PAPER

# EFFECT OF SOLID-STATE FERMENTATION WITH *RHIZOPUS OLIGOSPORUS* ON BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF RAW AND ROASTED BUCKWHEAT GROATS

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ABSTRACT

The effect of solid-state fermentation with *Rhizopus oligosporus* on the changes in the total phenolic compounds, rutin, vitamin B and C, tocopherol, phytic acid and antioxidant capacity of raw and roasted buckwheat groats was studied. The roasted groats contained reduced level of studied bioactive compounds as compared to raw groats. In this study was evidenced that the solid-state fermentation with *Rhizopus oligosporus* enhanced water soluble vitamins (thiamine, pyridoxine and L-ascorbic acid) as well as tocopherols contents. In contrast the decrease of the inositol hexaphosphate, phenolic compounds, the rutin content and antioxidant capacity determined by ACL and ABTS methods was noticed.

- Keywords: antioxidant capacity, bioactive compounds, buckwheat groats, *Rhizopus oligosporus*, solid-state fermentation -

## INTRODUCTION

Globally, there is a growing interest in buckwheat products as healthy foods. The major producers of buckwheat are China, Russia, Ukraine and Kazakhstan, but it is also cultivated in Slovenia, Poland, Hungary, Brazil and Austria (BONAFACCIA *et al.*, 2003). Rutin (quercetin-3-rutinoside) is the main buckwheat flavonoid, which poses antioxidant, anti-inflammatory and anticarcinogenic properties. Buckwheat is also rich in other antioxidant compounds such as phenolic acids, tocopherols, reduced glutathione, inositol phosphates and melatonin (WIJNGAARD and ARENDT, 2006). This pseudocereal is characterised also in a high content of thiamine (vitamin B1) and riboflavin (vitamin B2), proteins with a well balance amino acid composition, including a high lysine content, phytosterols, soluble carbohydrates, D-chiro-inositol, fagopyritols and thiamin-binding proteins. Its fatty acid composition is superior to that of cereal grains, with typically 80% unsaturated fatty acids, including more than 40% of linoleic acid, an essential polyunsaturated fatty acid (STEADMAN *et al.*, 2001).

Raw and roasted buckwheat groats are particularly popular in Central and Eastern Europe. Roasted buckwheat groats are usually served like rice after cooking, while raw buckwheat groat or flour is used as a substitute for wheat flour in products for people with allergy to gluten and can be a valuable ingredient in diets or food products for coeliac patients (WRONKOWSKA *et al.*, 2010). Roasting affects the chemical composition and functional properties of buckwheat groats. The reduction of parent antioxidants as well as the formation of Maillard reaction products after roasting was observed (ZIELIŃSKA *et al.*, 2007a).

Tempeh, or "tempe", if we use authentic Indonesian spelling, a traditional product originating from Indonesia, is usually made from soybeans. The traditional tempeh process involves soaking and cooking, cooling and dehulling of the beans, followed by 20-30 hours of solid-state fermentation with *Rhizopus oligosporus*. Tempeh products have high protein contents of 40-50% and can be serve as tasty protein complements to starchy staples, and can substitute meat or fish (NOUT and ROMBOUTS, 1990). The solid-state fermentation with *Rhizopus oligosporus* has several beneficial effects, for example enhances antioxidant properties such as free-radical scavenging activity. HANDOYO *et al.* (2006) used fermentation with *R. oligosporus* to produce a fermented buckwheat flour that not only had a higher content of amino acids and minerals than non-fermented buckwheat samples, but also a lower content of allergic proteins. However, the production of tempeh-like products from buckwheat groats themselves has not been investigated. Therefore, in order to promote the utilization of raw or roasted buckwheat groats as well as to create a new type of healthy food, the effects of solid-state fermentation with *Rhizopus oligosporus* on the changes in the total phenolic compounds, rutin, vitamin B and C, tocopherol, phytic acid and antioxidant capacity of raw and roasted buckwheat groats was addressed in this study.

## MATERIALS AND METHODS

### Chemicals

Acetonitrile and methanol (HPLC-grade) were provided by Merck (Darmstad, Germany). Rutin (quercetin-3-rutinoside), L-ascorbic acid, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), thiamine, riboflavin, pyridoxine, Taka-diastase from *Aspergillus oryzae* (EC No 232-588-1) and inositol hexaphosphoric acid (dodecasodium salt) from corn were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). Other reagents of reagent-grade quality were from POCh, Gliwice, Poland. Water was purified with a Milli-Q-system (Milipore, Bedford, USA). All solutions prepared for HPLC were passed through a 0.45 μm nylon filter before use.

### Solid-state fermentation (SSF)

The raw (B) and roasted common (*Fagopyrum esculentum Moench*) buckwheat groats (RB) were purchased from a local healthy food shop (Olsztyn, Poland). The SSF was performed according to the modified method of HANDOYO *et al.* (2006). Briefly, 50 grams of raw or roasted buckwheat groats were cooked with 200 mL of deionized water for 5 min at 100°C. After cooling to room temperature, the excess of water was discarded. The drained samples were then inoculated with *Rhizopus oligosporus* NRRL 2710 (approx. $10^4$ spores/g, Northern Regional Research Laboratory, Peoria, Illinois, USA) and incubated at 37°C for 24 h. The fermented raw groats (SSF-B) and fermented roasted groats (SSF-RB), as well as unfermented materials, were lyophilized using a Labconco Co. (Kansas City, Missouri, USA) laboratory freeze-dryer at a pressure of 13 Pa at -60°C for 24 h.

### Sample preparation for measurement of rutin, total phenolic compounds and antioxidant capacity

The lyophilized buckwheat samples were extracted in triplicate with 80% aqueous methanol (1/10; w/v) for 2 h, with shaking at 37°C (1400rpm, Comfort R, Eppendorf, Germany). Samples were then centrifuged at 2600xg at 4°C for 15 min in a Beckman GS-15R centri-

fuge (Beckman Instruments, Inc., Palo Alto, CL, USA). The samples were preserved at -20°C prior to further analysis.

### Preparation of hydrophilic and lipophilic extracts for the measurement of antioxidant capacity by photo-induced chemiluminescence (PCL) assay

For the hydrophilic extract, about 100 mg of lyophilized buckwheat samples were extracted for 3 min with 1 mL of HPLC-grade water using a Genie-2 type vortex (Scientific Industries, USA). Next, samples were centrifuged for 5 min at 4°C, at 16100xg (5415 R, Eppendorf, Germany) and the fresh supernatants were directly used to determine the antioxidant capacity formed by water-soluble antioxidants (ACW). For the lipophilic extracts, about 100 mg of lyophilized buckwheat samples were extracted for 3 min with a mixture of 200 μL of n-hexane and 800 μL of methanol using a Genie-2 type vortex (Scientific Industries, USA). Next, samples were centrifuged for 5 min at 4°C, at 16100xg (5415 R, Eppendorf, Germany) and the fresh supernatants were directly used to determine the antioxidant capacity formed by lipid-soluble antioxidants (ACL).

### Determination of total phenolic compounds

The content of total phenolic compounds (TPC) was determined according to SHAHIDI and NACZK (1995). Buckwheat extracts (0.25 mL) were mixed with 0.25 mL of Folin-Ciocalteu reagent (previously diluted with water, 1:1 v/v) and 0.5 mL of $Na_2CO_3$ solution, and 4 mL of water. The mixture was allowed to stand at room temperature for 25 min and then centrifuged at 2000xg for 10 min. The absorbance of the supernatant was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The data were calculated as rutin equivalents.

### Determination of rutin content by HPLC

Rutin content was analyzed in a HPLC system (Shimadzu, Kyoto, Japan) comprising two pumps (LC-10 AD), a UV detector (SPD-10A) set at 330 nm, an autosampler set for 5 μL injection (SIL-10 ADVP), a column oven (CTO-10 ASVP), and a system controller (SIL-10 ADVP) according to method described by ZIELIŃSKA *et al.* (2010).

### Determination of thiamine (B1) and riboflavin (B2) by HPLC

To determine the content of thiamine and riboflavin, the modified method of PRODANOV *et al.* (1997) was used. The 500 mg of lyophilized buckwheat were extracted by acid hydrolysis with 15 mL of 0.3 M HCl in autoclave (15 min at 120°C) and, after cooling, the pH was adjusted to 5-5.4 using 4 M sodium acetate. Then 5 mL of an aqueous solution of Taka-Diastase enzyme (100 U/mg) was added to the samples and incubated for 3 h at 45°C, in a water bath with shaking (120rpm, HS-B20, IKA Labortechnik, Germany). After enzymatic hydrolysis, the extracts were filtered by Whatman No. 40 filters and water was added to complete the volume to 25 mL. The samples were preserved at -20°C prior to HPLC analysis.

### Determination of pyridoxine (B6) and L-ascorbic acid by HPLC

Analysis of pyridoxine and L-ascorbic acid content was made by the modified method of ESTEVE *et al.* (1997). Approximately 100 mg of lyophilized buckwheat samples were added to 1 mL of 5% aqueous solution of metaphosphoric acid. The samples were extracted in triplicate, and then centrifuged at 12000xg for 10 min at 4°C (GS-15 R Beckman Instruments, Inc., Palo Alto, CL, USA). The supernatant were mixed with 100 μL of dithiotreitol (DTT), incubated for 1 h (without light) and then centrifuged at 13000xg for 5 min at 4°C. The samples were preserved at -20°C prior to further HPLC analysis.

### Determination of tocopherol content

Tocopherol (α-T, β-T, γ-T, δ-T) was extracted with methanol (0.5 g of sample/7 mL). After evaporation, extracts were redissolved in *n*-hexane. The HPLC analysis was run with a Shimadzu system (LiChrospher® Si-60, 5-μm particle size, 4 x 250-mm column) according to the method described by PEGG and AMAROWICZ (2009). The tocopherols contents were calculated from the peak areas using standard curves of tocopherols.

### Determination of inositol hexaphosphates and its lower forms

The analysis of the content of tri-, tetra-, penta-, and hexaphosphate inositols was made according to the method of HONKE *et al.* (1999). Inositol hexaphosphate was determined as follows: exactly 0.5 g of the buckwheat samples were extracted with 20 mL 0.5 M HCl for 5 h using a BM1 magnetic stirrer (IKA, Staufen, Germany). The extract was centrifuged at 3500xg for 40 min (Centrifuge MPW-360, Factory of Precise Mechanics, Warsaw, Poland) and the supernatants were decanted, frozen overnight (-20°C), thawed at room temperature and recentrifuged at 3500xg for 40 min. The supernatants (15 mL) were evaporated under reduced pressure to dryness at 40°C and dissolved in 15 mL of 0.025 M HCl. The samples were transferred to minicolumns filled with Dowex AG 1-X8 resin, from which the inositol phosphates were eluted using 2 M HCl (5 x 4 mL). After removal of the sol-

vent by evaporation with an air stream, the dry residue was dissolved in a mobile phase. Then the samples were analysed by HPLC. The inositol hexaphosphates contents were calculated from the peak areas using standard curves of inositol hexaphosphates.

### Measurement of the antioxidant capacity of buckwheat products against $ABTS^{+\bullet}$ and $O_2^{-\bullet}$

The antioxidant capacities of the 80% aqueous methanol extracts from lyophilized buckwheat samples were determined against $ABTS^{+\bullet}$ radical cation using a spectrophotometric assay. The $ABTS^{+\bullet}$ radical cation was prepared by mixing $ABTS^{+\bullet}$ stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture remained for 12-24 h until the reaction was complete and the absorbance was stable. Antioxidant capacity was determined following the procedure described by RE *et al.* (1999) with a minor modification. The $ABTS^{+\bullet}$ solution was diluted with 80% methanol to an 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 buckwheat extracts or Trolox standards were mixed and measured immediately and again after 6 min at 30°C and 734 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Kyoto, Japan). Appropriate solvent blanks were run in each assay. The antioxidant capacity was calculated on the basis of percentage inhibition of absorbance at 734 nm using a Trolox standard curve and was expressed as µmol Trolox/g of dry matter (d.m.).

The Photo-Induced Chemiluminescence Assay (PCL), carried out according to the method of POPOV and LEWIN (1999), was used to measure the antioxidant capacity of unfermented and fermented groat extracts against superoxide anion radicals ($O_2^{-\bullet}$), which were generated from luminol, a photosensitizer, under exposure to UV light. The antioxidant capacity of buckwheat water or methanol extracts were determined using ACW (antioxidative capacity in water-soluble substances) and ACL (antioxidative capacity in lipid-soluble substances) kits (Analytik Jena, Leipzig, Germany) reported in details by ZIELIŃSKA *et al.* (2010). Antioxidant capacity was expressed in terms of µmol Trolox/g d.m.

### Statistical analysis

The measurement were performed in triplicate for each of two independent fermentation batches. The data are the mean results with the standard deviation. The effects of the two parameters, type of product (P) and fermentation process (F) or their interactions (P x F) were tested using a two-way ANOVA (Statistica, ver. 7.1, USA). Fisher's Least Significant Difference Test at a significance level of $p<0.05$ was performed for *post-hoc* comparison.

## RESULTS AND DISCUSSION

### Total phenolic compounds (TPC) and rutin (Ru) contents

Table 1 shows total phenolic compounds (TPC) and rutin (Ru) contents of unfermented and fermented raw and roasted buckwheat groats. Raw buckwheat groats (7.2 mg Ru equiv/g d.m.) was almost two times richer in phenolic compounds

Table 1 - The phenolic, vitamin B1, B2, B6, C and α-, γ- and δ-tocopherol contents of SSF raw and roasted buckwheat groats.

| | B | SSF-B | RB | SSF-RB | ANOVA | | |
|---|---|---|---|---|---|---|---|
| | | | | | P | F | P x F |
| **The phenolic contents** | | | | | | | |
| rutin [µg/g d.m.] | 205.84±0.94a | 164.54±24.59b | 158.84±3.42b | 142.09±1.81b | ns | *** | *** |
| total phenolic compounds [mg Ru equiv/g d.m.] | 7.19±0.84a | 5.15±0.23b | 3.54±0.02c | 3.41±0.16c | *** | *** | *** |
| **Content of vitamin B1, B2, B6 and C** | | | | | | | |
| thiamine (B1) [µg/g d.m.] | 9.70±0.29b | 10.91±0.50a | 5.41±0.13d | 7.56±0.29c | *** | *** | *** |
| riboflavin (B2) [µg/g d.m.] | 1.65±0.12a | 1.70±0.08a | 1.29±0.09b | 1.18±0.01c | *** | *** | *** |
| pyridoxin (B6) [mg/g d.m.] | 0.12±0.01b | 0.33±0.01a | 0.11±0.02b | 0.12±0.01b | *** | *** | *** |
| L-ascorbic acid [mg/g d.m.] | 0.05±0.01c | 0.11±0.02a | 0.05±0.01c | 0.09±0.01b | *** | *** | *** |
| **Content of α-, γ- and δ-tocopherol** | | | | | | | |
| α-tocopherol | 0.73±0.06b | 1.10±0.12a | 0.27±0.17c | 0.64±0.07b | *** | *** | *** |
| γ-tocopherol | 106.20±1.97b | 126.88±3.18a | 60.40±0.59d | 95.08±1.01c | *** | *** | *** |
| δ-tocopherol | 2.93±0.30b | 3.45±0.28a | 3.78±0.32a | 2.10±0.07c | ns | *** | *** |

Control buckwheat groats: raw (B) and roasted (RB); fermented buckwheat groats: raw (SSF-B) and roasted (SSF-RB). Data expressed as mean±standard deviation (n=6). Different letters within the same line indicate statistically significant differences at $p<0.05$ in NIR Fisher test (for interactions PxF).
*** significant effects by kind of products (P), fermentation process (F) or their interactions (PxF) at $p<0.05$; ns, not significant.

than the roasted groat (3.5 mg Ru equiv/g d.m.). Our results confirms results of ZIELIŃSKA *et al.* (2007b).

SSF led to a statistically significant decrease in the rutin content of raw groats (by 20%) but did not affect the rutin content of roasted groats. Performed two-way ANOVA analysis showed that the content of rutin was closely associated with the used SSF process.

It is well known that buckwheat groats contain mainly rutin and a little amounts of isovitexin, depending on the cultivar and growth conditions (WIJNGAARD and ARENDT, 2006). Isovitexin was not detected in our buckwheat samples. The rutin content of raw buckwheat groats was 205.8 μg/g d.m. and 23% lower ($p<0.05$) for roasted groats. It is connected with the hydro-thermal processes used for roasting. DIETRICH-SZOSTAK and OLESZEK (1999) found that rutin content in buckwheat groat was affected by temperature and heating time adversely. WANG *et al.* (2011) found the reduction of rutin content after the fermentation of peanut flour with the different strains of lactic acid bacteria.

SSF led to a statistically significant decrease in TPC for raw groats (by 28%), but did not affect the TPC of SSF roasted groats. DORDEVIC *et al.* (2010) showed that the fermentation of buckwheat by *S. cerevisiae* and *L. rhamnosus* caused the increasing of TPC compared to non-fermented samples. DUEÑAS *et al.* (2012) found significant increase of phenolic acid content in the soybean fermented with different microorganisms (*Aspergillus oryzae, Rhizopus oryzae* and *Bacillus subtilis*).

**Vitamins B and C contents**

The contents of vitamins B1, B2, B6 and C before and after fermentation of raw and roasted buckwheat groats are presented in Table 1. The raw and roasted groats used for fermentation had different thiamine and riboflavin contents, but the levels of pyridoxine and L-ascorbic acid were similar. The level of vitamin C in whole buckwheat flour ranges from 3.9 to 7.3 mg/l00 g, our results are similar to those presented by other authors (WIJNGAARD and ARENDT, 2006). The concentrations of B1 and B2 in the raw groats were 80 and 28%, higher, respectively, than those of roasted groats. This finding is in accordance with reports regarding the presence of vitamins B in buckwheat (WIJNGAARD and ARENDT, 2006).

The SSF of raw groats caused a statistically significant increase of thiamine, pyridoxine and L-ascorbic acid ($p<0.05$) compared to the control samples. The pyridoxine content increased almost three-fold, the L-ascorbic acid content more than two-fold, whereas the thiamine content by 12%. Fermentation did not change the riboflavin content of raw groats. SSF of roasted groats caused the increasing of thiamine content by 40%, the L-ascorbic acid content almost doubled, the pyridoxine contents did not change and riboflavin content decreased.

The increase of the contents of some B-group vitamins obtained in this study is similar to those of previous reported by other authors. The increases in thiamine content that we obtained after fermentation of the raw and roasted buckwheat groats are in contrast to results reported for SSF of other substrates. NOUT and ROMBOUTS (1990) found that tempeh fermentation of soya caused the increase of content of vitamins, except for thiamine for which decrease of the content was observed. FADAHUNSI (2009) showed the effect of *Rhizopus oligosporus* on the vitamin content in the flour obtained from bambara nut. After the 24 h fermentation this author found the significant reduction in the thiamine content, while riboflavin, folacin, niacin and biotin content increased significantly. In traditional Turkish fermented wheat-flour-yoghurt mixture (tarhana) EKINCI (2005) found no significant differences in thiamine and pyridoxine contents with the increase of fermentation period. While, he observed significant increases of riboflavin, niacin, pantothenic acid, ascorbic acid and folic acid during the fermentation.

**Tocopherol content**

Table 1 shows the tocopherol content in raw and roasted buckwheat groats before and after fermentation. In buckwheat, γ-tocopherol is the major tocopherol homologue and the β-form is present in only trace amounts. In our study α-T, γ-T, δ-T were found in the raw and fermented buckwheat materials, whilst β-T was not detected. The raw groats had almost three- and two-fold higher contents of α-T and γ-T, respectively, compared to the roasted groats. GEMROT *et al.* (2006) found for gourd seeds the decrease of tocopherol content under the influence of roasting process. In the literature data there are no information concerning the tocopherol level in fermented buckwheat groats.

The SSF of raw and roasted groats caused a statistically significant increase of the α-T and γ-T. The content of δ-tocopherol was significantly associated with the used fermentation process, but not with the type of the product. In contrast to results obtained in this study the literature data showed the decrease of tocopherols content under the influence of fermentation. For soya fermented with *A. oryzae* decreased of α-T content was observed by ESAKI *et al.* (1994), but they do not observed a modification of tocopherol content for soya fermented by *B. subtilis* or *R. oligosporus*. DENTER *et al.* (1998) found that in soybean tempeh the tocopherols content slightly decreased as a consequence of fermentation with 14 varieties of *Rhizopus* studied.

### Contents of inositol hexaphosphate and its lower forms

Table 2 shows the contents of inositol hexaphosphate (IP-6) and its lower forms (IP-5, IP-4 and IP-3) in raw and roasted buckwheat groasts before and after fermentation. Phytic acid may be classified as a prohealthy or antinutritional compound, depending on its action. It can form an iron chelate that inhibits iron-mediated oxidative reactions and limits site specific DNA damage. Phytic acid inhibits tumor growth by suppressing the formation of damaging hydroxide free radicals and other reactive oxygen species (VUCENIK and SHAMSUDDINY, 2003).

The raw groats contained 18.3 mg/g d.m. of IP-6. After roasting, a decrease by 43% was noted. This finding was in accordance with the previously reported by other authors (ZIELIŃSKI *et al.*, 2006). In our study, IP-5, IP-4 or IP-3 were not found in raw groats, but they were detected after roasting.

After 24 h fermentation with *Rhizopus oligosporus*, the formation of IP-3, IP-4, and IP-5 was noted in fermented raw groats. Moreover, SSF caused the significant increase of IP-3 and IP-4 in fermented roasted groats (Table 2). These findings were related to the three- and two-fold decreased content of IP-6 in fermented raw and roasted groats. Fermentation, steaming and extrusion cooking were identified as processes causing the degradation of IP-6 to the lower forms (ZIELIŃSKI *et al.*, 2006). EGOUNLETY and AWORH (2003) showed that fermentation with *R. oligosporus* reduced the phytic content by 30.7% in soybean, 32.6% in cowpea and 29.1% in ground bean.

### Antioxidant capacity

In this study, the antioxidant capacity of raw and roasted groats before and after fermentation was measured against the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{+\bullet}$) and by the photo-induced chemiluminescence assay (PCL) against the superoxide anion radical ($O_2^{-\bullet}$).

The PCL method can be conducted by two different protocols, ACW and ACL, which allowed for measurement of antioxidant capacity of the water- and lipid-soluble components, respectively. Finally, it was possible to calculate the total antoxidant capacity as a sum of ACW and ACL values. The results are compiled in Table 3. The antioxidant capacity of raw groats against $ABTS^{+\bullet}$ and $O_2^{-\bullet}$, expressed as ACW+ACL, was 34 and 20% higher compared to the roasted groats. It should be also noted that lipophilic antioxidants (ACL) were the main contributor (up to 80%) to the total antioxidant capacity of raw and roasted groats. ZIELIŃSKA *et al.* (2007a) observed decrease by 27% of ABTS value for buckwheat groats after using roasting process. Also ZHANG *et al.* (2010) found that the scavenging activity of tartary buckwheat flour against $O_2^{-\bullet}$ was reduced by roasting.

The SSF of raw and roasted groats caused statistically significant decreases ($p<0.05$), by 32 and 15%, respectively, of antioxidant capacity measured against $ABTS^{+\bullet}$. Also decrease of total antioxidant capacity evaluated by PCL (ACW+ACL) was noticed under the influence of SSF. Similar finding were presented by BERGHOFER *et al.* (1998) for the fermented product obtained from faba bean, soybean and oat. In our study lipophilic antioxidants were significantly reduced after fermentation of raw and roasted groats (by 35 and 13%). On the other hand, these lipophilic antioxidants highly contributed, up to 75%, to the total antioxidant capacity of the both fermented buckwheat products.

The observed decrease in antioxidant capacity of fermented raw and roasted groats could be connected with the increasing of water soluble

Table 2 - Content of inositol phosphates of SSF buckwheat groats.

| Sample | Inositol phosphates [mg/g d.m.] | | | |
|---|---|---|---|---|
| | IP-3 | IP-4 | IP-5 | IP-6 |
| B | nd | nd | nd | 18.33±0.24a |
| SSF-B | 0.33±0.04b | 1.65±0.15b | 2.01±0.18b | 5.65±0.19c |
| RB | 0.29±0.01b | 2.22±0.07a | 3.80±0.08a | 10.51±0.07b |
| SSF-RB | 0.44±0.05a | 1.86±0.18b | 3.62±0.09a | 5.66±0.10c |
| **ANOVA effects** | | | | |
| P | *** | *** | *** | *** |
| F | *** | *** | *** | *** |
| P x F | *** | *** | *** | *** |

Control buckwheat groats: raw (B) and roasted (RB); fermented buckwheat groats: raw (SSF-B) and roasted (SSF-RB). Data expressed as mean±standard deviation (n=6). Different letters within the same column indicate statistically significant differences at $p<0.05$ in NIR Fisher test (for interactions PxF).
*** significant effects by kind of products (P), fermentation process (F) or their interactions (PxF) at $p<0.05$; ns, not significant; nd, non detected.

Table 3 - The antioxidant capacity of SSF buckwheat groats provided by ABTS and PCL assay.

| Sample | Antioxidant capacity (μmol Trolox/g d.m.) | | | |
|---|---|---|---|---|
| | against $ABTS^{+\bullet}$ | against $O_2^{-\bullet}$ | | |
| | | ACW | ACL | ACW + ACL |
| B | 22.93±1.24a | 3.97±0.59b | 17.32±0.59a | 21.29±0.11 |
| SSF-B | 15.68±0.94b | 4.57±0.59a | 11.18±0.59d | 15.75±0.12 |
| RB | 15.15±2.34b | 3.76±0.59c | 13.19±0.59b | 16.95±0.74 |
| SSF-RB | 12.82±0.51c | 3.84±0.59c | 11.50±0.59c | 15.34±0.20 |
| **ANOVA effects** | | | | |
| P | *** | *** | *** | *** |
| F | *** | *** | *** | *** |
| P x F | *** | *** | *** | *** |

Control buckwheat groats: raw (B) and roasted (RB); fermented buckwheat groats: raw (SSF-B) and roasted (SSF-RB). Data expressed as mean±standard deviation (n=6). Different letters within the same column indicate statistically significant differences at p<0.05 in NIR Fisher test (for interactions PxF).
*** significant effects by kind of products (P), fermentation process (F) or their interactions (PxF) at p<0.05; ns, not significant.

antioxidants (e.g. vitamins B, L-ascorbic acid) and lipid-soluble antioxidant (e.g. increased tocoperols level) and decreasing of the phenolic compounds, including rutin. In our study, the total content of vitamins B was positively correlated with the antioxidant capacity of buckwheat groats before and after fermentation, when evaluated by ABTS test (r = 0.43) and by ACW assay (r = 0.86). The level of ascorbic acid was also positively correlated with antioxidant capacity, as measured by ACW assay (r = 0.74). Moreover, a very high correlation was calculated between rutin contents and antioxidant capacity, as determined by the ABTS test (r = 0.99). The same observation was made in relation to TPC contents and values provided by ABTS. Phenolic compounds, including rutin, could be extracted by medium used for the ACL assay since a high correlation was also noted between Ru and TPC vs ACL (r = 0.90 and r = 0.77, respectively). In our study, no correlation existed between total tocopherols and ACL values, what could indicate that this group of compounds has no impact on the formation of antioxidant capacity of non-fermented and fermented buckwheat groats, both raw and roasted. Therefore, the antioxidant capacity of fermented groats clearly depended on the antioxidant activity of vitamins B, vitamin C and rutin. It is a known that vitamins B have little or no antioxidant activity (GLISZCZYŃSKA-ŚWIGŁO, 2006). On the other hand, the antioxidant activity of rutin provided by ABTS and PCL ACL assay (1.16±0.02 and 1.38±0.07 mmol Trolox, respectively) as reported by ZIELIŃSKA *et al.* (2010), was higher than antioxidant activity of L-ascorbic acid (1.05±0.02 mmol Trolox by ABTS and 1.0 mmol Trolox in PLC ACW) (RE *et al.*, 1999). Therefore, the noted decrease of the antioxidant capacity evaluated by ABTS and PCL assay in SSF raw and roasted groats was mainly related to the decreased rutin content and could not be ameliorated by increased content of both vitamins B and C.

### General remarks

Two-way ANOVA used for statistical analysis of the obtained data indicated that both analysed parameters: type of product (raw and roasted), fermentation process (fermented and unfermented) and their interactions significantly influenced on the obtained results. Only in the case of rutin and δ-tocopherol content, the type of product had not significant effect. Also principal component analysis (PCA) was performed on the covariance matrix of the samples with no rotation (data not showed). Two principal components were extracted (PC1 and PC2) and together explained 83.66% of the total variance. The PC1 was differentiated by almost all investigated compounds except rutin, IP-5, IP-6, δ-tocopherol and antioxidant capacity determined by ACL and ABTS methods.

## CONCLUSIONS

Solid-state fermentation with *Rhizopus oligosporus* was used to obtain tempeh-type products from raw and roasted buckwheat groats. The used SSF enhanced water soluble vitamins (thiamine, pyridoxine and L-ascorbic acid), as well as and α-, δ- and γ-tocopherol contents. After fermentation, a decrease in total phenolic compounds as well as rutin contents was observed. These changes had an impact on $ABTS^{\bullet+}$ radical cation and superoxide anion radical ($O_2^{-\bullet}$) scavenging activity of fermented raw and roasted buckwheat groats. Based on the correlation studies and knowledge on the antioxidant activ-

ity of analysed bioactive components it should be noted that rutin content was the main factor responsible for the antioxidant capacity of fermented products. On the other hand, the fermented products were rich source of vitamins B and C and therefore SSF with *Rhizopus oligosporus* can be recommended for production of tempeh-like functional buckwheat-based foods with reduced antinutritional factor.

ACKNOWLEDGMENTS

This research was supported by statutory found from the Department of Chemistry and Biodynamics of Food of the Institute of Animal Reproduction of the Polish Academy of Sciences in Olsztyn, Poland. The authors thank PhD Agnieszka Ornatowska for technical help in the analysis of bioactive compounds. The Authors declares that they have no conflict of interest.

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Paper Received July 16, 2014 Accepted December 1,2014