

## EFFECT OF FLOUR EXTRACTION RATE ON BIOACTIVE COMPOUNDS CONTENT OF TWO RYE VARIETIES

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Rye flours with extraction rate of 100% (wholemeal flour), 95% (brown flour), 90% (brown flour) and 70% (light flour) originated from Warko and Dańkowskie Złote rye varieties were prepared in order to compare the relation between flour extraction rates and content of bioactive compounds. The following compounds were analysed: total phenolic compounds (TPC), total flavonoids (TF), inositol hexaphosphate (IP6), reduced (GSH) and oxidised (GSSG) glutathione, tocopherols (T) and tocotrienols (T3). The reduced/oxidised glutathione status (GSH/GSSG) of the flours was examined as a potential index of flour resistance against oxidative stress. The following observations were made in relation to the flour types and bioactive compounds content of each variety: (a) milling process caused a decrease in the content of TPC, TF, IP6, GSH and GSSG, T and T3; (b) the most resistant to oxidation processes appeared to be brown flours, then light and finally wholemeal flour; (c) the ratio of tocotrienols to tocopherols (T3/T) was higher than that in rye flours with extraction rate of 100–90% whereas light flour was the poorest source of tocopherols and tocotrienols. The provided data on the contents of bioactive compounds in flour with different extraction rates strongly support the use of rye flours with extraction rates up to 90% in the bakery industry. It can be suggested that in the nearest future more and more rye products will be available on the market, especially those originated from wholemeal or brown flours.

### INTRODUCTION

Rye (*Secale cereale* L.) is not such a common crop like wheat or rice, but its cultivation in Middle East Europe gives traditional rye bread and becomes the main dietary component in these countries [Glitsso & Bach Knudsen, 1999]. As far as production is concerned, nowadays Russia, Poland and Germany are one of the largest rye producers [Bushuk, 2001]. Rye grain, like other cereals, provides principal quantities of energy, protein, selected micronutrients and non-nutrient substances to our diet. The whole grain contains a variety of bioactive substances, especially those with antioxidant properties (free radical scavengers, reducing agents, potential chelating agents of prooxidant metals and quenchers of the formation of a singlet oxygen) [Zieliński, 2002]. These substances include polyphenols, phytic acid, tocopherols and tocotrienols, microelements and water soluble vitamins [Adlercreutz & Mazur, 1997]. Milling and baking are the most common techniques used in grains processing. During the milling process of rye kernel, flour with different extraction rate is obtained. The extraction rate is the proportion of rye flour, delivered during the milling process, from a known quantity of grain. The extraction rate defines various types of flours *e.g.* extraction rate 100% characterise whole grain flour (wholemeal flour) [Heinio *et al.*, 2003]. Typical values for white flours are between 72% and 80%, whereas for brown flours - between 85% and 95%. The most popular rye flour for baking is wholemeal flour, nevertheless rye flour with an extraction rate of approximately 80% is also widely used. In

addition to the traditional use of different types of rye flour, various types of rye flakes, breakfast cereals with rye content up to 55% are available as well [Steller, 1995].

At present, little is known about the relationship between extraction rates of flour and contents of bioactive compounds in different types of rye flours. Because of the fact that flours with different extraction rates are commonly used in preparation of bakery products, the aim of this work was to study the relation between flour extraction rates and contents of selected bioactive compounds. To this end, the total phenolic compounds, flavonoids, inositol phosphate, oxidised and reduced glutathione, tocopherols and tocotrienols were determined in rye flours with extraction rates of 100%, 95%, 90% and 70%.

### MATERIALS AND METHODS

Glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH), inositol hexaphosphoric acid (dodecasodium salt) from corn, oxidised glutathione (GSSG), ( $\pm$ ) catechin, ferulic acid were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). Standards of tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) and tocotrienols ( $\alpha$ -T3,  $\beta$ -T3,  $\gamma$ -T3) were obtained from Merck and Sigma. All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

Two varieties of rye grain (Warko and Dańkowskie Złote) were selected from breeding materials grown in central Poland (DANKO, Plant Breeding Co., Laski) in 2004. Samples were tempered to 14.0% moisture and milled on a Quadrumat Senior laboratory mill (Brabender) to obtain a

straight grade flour with extraction rates of 100%, 95%, 90% and 70%, respectively. Samples from three replications were chosen for analysis. Flour samples were stored at 4°C until analysis.

Flour was characterised with the following analyses: moisture, ash, protein and starch content as well as Amylograph (Brabender) and Falling Number. Protein content was measured following the AACC method using a Foss Tecator apparatus whereas starch content was determined by the polarimetric method [AACC, 2000]. Moisture and ash contents of flours were analysed according to AOAC 15.950.01 and 15.955.03, respectively [AOAC, 1990]. Falling Number values were determined with a Falling Number apparatus (Perten, Sweden) using the AACC method 56-81B [AACC, 2000]. All analyses were made in triplicate.

The flour was extracted in triplicate with phosphate-buffered saline (PBS) pH 7.4 (15 mL per 1 g of flour) or with 80% aqueous methanol (1/10; w/v) (10 mL per 1 g of flour) for 2 h of shaking at 37°C. Then, the samples were centrifuged at 12000 × *g* for 15 min in a Beckman GS-15 R centrifuge (Beckman Instruments, Inc., Palo Alto, CL., U.S.A.). The fresh extracts were used to determine contents of total phenolic compounds (TPC) and total flavonoids (TF).

The content of TPC was determined in PBS and 80% methanolic extracts according to Shahidi & Naczki [1995]. Exactly 0.25 mL of an aliquot of the respective extract were mixed with 0.25 mL of Folin-Ciocalteu reagent (previously diluted with water 1:1 v/v) and 0.5 mL of saturated sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution and 4 mL of water. The mixture was allowed to stand at room temperature for 25 min and then was centrifuged at 2000 *g* for 10 min. Supernatant's absorbance was measured at 725 nm using a spectrophotometer UV-160 IPC (Shimadzu, Japan). The data were calculated on ferulic acid equivalents.

Total flavonoids (TF) content was determined with a colorimetric method described by Jia *et al.* [1998]. Briefly, 0.25 mL of the PBS or 80% methanolic extract were diluted with 1.25 mL of distilled water. Then, 75 µL of a 5% NaNO<sub>2</sub> solution were added, and the mixture was allowed to stand at room temperature. After 6 min, 150 µL of a 10% AlCl<sub>3</sub> × 6 H<sub>2</sub>O solution were added, and the mixture was allowed to stand for another 5 min. After that, 0.5 mL of 1 mol/L NaOH were added. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm in comparison with the standards prepared similarly with known (±) catechin concentrations. Next, the results were expressed as mg of catechin equivalents per gram of dry matter basis of the respective type of flour. Data were reported as means and standard deviation for at least three replications.

Inositol hexaphosphate (IP6) was determined as follows: flours were extracted with 20 mL of 0.5 mol/L HCl for 5 h using a BM1 magnetic stirrer. The extract was centrifuged at 3500 *g* for 40 min (Centrifuge MPW-360) and the supernatants were decanted, frozen overnight (-18°C), thawed at room temperature and recentrifuged at 3500 *g* for 40 min. The supernatants (15 mL) were vacuum evaporated to dryness at 40°C and dissolved in 15 mL of 0.025 mol/L HCl. The samples were then transferred to the mini-columns filled with Dowex AG 1-X8 resin, from which the inositol phosphates were eluted using 2 mol/L HCl (5 × 4 mL). After

the solvent had been removed by evaporation, the dry residue was dissolved in a mobile phase. Then the samples were analysed with HPLC according to the methods of Sandberg & Ahderinne [1986] and Sandberg *et al.* [1989] using a Shimadzu chromatograph (LC-10 AD pump, refractometric detector RID-6A, CTO 6A column oven) and a Nova-Pak C<sub>18</sub> column 150 × 4 mm. The mobile phase was a mixture of methanol and 0.05 mol/L formic acid (51:49, v/v) and 1.5 mL/100 mL tetrabutylammonium hydroxide (TBA-OH). The flow rate was 0.4 mL/min. Inositol hexaphosphoric acid (dodecasodium salt) from corn was the external standard and injections were made with a 20-µL loop.

Reduced (GSH) and oxidised glutathione (GSSG) was extracted from the samples according to Smith *et al.* [1988]. Both forms of glutathione were determined according to the spectrofluorometric method of Hissin & Hilf [1976]. The detailed procedure was described previously [Zieliński *et al.*, 1999]. The assays were performed using a Perkin-Elmer LS 50 B Luminescence Spectrometer. The data were calculated on gram of dry matter basis of the respective type of flour.

Tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) were extracted with methanol (0.5 g of flour/7 mL) for one minute using a Polytron homogeniser at full speed, at room temperature. The solvent was decanted after centrifuging (2000 *g*, 10 min), and the extraction was repeated on the residue using the same volume of solvent. The combined supernatants were evaporated in a rotary evaporator under vacuum, and then evaporated extracts were redissolved in 2 mL of *n*-hexane. The tocols were separated by HPLC on Lichrospher Si 60 5-µm particle size, 4 × 250-mm column (Merck, Germany), according to the method described by Paterson & Qureshi [1993]. Twenty microliters of each sample were 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. Flow rate was 1 mL/min, and compounds were detected using an excitation wavelength of 295 nm and emission wavelength of 330 nm. The contents of tocols 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) obtained from Merck and Sigma.

Data were subjected to multifactor analysis of variance (ANOVA) using the least-squared difference test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, Md., USA) and multiple correlation using Statistica 5.1 Program (Statsoft, Tulsa, Okla, USA) for Windows using a PC-Pentium.

## RESULTS AND DISCUSSION

The proximate chemical composition and functional properties of two varieties of rye flour with extraction rates of 100%, 95%, 90% and 70% are compiled in Table 1 and Table 2, respectively. The effect of flour extraction rate was found in respect to the protein, starch and ash content as well as to starch viscosity and the Falling Number value. The light flour with an extraction rate of 70% contained the highest amount of starch with the maximal viscosity when compared to the wholemeal flour and two kinds of brown flours. In contrast, with the decreasing percent of extraction rate from 100% up to 70%, a decrease was noted in protein and ash content,

TABLE 1. Proximate chemical composition of flours with different extraction rates originated from Warko (W) and Dańkowskie Złote (DZ) rye varieties.

Rye variety / flour extraction rate	Dry matter (%)	Protein content (%)	Ash content (%)	Starch content (%)
W/100%	88.5 ± 0.04 <sup>a</sup>	11.02 ± 0.02 <sup>a</sup>	1.8 ± 0.01 <sup>a</sup>	53.3 ± 0.28 <sup>a</sup>
W/95%	88.3 ± 0.02 <sup>a</sup>	10.55 ± 0.09 <sup>bc</sup>	1.7 ± 0.01 <sup>b</sup>	54.0 ± 0.14 <sup>bc</sup>
W/90%	88.3 ± 0.02 <sup>a</sup>	10.44 ± 0.02 <sup>ce</sup>	1.6 ± 0.06 <sup>c</sup>	54.2 ± 0.28 <sup>ce</sup>
W/70%	88.0 ± 0.01 <sup>b</sup>	7.93 ± 0.12 <sup>d</sup>	0.7 ± 0.02 <sup>d</sup>	56.6 ± 0.14 <sup>d</sup>
DZ/100%	88.5 ± 0.01 <sup>a</sup>	10.54 ± 0.22 <sup>a</sup>	1.8 ± 0.12 <sup>a</sup>	54.0 ± 0.14 <sup>a</sup>
DZ/95%	88.5 ± 0.01 <sup>a</sup>	10.31 ± 0.22 <sup>ad</sup>	1.7 ± 0.07 <sup>a</sup>	54.3 ± 0.28 <sup>ad</sup>
DZ/90%	88.4 ± 0.01 <sup>b</sup>	9.96 ± 0.19 <sup>bd</sup>	1.6 ± 0.02 <sup>b</sup>	54.6 ± 0.28 <sup>bd</sup>
DZ/70%	88.1 ± 0.01 <sup>c</sup>	7.95 ± 0.09 <sup>c</sup>	0.7 ± 0.02 <sup>c</sup>	56.7 ± 0.28 <sup>c</sup>

Data expressed as mean ± standard deviation (n = 3). Within each column for the each rye variety, means with the same letter are not significantly different (p ≤ 0.05)

TABLE 2. Functional properties of flours with different extraction rates originated from Warko (W) and Dańkowskie Złote (DZ) rye varieties.

Rye variety / flour extraction rate	Starch		Falling Number (s)
	Max. temp. gelation (°C)	Max. viscosity (UA)	
W/100%	66.0 <sup>a</sup>	420 ± 28 <sup>a</sup>	251 ± 7 <sup>a</sup>
W/95%	69.0 <sup>b</sup>	500 ± 28 <sup>bc</sup>	233 ± 2 <sup>b</sup>
W/90%	67.0 <sup>a</sup>	520 ± 28 <sup>ce</sup>	227 ± 3 <sup>c</sup>
W/70%	70.0 <sup>bc</sup>	860 ± 42 <sup>d</sup>	245 ± 5 <sup>d</sup>
DZ/100%	63.0 <sup>a</sup>	390 ± 42 <sup>a</sup>	209 ± 4 <sup>a</sup>
DZ/95%	64.0 <sup>a</sup>	560 ± 14 <sup>b</sup>	191 ± 1 <sup>bc</sup>
DZ/90%	64.0 <sup>a</sup>	640 ± 42 <sup>ce</sup>	181 ± 4 <sup>ef</sup>
DZ/70%	64.0 <sup>a</sup>	670 ± 28 <sup>de</sup>	188 ± 6 <sup>def</sup>

Data expressed as mean ± standard deviation (n = 3). Within each column for the each rye variety, means with the same letter are not significantly different (p ≤ 0.05)

and Falling Number values. The main differences between the flour with the same extraction rate of both varieties were found in the maximal starch viscosity and the Falling Number value.

In this study, the content of total phenolic compounds (TPC) and total flavonoids (TF) was analysed in PBS and 80% methanol extracts originated from rye flours with extraction rates of 100%, 95%, 90% and 70%. Results indicated a higher concentration of TPC in the PBS extracts than in the 80% methanol extracts (Table 3). The content of TPC in rye flours with extraction rates of 100%–90%, based on PBS extracts originated from Warko and Dańkowskie Złote rye varieties, was higher by 30% and 40% than the contents provided by 80% methanol extracts, respectively. The content of TPC in flour with an extraction rate of 70% was approximately two-fold lower in respect to the flours with extraction rates of 100%–90%, for both rye variety and solvents used. It was also found for Warko variety that total flavonoids content (TF) in the four types of flours ranged only from 3% to 7% of TPC extracted by PBS and from 21% to 42% of TPC extracted by 80% methanol. Whereas the same percentage contribution of TF to TPC was observed for flour PBS extracts originated from Dańkowskie Złote, the contribution of TF to TPC noted in 80% methanol extracts was lower when compared to the Warko flours. Rye flours originated from both varieties with an extraction rate ranging from 100% to 90% had a similar level of TF, whereas the extraction rate of 70% was observed to decrease their content. These findings confirm that the milling process into light flours removes outer layers of the grain, in which phenolic compounds are mostly concentrated, and for this reason it is a negligible process with respect to the content of TPC and TF in rye flours with extraction rates of 100%–90%. In this study it was also found for both rye varieties that the relative percentage ratio of TPC content in 80% methanol to TPC content in PBS extract of flours was decreased when the applied extraction rate was from 100% to 70% (Figure 1). It was concluded that despite the losses of TPC due to the milling process, the contribution of aqua soluble phenolics increased, whereas those of the lipid soluble decreased, affecting the quality of these compounds in brown and light flours.

The phenolic compounds are of concern in food technology since they may affect colour, flavour and nutritional value

TABLE 3. The content of total phenolic compounds (TPC) and total flavonoids (TF) in PBS and 80% methanol extracts of flours originated from Warko (W) and Dańkowskie Złote (DZ) rye varieties.

Rye variety / flour extraction rate	(TPC)* (mg/g d.m.)		(TF)** (mg/g d.m.)	
	PBS	80% MeOH	PBS	80% MeOH
W/100%	2.43 ± 0.16 <sup>a</sup>	1.96 ± 0.31 <sup>a</sup>	0.170 ± 0.002 <sup>a</sup>	0.416 ± 0.013 <sup>a</sup>
W/95%	2.48 ± 0.08 <sup>a</sup>	1.67 ± 0.08 <sup>a</sup>	0.182 ± 0.002 <sup>b</sup>	0.420 ± 0.026 <sup>ad</sup>
W/90%	2.43 ± 0.05 <sup>a</sup>	1.53 ± 0.02 <sup>b</sup>	0.164 ± 0.002 <sup>c</sup>	0.448 ± 0.016 <sup>bd</sup>
W/70%	1.56 ± 0.02 <sup>b</sup>	0.85 ± 0.01 <sup>c</sup>	0.055 ± 0.004 <sup>d</sup>	0.355 ± 0.003 <sup>c</sup>
DZ/100%	2.50 ± 0.17 <sup>a</sup>	1.51 ± 0.02 <sup>a</sup>	0.153 ± 0.008 <sup>a</sup>	0.367 ± 0.026 <sup>a</sup>
DZ/95%	2.26 ± 0.05 <sup>b</sup>	1.51 ± 0.02 <sup>a</sup>	0.185 ± 0.015 <sup>b</sup>	0.432 ± 0.007 <sup>b</sup>
DZ/90%	2.75 ± 0.33 <sup>a</sup>	1.49 ± 0.02 <sup>a</sup>	0.141 ± 0.004 <sup>c</sup>	0.334 ± 0.016 <sup>a</sup>
DZ/70%	1.86 ± 0.13 <sup>c</sup>	0.91 ± 0.01 <sup>b</sup>	0.074 ± 0.013 <sup>d</sup>	0.103 ± 0.013 <sup>c</sup>

\* The data were calculated as mg of ferulic acid equivalents. \*\*The data were calculated as mg of (±) catechin equivalents. Within each column for the each rye variety, means with the same letter are not significantly different (p ≤ 0.05)

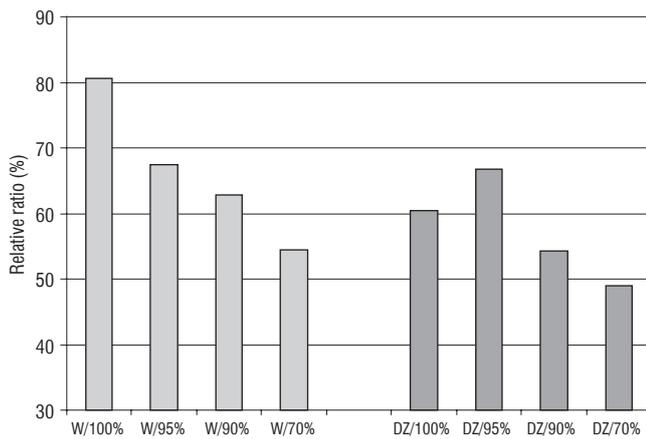


FIGURE 1. The impact of the extraction rate of Warko (W) and Dańkowskie Złote (DZ) rye variety on the relative percentage ratio (%) of total phenolic compounds (TPC) content in 80% methanolic extracts to TPC content in respective PBS extracts.

[Weidner & Paprocka, 1996; Klepacka *et al.*, 1999]. Epidemiological studies strongly suggest that phenolic compounds, including phenolic acids and flavonoids, have a protective effect against coronary diseases and possess free radical scavenging properties which antibacterial, anticarcinogenic, anti-inflammatory and antiallergic properties may originate from [Adams & Engstrom, 2000; Kris-Etherton *et al.*, 2002; Herald & Davidson, 1983; Wu *et al.*, 2003 Andlauer & Furst, 1998]. It was reported that rye was a good source of hydroxycinnamates, especially ferulic acid and ferulic acid dehydrodimers [Andreasen *et al.*, 2000] and for this reason, the milling process of rye grains using extraction rates of 100%-90% might stored these compounds in flours.

The results of quantitative analyses of inositol hexaphosphate (IP6) and glutathione (GSH, GSSG) in rye flours are shown in Table 4. The content of IP6 found in wholemeal flour was comparable with data reported by Lantzsch [1990]. The highest content of IP6 was noted in wholemeal flour whereas the lowest was found in rye light flour with an extraction rate of 70%. The remaining IP6 content in this flour constitutes only 1.7% and 2.3% of IP6 found in wholemeal flour of Warko and Dańkowskie Złote variety, respectively. The milling process into light flours generally caused a significant decrease in IP6 content with one exception made

to the brown flour with an extraction rate of 95%. In this case, the level of IP6 was statistically significantly increased by 22% and 12% when compared to that noted in wholemeal flours of Warko and Dańkowskie Złote variety, respectively. These data, similarly to other reports [Harland *et al.*, 2004], showed a negative effect of the milling process on IP6 content in different kinds of rye flours since phytic acid may play a significant role in the prevention from colon cancer and protection against inflammatory diseases resulted from chelating activity and suppressing iron-catalysed redox reactions [Graf & Eaton, 1990; Shamsuddin, 1995; Slavin, 2000].

The level of GSH and GSSG noted in the four kinds of rye flour is shown in Table 4. The milling process caused an increase in GSH content by 45% on average in flours with an extraction rate of 95% and 90% of Dańkowskie Złote variety when compared to the content in wholemeal flour. This GSH increase was not noted in relation to the respective flours of Warko variety. In contrast, the milling process of Warko grain into the light flour with an extraction rate of 70% caused a decrease in GSH content by 16% and GSSG by 21% only in Warko but not in Dańkowskie Złote variety was found in respect to the flour with the extraction rate of 70%. Little is known about the role of glutathione in rye flours. Even though the level of GSH is low in rye flours (300–500 nmol/g), this compound is considered to play a crucial role in redox reactions in flour and baking technolo-

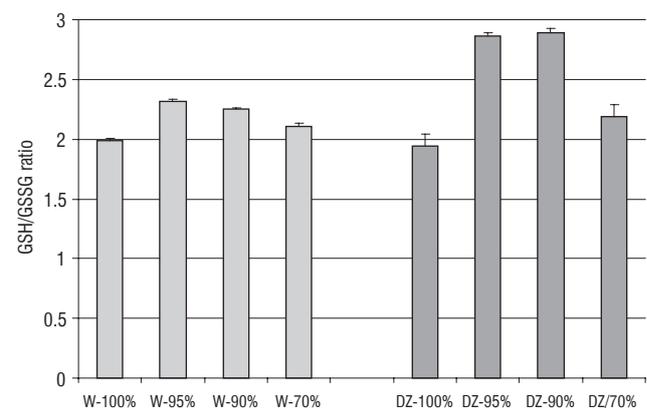


FIGURE 2. The effect of extraction rate of Warko (W) and Dańkowskie Złote (DZ) rye variety on the ratio of reduced to oxidized glutathione (GSH/GSSG) in flours with extraction rate of 100%, 95%, 90% and 70%.

TABLE 4. Content of inositol hexaphosphate (IP6), reduced (GSH) and oxidised (GSSG) glutathione, and GSH/GSSG ratio in flours originated from Warko (W) and Dańkowskie Złote (DZ) rye varieties.

Rye variety / flour extraction rate	IP6 ( $\mu\text{mol/g d.m.}$ )	GSH ( $\mu\text{mol/g d.m.}$ )	GSSG ( $\mu\text{mol/g d.m.}$ )	GSH/GSSG
W/100%	9.66 $\pm$ 0.46 <sup>a</sup>	0.434 $\pm$ 0.015 <sup>a</sup>	0.219 $\pm$ 0.010 <sup>a</sup>	1.99 $\pm$ 0.02 <sup>a</sup>
W/95%	11.77 $\pm$ 0.11 <sup>b</sup>	0.456 $\pm$ 0.018 <sup>a</sup>	0.197 $\pm$ 0.007 <sup>bc</sup>	2.32 $\pm$ 0.01 <sup>b</sup>
W/90%	5.56 $\pm$ 0.38 <sup>c</sup>	0.434 $\pm$ 0.017 <sup>a</sup>	0.193 $\pm$ 0.009 <sup>cc</sup>	2.25 $\pm$ 0.01 <sup>c</sup>
W/70%	0.16 $\pm$ 0.01 <sup>d</sup>	0.365 $\pm$ 0.013 <sup>b</sup>	0.173 $\pm$ 0.007 <sup>d</sup>	2.11 $\pm$ 0.02 <sup>d</sup>
DZ/100%	7.76 $\pm$ 0.22 <sup>a</sup>	0.322 $\pm$ 0.010 <sup>a</sup>	0.166 $\pm$ 0.004 <sup>a</sup>	1.94 $\pm$ 0.10 <sup>a</sup>
DZ/95%	8.68 $\pm$ 0.33 <sup>b</sup>	0.468 $\pm$ 0.021 <sup>bd</sup>	0.164 $\pm$ 0.006 <sup>ac</sup>	2.86 $\pm$ 0.03 <sup>bc</sup>
DZ/90%	4.24 $\pm$ 0.12 <sup>c</sup>	0.462 $\pm$ 0.013 <sup>cd</sup>	0.165 $\pm$ 0.007 <sup>ad</sup>	2.81 $\pm$ 0.04 <sup>cc</sup>
DZ/70%	0.18 $\pm$ 0.02 <sup>d</sup>	0.329 $\pm$ 0.007 <sup>a</sup>	0.150 $\pm$ 0.010 <sup>bcd</sup>	2.19 $\pm$ 0.10 <sup>d</sup>

\* Data expressed as mean  $\pm$  standard deviation (n=3). Within each column for the each rye variety, means with the same letter are not significantly different ( $p \leq 0.05$ ).

gy [Li *et al.*, 2004]. The calculated GSH/GSSG ratio showed the highest values for flours of both varieties with extraction rates of 95% and 90%, whereas the lowest ratio was found for whole-meal flour (Figure 2). This finding may indicate that the most resistant against oxidation processes during flour storage are brown flours with extraction rates of 95% and 90%, then light flour and finally wholemeal flour.

In this study, tocopherols and tocotrienols profile of the four kinds of rye flours of Warko and Dańkowskie varieties was also investigated. The results indicate the highest level of tocopherols and tocotrienols in rye flours with the extraction rate of 100%. It was found that  $\alpha$ -tocopherol was the major fraction of tocopherols whereas  $\alpha$ -tocotrienol and  $\beta$ -tocotrienol formed the main pool of tocotrienols (Table 5). The milling process into flour with the extraction rate of 70% caused a statistically significant decrease in contents of total tocopherols and total tocotrienols, *i.e.* by 52% and 68% for rye grain variety Warko, and by 39% and 67% for rye grain variety Dańkowskie Złote, respectively. However, the extent of this decrease noted in flours with extraction rates up to 90% of Warko and Dańkowskie Złote varieties was at least three- and four times lower when compared to the respective wholemeal flour. The higher losses of tocopherols than tocotrienols during the milling process confirmed that tocotrienols are mainly distributed within the outer layer of the grain [Andlander & Furst, 1999]. The role of tocopherols and tocotrienols in a diet is essential because of the multiprotective effect of these bioactive compounds. They destroy nitrite, a component whose pres-

ence in the food chain is associated with some type of stomach cancers [Kris-Etherton *et al.*, 2002]. Moreover, tocotrienols are inhibitors of cholesterol synthesis [Andlauer & Furst, 1998].

Our data demonstrated that the ratio of tocotrienols to tocopherols (T3/T) was higher than that in rye flours with the extraction rate of 100%-90%. The light flour with the extraction rate of 70% was the poorest source of tocopherols and tocotrienols (Figure 3) and that is why supplementation of tocopherols and tocotrienols is usually necessary since bakery industries prefer rather light flours than the whole-grain flour.

## CONCLUSIONS

The growing public awareness of the role of rye products containing nutrients, bioactive compounds as well as antioxidants in diet could support consumption of this crop. It can be suggested that in the nearest future more and more rye products will be available on the market, especially those originated from wholemeal or brown flours. The provided data on the bioactive compound contents in flour with different extraction rates strongly support the use of rye flours with extraction rates up to 90% in the bakery industry.

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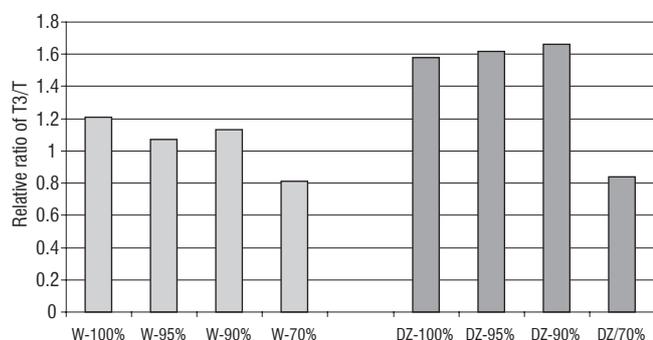


FIGURE 3. The effect of extraction rate on the ratio of tocotrienols to tocopherols (T3/T) in rye flours with extraction rate of 100%, 95%, 90% and 70%.

TABLE 5. Content of tocopherols and tocotrienols in rye flours originated from Warko (W) and Dańkowskie Złote (DZ) rye varieties ( $\mu\text{g/g d.m.}$ ).

Rye variety / flour extraction	Tocopherols (T)					Tocotrienols (T3)			
	$\alpha$	$\beta$	$\gamma$	$\delta$	Total	$\alpha$	$\beta$	$\gamma$	Total
W/100%	7.21 ± 0.34	1.15 ± 0.04	0.05 ± 0.01	-	8.41 ± 0.38 <sup>a</sup>	6.22 ± 0.29	3.98 ± 0.12	-	10.19 ± 0.40 <sup>a</sup>
W/95%	6.66 ± 0.59	1.22 ± 0.06	0.06 ± 0.01	-	7.94 ± 0.66 <sup>a</sup>	4.41 ± 2.60	4.11 ± 0.09	-	8.52 ± 2.52 <sup>ad</sup>
W/90%	5.97 ± 0.96	1.22 ± 0.13	0.07 ± 0.01	-	7.26 ± 1.07 <sup>a</sup>	4.57 ± 0.75	3.64 ± 0.47	-	8.21 ± 1.18 <sup>bd</sup>
W/70%	3.14 ± 0.19	0.82 ± 0.07	0.05 ± 0.01	-	4.01 ± 0.27 <sup>b</sup>	1.14 ± 0.05	2.12 ± 0.11	-	3.26 ± 0.15 <sup>c</sup>
DZ/100%	8.54 ± 1.51	1.51 ± 0.23	0.09 ± 0.02	-	10.14 ± 1.75 <sup>a</sup>	9.31 ± 1.39	6.72 ± 0.93	-	16.02 ± 2.29 <sup>a</sup>
DZ/95%	7.80 ± 0.57	1.53 ± 0.11	0.08 ± 0.01	-	9.41 ± 0.68 <sup>a</sup>	8.45 ± 0.54	6.80 ± 0.49	-	15.25 ± 1.02 <sup>a</sup>
DZ/90%	6.82 ± 0.29	1.41 ± 0.05	0.08 ± 0.01	-	8.31 ± 0.34 <sup>a</sup>	7.44 ± 0.44	6.34 ± 0.33	-	13.77 ± 0.76 <sup>a</sup>
DZ/70%	5.01 ± 0.11	1.13 ± 0.03	0.07 ± 0.01	-	6.21 ± 0.13 <sup>b</sup>	1.99 ± 0.07	3.26 ± 0.09	-	5.24 ± 0.16 <sup>b</sup>

Data expressed as mean ± standard deviation (n=3). Within each column for the each rye variety, means with the same letter are not significantly different ( $P \leq 0.05$ ).

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## WPLYW WYCIĄGU MĄKI DWÓCH ODMIAN ŻYTA NA ZAWARTOŚĆ ZWIĄZKÓW BIOAKTYWNYCH

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W pracy badano zależność pomiędzy wielkością wyciągu mąki a zawartością w niej związków bioaktywnych. Materiał stanowiły mąki żytnie o wyciągu 100%, 95%, 90% i 70% pochodzące z przemiału ziarna żyta odmiany Warko i Dańkowskie Złote. W mąkach analizowano zawartość związków fenolowych ogółem (TPC), flawonoidów ogółem (TF), heksafosforanu inozytolu (IP6), zredukowanego (GSH) i utlenionego (GSSG) glutationu oraz tokoferoli (T) i tokotrienoli (T3). Prześledzono ponadto zmiany indeksu odporności mąk żytnich na stres oksydacyjny (GSH/GSSG) w zależności od stopnia wyciągu mąki.

Uzyskane wyniki pozwoliły na wysunięcie następujących wniosków: proces przemiału ziarna w kierunku mąk jasnych o wyciągu 70% obniżał zawartości związków bioaktywnych, tj. TPC, TF, IP6, GSH i GSSG, T i T3 (tab. 3, 4 i 5). Odporność badanych mąk na stres oksydacyjny wyrażona poprzez wartości współczynnika GSH/GSSG kształtowała się w następującej kolejności: ciemne mąki o wyciągu 95% i 90%, następnie jasne mąki (wyciąg 70%) i mąki pochodzące z pełnego przemiału (wyciąg 100%) (tab. 4, rys. 2). Najwyższy stosunek tokoferoli do tokotrienoli stwierdzono w mąkach o wyciągu od 100% do 90%, najniższy zanotowano dla mąk jasnych (rys. 3). Przedstawione zależności pomiędzy wyciągiem mąki a zawartością związków bioaktywnych były charakterystyczne dla obydwu badanych odmian żyta. Uzyskane wyniki i wnioski wskazują na celowość wykorzystania mąk żytnich o stopniu wyciągu od 100% do 90% w przemyśle piekarniczym.

