antioxidants content and antioxidant gap, the estimated TEAC values of buckwheat groats based on relative abilities of 80% methanolic extracts to scavenge the $ABTS^{\bullet+}$ in comparison with Trolox showed high antioxidant capacity of this material (25.7 μmol Trolox/g dry mass). The TEAC value was at least twice higher when compared to barley grain, almost four times higher compared to oat and rye grains, and over eight times higher in comparison to wheat grain [42]. The extrusion cooking process caused no statistically significant decrease in the antioxidant capacity of the extruded buckwheat groats. Moreover, the estimated TEAC values of buckwheat extrudates were not statistically different. It was found that TEAC values of extrudates were lower by 7, 13, and 16% for the material processed at 120, 160, and 200°C, respectively, when compared to raw material (Fig. 3). This finding was supported by a recent observation by Sensoy *et al.* [28], who did not find any statistically significant change in the antioxidant activity of different kinds of extruded buckwheat flours when compared to raw flours. They found only a slightly decreased antioxidant activity after roasting when DPPH radical scavenging activity test was used in both thermal processes.

In the present study, the average antioxidant capacity of buckwheat extrudates was generally higher when compared to previously published data on antioxidant capacity of extrudates of barley, rye, oat, and wheat grains under the same conditions [42]. Buckwheat extrudates had about twice, four, and eight times higher antioxidant capacity than those noted in extruded barley and rye, oat, and wheat grains, respectively.

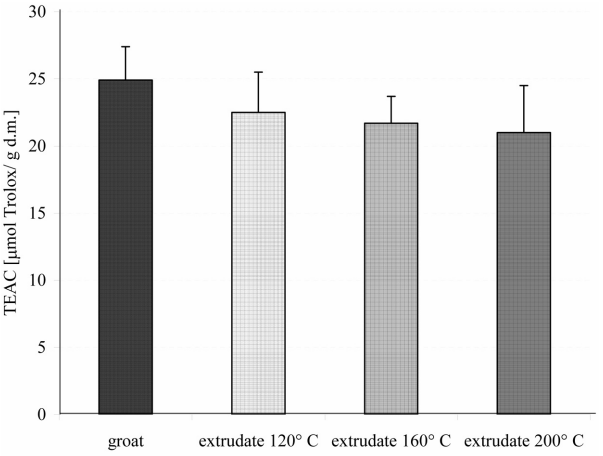

Figure 3. TEAC due to the extrusion cooking of buckwheat groats in different temperature profiles.

The contribution of TPC, including phenolic acids, as well as of tocopherols and tocotrienols, glutathione, and MLT to the TEAC of raw and extruded buckwheat groats was calculated. For this purpose, the TAEC values of rutin, phenolic acids, α-T, GSH, and MLT were used to calculate the contribution of these compounds to the TEAC of raw and extruded buckwheat groats. In order to perform the calculations, the following TEAC values of individual compounds were taken: 1.43 for vanilic acid, 1.36 for syringic acid, 1.90 for ferulic acid, 2.22 for coumaric acid, 2.40 for rutin, 0.97 for α-T, 0.90 for GSH, and 1.34 for MLT [43, 44]. The TEAC values used for individual compounds were confirmed experimentally. Calculation of the fraction of antioxidant capacity derived from tocopherols was based upon the presumption that as each tocopherol and Trolox possess a single aromatic –OH, they quench reactive oxygen species (ROS) on an equimolar basis *in vitro* [45]. The contribution of buckwheat groats antioxidants to the TEAC of raw and extruded buckwheat groats was calculated by multiple mean values for the content of investigated compounds shown in Tables 1 – 3 and in Figs. 1, 2 (expressed as μmol of individual compound *per* gram of dry matter) and their relative potential with respect to Trolox. After that, the result was divided by the TEAC values of raw and extruded buckwheat groats, and finally expressed as percentage of contribution.

The contribution of the TPC to the capacity of buckwheat groats was 62.4%, but after extrusion cooking at 120, 160, and 200°C it decreased to 19.2, 14.7, and 25.5%, respectively. It also covers the contribution of phenolic acids which was estimated to be 0.11% for groats, but after extrusion cooking at 120, 160, and 200°C its input increased to 0.43, 0.44, and 0.45%, respectively. In contrast to phenolic acids, the total contribution of α-T, β-T, and α-T3 to the TEAC from initial 0.28% for groats, decreased after extru-

sion to the range 0.11 – 0.12%. The glutathione contribution to the TEAC was 3.91% for groats and 2.68, 2.52, and 2.48% for respective extrudates. Finally, the calculated MLT contribution was negligible because of the very low content of this compound in buckwheat groats and extrudates.

The TEAC values of buckwheat extrudates may reflect the general changes occurring during thermal processing of cereal and pseudocereal grains, which comprises the losses of naturally occurring antioxidants, formation of novel compounds having pro-oxidant activity and/or antioxidant activity (*i. e.*, Maillard reaction products), and interactions among different compounds (*e. g.*, lipids and natural antioxidants, lipids and Maillard reaction products) [46]. It was shown that for short heat treatments, a reduction in the overall antioxidant properties was due to the loss of naturally occurring antioxidants and/or the formation of Maillard reaction products [47]. For all the compounds investigated, with one exception made to IPs, the statistically significant differences were found between raw and extruded materials but not between extrudates obtained at 120, 160, and 200°C. The losses of bioactive compounds during extrusion cooking of buckwheat groats were similar to those previously reported for other cereals (wheat, rye, barley, and oat) when the same conditions of extrusion cooking were applied [24, 31].Since extrusion cooking is a high temperature and short time process in which food material is cooked by a combination of moisture, pressure, temperature, and mechanical shear, the data provided in this study strongly support this observation.

4 Concluding remarks

Buckwheat products obtained after thermal treatment, in our case extrusion cooking process, still had significantly higher antioxidant capacity when compared to the extruded cereal grains and should be more widely recommended in human nutrition.

5 References

[1] Watanabe, M., *J. Agric. Food Chem.* 1998, *46*, 839 – 845.

[2] Dietrych-Szostak, D., Oleszek, W., *J. Agric. Food Chem.* 1999, *47*, 4384 – 4387.

[3] Horbowicz, M., Obendorf, R. F., *J. Agric. Food Chem.* 1992, *40*, 742 – 752.

[4] Obendorf, R. L., Steadman, K. J., Fuller, D. J., Horbowicz, M., Lewis, B. A., *Carbohyd. Res.* 2000, *328*, 623 – 627.

[5] Horbowicz, M., Brenac, P., Obendorf, R. L., *Planta* 1998, *205*, 1 – 11.

[6] Watanabe, K., Shimizu, M., Adachi, T., Mitsunaga, T., *J. Nutrit. Sci. Vitaminol.* 1999, *44*, 323 – 328.

[7] Li, S., Zhang, O. H., *Crit. Rev. Food Sci. Nutr.* 2001, *41*, 451 – 464.

[8] Edwardson, S., in: Janick, J. (Ed.), *Progress in New Crops*, ASHS Press, Alexandria, VA 1996, pp. 195 – 207.

[9] Przybylski, R., Lee, Y. C., Eskin, N. A. M., *JAOCS* 1998, *75*, 1595 – 1601.

[10] Holasaova, M., Fiedlerowa, V., Smrcinova, H., Orsak, M., *et al.*, *Food Res. Int.* 2002, *35*, 207 – 211.

[11] Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis*, 15th Edn., Washington, DC, USA 1990, *2*, p. 777.

[12] Zadernowki, R., *Acta Acad. Agricult. Techn. Olst.* 1987, *21(Suppl. F)*, 5 – 11.

[13] Shahidi, F., Naczk, M., in: Shahidi, F., Naczk, M. (Eds.), *Food Phenolic: Sources, Chemistry, Effects and Applications*, Technomic Publishing Company, Lancaster, Pennsylvania 1995, pp. 287 – 293.

[14] Peterson, D. M., Qureshi, A. A., *Cereal Chem.* 1993, *70*, 157 – 162.

[15] Mc Laughlin, P. J., Weihrauch, J. L., *J. Am. Dietet. Assoc*. 1979, *75*, 647 – 651.

[16] Sandberg, A. S., Ahderinne, R., *J. Food Sci.* 1986, *51*, 547 – 550.

[17] Sandberg, A. S., Carlsson, N. G., Svanberg, U., *J. Food Sci.* 1989, *54*, 159 – 161.

[18] Smith, I. K., Vierheller, T. L., Thorne, C. A., *Anal. Biochem.* 1988, *175*, 408 – 413.

[19] Hissin, J., Hilf, R., *Anal. Biochem.* 1976, *74*, 214 – 226.

[20] Fraser, S., Cowen, P., Franklin, M., Franey, C., Arendt, J., *Clin. Chem*. 1983, *29*, 396 – 397.

[21] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., *et al.*, *Free Rad. Biol. Med.* 1999, *26*, 1291 – 1237.

[22] Smith, P. K., Krohn, R. I., Hermanson, G. T., Macia, A. K., *et al.*, *Anal. Biochem.* 1985, *150*, 76 – 85.

[23] Meuser, F., Pfaller, W., Van Lengerich, B., *Extrusion Technology for the Food Industr.* Eelsevier, London 1987, pp. 35 – 53.

[24] Zielinski, H., Kozlowska, H., Lewczuk, B., *Inn. Food Sci. Emerg. Technol.* 2001, *2/3*, 159 – 169.

[25] Quettier-Deleu, C., Gressier, B.,Vasseur, J., Dine, T., *et al.*, *J. Ethnopharmacol*. 2000, *72*, 35 – 42.

[26] Dewanto, V., Wu, X., Adom, K. K., Liu, R. H., *J. Agric. Food Chem.* 2002, *50*, 3010 – 3014.

[27] Kreft, I., Fabjan, N., Yasumoto, K., *Food Chem.* 2006, *98*, 508 – 512.

[28] Sensoy, I., Rosen, R. T., Ho, Ch.-T., Karwe, M. V., *Food Chem*. 2006, *99*, 388 – 393.

[29] Sun, T., Ho, Ch.-T., *Food Chem.* 2005, *90*, 743 – 749.

[30] Holasowa, M., Velisek, J., Davidek, J., *Czech J. Food Sci.* 1995, *13*, 409 – 417.

[31] Honke, J., Zielinski, H., Troszynska, A., Kozlowska, H., *Czech J. Food Sci.* 2000, *18*, 251 – 253.

[32] Saastamoinen, M., Plaami, S., Kumpulainen, J., *Acta Agric. Scand., Sect. B, Soil Plant Sci.* 1992, *42*, 6 – 11.

[33] Lasztity, R., *Food Rev. Int.* 1998, *14*, 99 – 119.

[34] Li, W., Bollecker, S. S., Schofield, J. D., *J. Cereal Sci.* 2005, *39*, 205 – 212.

[35] Jones, D. P., Coates, R. J., Flagg, E. W., Eley, J. W., *et al.*, *Nutr. Cancer.* 1992, *17*, 57 – 75.

[36] Murch, S. J., Simmons, C. B., Saxena, P. K., *Lancet.* 1997, *350*, 1598.

[37] Hattori, A., Migitaka, H., Iigo, M., Itoh, M., *et al.*, *Biochem. Mol. Biol. Int.* 1995, *35*, 627 – 634.

[38] Poeggeler, B., Reiter, R. J., Hardeland, R., Sewerinek, E., *et al.*, *Neuroendocrinol. Lett.* 1995, *17*, 87 – 92.

[39] Livrea, M. A., Luisa, T., D'Arpa, D., Morreale, M., *Free Rad. Biol. Med*. 1997, *23*, 706 – 711.

[40] Zielinski, H., Kozlowska, H., *Pol. J. Food Nutr. Sci.* 2000, *9/50*, 85 – 90.

[41] Ghiselli, A., Serafini, M., Natella, F., Scaccini, C., *Free Rad. Biol. Med*. 2000, *29*, 1106 – 1114.

[42] Zielinski, H., Troszynska, A., *Pol. J. Food Nutr. Sci.* 2000, *9/50*, 79 – 83.

[43] Rice-Evans, C. A., Miller, N. J., *Meth. Enzymol.* 1994, *234*, 279 – 293.

[44] Rice-Evans, C. A., Miller, N. M., Paganda, G., *Free Radic. Biol. Med.* 1996, *20*, 933 – 956.

[45] Handelman, G. J., Cao, G., Walter, M. F., Nightingale, Z. D., *et al.*, *J. Agric. Food Chem.* 1999, *47*, 4888 – 4893.

[46] Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., Lerici, C. R., *Trends Food Sci. Tech.* 2001, *11*, 340 – 346.

[47] Nicoli, M. C., Anese, M., Parpinel, M., *Trends Food Sci. Tech.* 1999, *10*, 94 – 100.

