THE INFLUENCE OF COOKING ON THE ANTIOXIDANT PROPERTIES AND POLYPHENOL CONTENT IN BUCKWHEAT, BARLEY AND MILLET GROATS AND THE TRANSFER OF THE COMPOUNDS TO THE WATER

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ABSTRACT
The research subject was the influence of the cooking process on the polyphenol content and antioxidant properties of groats (buckwheat groats, barley groats, millet groats) and on the colour parameters of the products. After groats cooking, also the water was tested for the polyphenol content and antioxidant properties that permeated into the decoction of the cooked raw material. The evaluation of the antioxidant properties of groats was conducted with the DPPH radical assay, the polyphenol content was determined with the Folin-Ciocalteu reagent, and the colour was determined with the trichromatic colorimetry method using the Konica Minolta CM-5 colorimeter (Osaka, Japan). The cooking process significantly lowered the content of polyphenolic compounds and antioxidant properties of the ready products. The best antioxidant properties after cooking were found in buckwheat groats and the weakest in millet groats. The polyphenol content in cooked products depended on the type of groats. Cooking significantly decreased the polyphenol content, but only in buckwheat and barley groats, as well as causing a change of groats colour as compared to uncooked samples. The most antioxidant properties were found in the water from cooking barley groats, and the least – from cooking millet groats. The most polyphenols permeated into the water from cooking buckwheat groats, and the least from cooking millet groats. All groats, except millet groats, darkened after cooking. In all types of groats, the correlation coefficients between colour parameter and general polyphenol content and the ability to scavenge DPPH radicals showed very strong negative dependences. The conducted research can help in designing the technological process of cooking buckwheat, barley and millet groats, and a way of using the water from cooked groats for consumption.

Keywords: buckwheat groats; barley groats; millet groats; antioxidant properties; cooking; polyphenol

INTRODUCTION
Due to their health properties, barley, buckwheat and millet groats are a vital element of human diet (Subramanian and Viswanathan, 2007; Sedej et al., 2010; Paszkiewicz et al., 2012; Liu et al., 2012; Danihelová and Šturdík, 2013; Hęś et al., 2014; Wronko et al., 2015; Mateo-Gallego et al., 2017; Kerienė et al., 2015; Hung, 2016; Skotnicka Ociezeck and Małgorzewicz, 2018; Kuznetsova et al., 2018). They contain a number of valuable nutrients, such as: high-value protein, fats, mineral salts, and vitamins (Ragaee, Abdel-Aal and Noaman, 2006; Singh, Mishra and Mishra, 2012; Aoe et al., 2017; Mateo-Gallego et al., 2017). Groats have a variety of applications in human nutrition due to the availability of different preparation techniques. They can be consumed with meats, fish, vegetables, as well as sweet desserts, warm or cold. They are recommended in human nutrition, especially to persons suffering from acute gastroenteritis, convalescents with strict diet plans, and children (Liu et al., 2012; Subramanian and Viswanathan, 2007; Ragaee, Abdel-Aal and Noaman, 2006; Kalinova and Moudry, 2006; Skotnicka Ociezeck and Małgorzewicz, 2018). According to Görecka et al. (2009) and Huth et al. (2000), the fibre contained in these products is capable of binding water and bile acids, absorbing metals, encourages bowel movement, lowers glucose and cholesterol levels in blood, increases fecal weight. Groats are also the source of bioactive ingredients, including polyphenols. Such compounds include: polyphenols (phenolic acids and a large group of flavonoids with anthocyanins), vitamins A and C, tocoferols, carotenoids, as well as organic acids, macro- and micro-elements, including selenium, chlorophyllins, glutathione, indoles, phytates and thiocyanates. Antioxidant properties are also found in the products of transformations in technological processes (Singh, Mishra and Mishra, 2012; Skotnicka Ociezeck...
and Małgorzewicz, 2018). According to Hollman, Geelen and Kromhout (2010); Karamać (2010) such compounds can improve health, facilitating mainly the treatment and prevention of cardiovascular diseases. According to Marchand (2002); Mateo-Gallego et al. (2017) and Skotnicka Ocieczek and Małgorzewicz, (2018) polyphenols also play an important role in preventing cancer and neurodegenerative diseases, and the content of those compounds in food improves the antioxidant potential of food, which is an additional protection of the organism against the activity of an excess of free radicals accumulated due to oxidative stress. However, when prepared for consumption, groats vary significantly in terms of the content of these ingredients. One of the ways of preparing groats for consumption is cooking. It is generally believed that cooking can have a positive effect on the polyphenol content and antioxidant activity, but some authors have a different opinion and argue that during the cooking process, some of the ingredients transfer to the stock, decreasing the nutritional value of groats, whereas the water from cooked groats is usually disposed of into the drain (Ismail, Marjan and Foong, 2004; Jeong et al., 2004; Jiratman and Liu, 2004; Puupponnen-Pimia et al., 2003; Sharma, Gujral and Singh, 2012; Hęś et al., 2014; Kerienė et al., 2015). Cooking also causes a change of colour, which is often a quality that determines consumer desirability of the product. Considering the aforementioned factors affecting the quality of cooked groats, research was conducted in order to determine the influence of the cooking process on the polyphenol content and antioxidant properties of groats, as well as their colour parameters. Moreover, the polyphenol content and antioxidant properties were tested in the water from cooked groats.

Scientific hypothesis

The aim of this study was to ascertain that the cooking process reduces the polyphenol content and antioxidant properties in buckwheat, barley and millet groats, and that it results in a colour change of cooked groats. Furthermore, the aim of the study was to determine the amount of polyphenols and antioxidant properties that transfer to the water when cooking buckwheat, barley and millet groats, and whether there is any correlation between the colour of the groats and the general polyphenol content and antioxidant properties.

MATERIAL AND METHODOLOGY

Materials

The research material included three types of groats: barley, buckwheat and millet purchased in 2017 at one of the supermarket chains in Krosno, Poland (49°49’ N, 21°46’ E).

Preparation of sample extracts

Groats samples were ground in a grinder, then 1 g of each sample was extracted in 10 mL of 70% ethanol for 15 minutes on the Model Sonic 6 (Polsonic, Poland) ultrasonic bath. Following the extraction, the samples were filtrated, and the obtained filtrate was used to determine the polyphenol content and antioxidant properties of groats (Sánchez-Rangel et al., 2013; Brand-Williams, Cuvelier and Berset, 1995). The water from cooking was tested for antioxidant properties and polyphenol content (Kerienė et al., 2015; Hęś et al., 2014).

DPPH radical scavenging assay

The antioxidant activity of the extracts was determined with the DPPH radical assay (AOAC International, 2009), and the results were expressed in the percentage of DPPH radical scavenging. For this purpose, the extracts were diluted to 1:1, and then, a DPPH ethanolic solution was prepared with a concentration of 0.05 mg.mL⁻¹, whose absorbance prior to analysis was 1.5. Then, 500 μL of the diluted sample was combined with 500 μL of the DPPH in three replicates. The samples were vortexed and left in a dark place for 30 minutes at room temperature. The control sample contained 500 μL of 70% ethanol and 500 μL of the DPPH solution. After that time, the absorbance of the samples was measured in relation to ethanol at a wave length of 515 nm. The results were expressed as the percentage of antioxidant activity.

%AA = ((Ak – Ap/Ak))∗100%


The antioxidant activity of the tested groats was determined with the use of the Jenway 6850 UV/VIS spectrophotometer (Jenway, England).

Determination of total polyphenol content

The total polyphenol content in the groats extracts was determined with the use of the Folin–Ciocalteu reagent (Aanchez-Rangel et al., 2013). For this purpose, calibration solution of gallic acid in 70% ethanol was prepared with specific concentrations (0.45 – 0.01 mg.mL⁻¹) in order to determine the calibration curve. Then, 20 μL of each solution of the standard of gallic acid and the tested extract were sampled in three replicates, 1.58 mL of distilled water and then 100 μL of the Folin-Denis reagent were added to each of them. The samples were mixed and after 1 minute, 300 μL of a 20% sodium carbonate solution was added to each; the samples were vortexed and placed in a thermostat set at 40 °C for 30 minutes. Afterwards, the absorbance of the extracts and standards was measured at a wave length of 765 nm against the reference sample (instead of 20 μL of the extract/standard, 20 μL of 70% ethanol was added). The polyphenol content in the extracts was determined on the basis of the calibration curve for gallic acid [mg GAE.mL⁻¹]: y = 1.0425x + 0.1277, R² = 0.888. Analyses were carried out in three replicates.

Polyphenol determination in a water sample after cooking

Groats were cooked according to the manufacturer’s instructions on the packagings. The polyphenol content in the water from the cooked groats (buckwheat, barley, millet) was determined with the spectrophotometric method with the use of the Folin–Ciocalteu reagent, by adding to 100 μL of the water extract from cooked groats: 7.9 mL of distilled water, 0.5 mL of the Folin–Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate. The samples were thoroughly mixed and incubated at room...
The total polyphenol content in the water after cooking different groat types (mg 100g⁻¹ DM of groats).

<table>
<thead>
<tr>
<th>Groat type</th>
<th>Type of groat treatment</th>
<th>Mean</th>
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<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>127.04 ±15.28</td>
<td>59.58</td>
</tr>
<tr>
<td>Millet</td>
<td>0.37 ±0.56</td>
<td>0.01</td>
</tr>
<tr>
<td>Barley</td>
<td>109.80 ±7.04</td>
<td>26.98</td>
</tr>
<tr>
<td>Mean</td>
<td>79.07</td>
<td>28.86</td>
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HSDₐ₀.₀₅ Kind of groats (R) – 3.29; type of groats processing (F) – 2.19; interaction R x F – 6.58

<table>
<thead>
<tr>
<th>Groat type</th>
<th>Type of groat treatment</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Water</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>127.04 ±15.28</td>
<td>28.93 ±0.42</td>
</tr>
<tr>
<td>Millet</td>
<td>0.37 ±0.56</td>
<td>1.75 ±0.64</td>
</tr>
<tr>
<td>Barley</td>
<td>109.80 ±7.04</td>
<td>4.49 ±0.26</td>
</tr>
<tr>
<td>Mean</td>
<td>79.07</td>
<td>11.73</td>
</tr>
</tbody>
</table>

HSDₐ₀.₀₅ Kind of groats (K) – 2.50; groats form (F) – 1.66; interaction K x F – 4.98

determined by a water sample after cooking

The ability of water extracts from cooked groats to scavenge free radicals was determined with the spectrophotometric method using a stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). For this purpose, the tested extracts were diluted as mg of the equivalent of gallic acid in 100 mL of water from cooked groats based on the calibration curve for gallic acid, with the following formula: y = 1.1799x + 0.0847, where R² = 0.9996. The concentration range of gallic acid in 100 mL of water from cooked groats and 2 mL of ethanol, then 0.25 mL of DPPH ethanolic solution with a concentration of 1 mM was added to them, and they were incubated in darkness at room temperature for 30 minutes. After that time, the absorbance of the samples was measured for ethanol at a wave length of 517 nm and % AA was calculated in relation to the absorbance of the control sample, which contained ethanol instead of water extract (Hēš et al., 2014). The antioxidant activity of the tested groats was determined with the use of the Jenway 6850 UV/VIS spectrophotometer.

%AA = ((A0 – Ap)/ A0)*100%
A0 – absorbance of the control sample
Ap – absorbance of the tested samples

Colour determination

The colour of the samples of raw and cooked groats was determined with the trichromatic colorimetry method using the Konica Minolta CM-5 colorimeter (Osaka, Japan). The colour was expressed in the CIE L*a*b* system. Positive a* parameter stands for the amount of red, and negative for green. Positive b* parameter stands for the amount of yellow, and negative for blue. Parameter L* has a value from 0 (black) to 100 (white) (CIE, 2007).

Statistical analysis

All analyses were carried out in three replicates and the results were subject to the analysis of variance (ANOVA) using the Statistica package, ver. 10. The significance of differences between the averages was calculated in Tukey’s test at a level of significance p < 0.05. Further analysis involved Pearson linear correlation coefficient and multiple regression (StatSoft, USA).

RESULTS AND DISCUSSION

The commodity-science-based evaluation of groats did not show any deviations from the relevant norms. All groats had a characteristic odour and a characteristic colour: brown in the case of roasted buckwheat groats, light grey with a greenish shade – of barley groats, and green-and-yellow – of millet groats. Moisture content in groats was in line with the norm and within a range from 8.9% (barley groats) to 10.40% (millet groats) (PN–EN ISO 712:2012).

The polyphenol content depended on the type of groats and varied from 0.19 to 93.31 mg 100g⁻¹ DM of groats (Table 2). Thermal processing (cooking) significantly lowered the polyphenol content, but only in buckwheat (Table 1).

Groats demonstrated various antioxidant activity measured by the ABTS+ free radical scavenging assay, ranging from 9.11% to 82.00% for raw groats, and from 5.22% to 67.55% for cooked groats. The type of groats significantly influenced the antioxidant properties of the product. The best antioxidant properties were found in buckwheat groats, and the lowest in millet groats. Barley groats had better antioxidant properties than millet groats, but significantly worse than buckwheat groats (Table 2).

The polyphenol content in the water from cooking varied from 1.75 to 28.93 mg.100g⁻¹. Volume 13 761 No. 1/2019
The most polyphenols permeated into the water from cooked buckwheat groats, and the least from cooked millet groats. The water from cooked barley groats had a higher polyphenol content than from cooked millet groats (Table 2).

Thermal processing resulted in decreased antioxidant activity of groats ready for consumption as compared to raw groats. The greatest decrease of antioxidant capability — 3.5 times — in comparison to the raw product was observed in barley groats. Cooked millet groats showed a decrease in the antioxidant value as compared to the raw product. Cooked buckwheat groats demonstrated the least change in antioxidant properties — 1.2 times smaller than the value obtained before cooking (Table 3).

The type of groats significantly influenced the antioxidant properties of the water from cooking. The most antioxidant properties were found in the water from cooking barley groats, and the least from cooking millet groats (Table 4).

Cooking had a significant influence on the change of selected colour parameters of groats. The highest L* value, i.e. the lightest colour among raw groats was observed in millet groats, and the lowest in buckwheat groats. Barley groats were lighter than buckwheat groats, but significantly darker than millet groats. After cooking, the value of this parameter decreased significantly (Table 5).

In each case, cooking resulted in a change of the a* colour parameter towards green, as compared to raw groats samples. The highest a* value was found in buckwheat groats, and the lowest (from blue to yellow) in millet groats (Table 5).

After cooking, the L* parameter was slightly higher in millet groats, i.e. they went brighter, and in the case of buckwheat and barley groats, the value decreased by 1.9% and 14% respectively. A significant change, however, was only observed in barley groats as compared to the colour determined prior to cooking (Figure 1).

After cooking, in all of the examined groats, the a* parameter was lower as compared to raw groats. The significantly lowest value of this parameter was observed in millet groats (Figure 2).

The highest value of the b* parameter, i.e. the share of the red colour after cooking, was demonstrated by buckwheat groats. In the other groats, the b* parameter decreased towards blue (Figure 3).

In all types of groats, the Pearson’s correlation coefficients between colour parameter and general polyphenol content and the ability to scavenge DPPH radicals showed very strong negative dependences. Only the a* parameter value was found to be significantly positively correlated with groats colour (Table 5).
The groats colour showed the strongest negative correlation with antioxidant properties, expressed as the capability to neutralise the free radical ABTS$^+$ ($r = -0.79$), and with the b* colour parameter ($r = -0.78$) (colour change in the range from blue to yellow) (Table 6).

The polyphenol content turned out to be positively, highly significantly correlated with the L* parameter value ($r = 0.95$), and the b* parameter value ($r = 0.90$), and with the antioxidant properties of groats ($r = 0.88$), which means that an increase in the polyphenol content coincides with an increase in the antioxidant properties and in the shade of the examined products (Table 6).

Polyphenols are compounds of plant origin belonging to the group of basic antioxidants. Along with other antioxidants (e.g. phytates, microelements and vit. E), they are the so-called natural non-nutritive substances, and their function is to scavenge free radicals initiating the oxidation process and the chelation of metal ions catalysing oxidation, reducing the activity of oxidative enzymes and antioxidant enzymes, and acting as reducing substances and compounds binding singlet oxygen (Filipiak-Florkiewicz and Florkiewicz, 2016). The cooking process in the conducted research significantly lowered the content of polyphenolic compounds in groats. The polyphenol content in cooked buckwheat groats decreased 2.1 times, and in the case of barley groats – over 4 times as compared to the raw product. Raw millet groats showed a minimum content of phenolic compounds, whereas after cooking, there were practically no such compounds at all. This might have resulted from the permeation of water-soluble polyphenols into the solution during cooking. The results of the research are concurrent with the research by Majkowska, Klepacka and Rafałowski (2015), who believes that the content of polyphenolic compounds is the characteristic differentiating types of groats.

The groats cooking process had an influence on a significant decrease in the polyphenol content as compared to raw groats. According to Gumula, Korus and Achemowicz (2005), most compounds belonging to antioxidants show high lability and small resistance to environmental factors, that is why processing results in significant losses of natural antioxidants. In the in-house research, the polyphenol content in cooked buckwheat groats decreased by approximately 53%, and in the case of barley groats – by 75% as compared to the raw product. In cooked millet groats, practically no polyphenolic compounds were detected. Similar results were obtained in the research by Worobiej and Koleński, (2013), where the cooking process of buckwheat groats caused a decrease in the determined content of polyphenolic compounds by 53% and 27% respectively as compared to raw groats. According to Worobiej et al. (2017), this may be a result of the permeation of water-soluble polyphenols into the solution during cooking, when the product has contact with water at a high temperature, which was confirmed in the in-house research. Dietrzych-Szóstak and Oleszek, (2001) argue that as temperature and hydrothermal processing time increase, the content of bioactive compounds decreases, whereas Zieliński et al. (2006) showed that depending on the processing temperature, their content may change to a varying degree. The authors claim that the changes depend on the form of polyphenols in food products and the consequential thermal stability. In the in-house research, the greatest antioxidant capability of raw groats was found in buckwheat groats, and the smallest in millet groats. In the case of all groats types, the cooking process decreased the antioxidant activity of the product. The best
antioxidant properties were found in buckwheat groats, both roasted and cooked. Similar results for buckwheat groats were obtained by Górecka et al. (2009), where the ethanolic extract made up 80.8%, with a high capability of scavenging free radicals DPPH•. Thermal processing resulted in a decreased antioxidant activity of groats ready for consumption as compared to the raw products. The greatest decrease of antioxidant capability – by 72% – in comparison to the raw product was observed in barley groats. Buckwheat groats demonstrated the smallest change in antioxidant properties after cooking – a decrease of 19% as compared to the value obtained before cooking. According to Şensoy et al. (2006), most compounds belonging to antioxidants show high lability and small resistance to environmental factors, that is why processing results in their significant losses. According to Gumula, Korus and Achremowicz (2005), heating of plant products in water results in a relatively quick heat transfer into the tissues, which in turn causes a longer exposure to that factor of the entire volume of the heated product and high antioxidant losses. Other processing types which slightly increase or do not affect the antioxidant activity include: blanching, freezing or short thermal processing. Stępnińska et al. (2007) conducted research using the cationic radical ABTS+ and found out that after thermal processing, buckwheat seeds had significantly lower antioxidant capability than unprocessed seeds. The research results show that cooking causes a decrease in the antioxidant capability of groats extracts obtained in 70% ethanol. Şensoy et al. (2006) concluded that the roasting of food products at 200 °C for 10 minutes decreases the antioxidant activity of buckwheat seeds. Zieliński et al. (2006) examined the antioxidant capability of buckwheat seeds prior to and after hydrothermal processing. They applied two methods of testing the capability of buffer and methanolic (80%) extracts to neutralise superoxide anion radicals, and both experiments showed a decreased antioxidant capability of the products. According to Filipiak-Florkiewicz and Florkiewicz (2016), during heating in the air, the internal temperature of the product is lower than the surface temperature, which results in lower antioxidant losses, so it is recommended to steam plant products, which involves better heat transfer and shorter thermal processing time. In the in-house research, the greatest antioxidant capability of raw groats was fund in buckwheat groats, and the smallest in millet groats. In the case of all types of groats, the cooking process decreased their antioxidant activity. This was confirmed by Filipiak-Florkiewicz and Florkiewicz (2016).

CIELab is presently the most popular way of describing colours and is the foundation of modern colour management systems. The difference between two colours in the CIELab colour space is a simple Euclidean distance between two points in a three-dimensional space. The conducted research showed that the brightest colour was characteristic for millet groats, and the darkest for buckwheat groats. Cooking of groats significantly differentiated their colours. The analysis of relationships between two determined features pointed to statistically significant correlations between groats colour and the total polyphenol content and the ability to scavenge radicals DPPH. However, in order to determine the exact influence of cooking on the polyphenol content, antioxidant properties and colour of selected types of groats, further research is required, considering various time combinations of thermal processing.

### CONCLUSION

The cooking process significantly lowered the content of polyphenolic compounds and antioxidant properties of groats. The best antioxidant properties after cooking were found in buckwheat groats – 67.55 mg.100g⁻¹, and the weakest in millet groats – 5.22 mg.100g⁻¹. The most antioxidant properties were found in the water from cooking barley groats, and the least from cooking millet groats. The most polyphenols permeated into the water from cooking buckwheat groats, and the least from cooking millet groats. The cooking process resulted in a colour change of groats as compared to raw products. After cooking, all groats, except millet groats, had a darker colour. It was shown that there are strong negative relations between the colour parameter and the total polyphenol content and the ability to scavenge radicals DPPH. Only the a* parameter value turned out to be significantly positively correlated with groats colour. Moreover, the polyphenol content was highly positively correlated with the value of the L* parameter and the b* parameter, as well as the antioxidant properties of groats, which proves that the antioxidant properties increase as the polyphenol content increases. The conducted research can help in designing the technological process of cooking buckwheat, barley and millet groats, and a way of using the water from cooked groats for consumption.
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