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THE INFLUENCE OF EXTRUSION ON THE CONTENT OF POLYPHENOLS AND ANTIOXIDANT/ANTIRADICAL ACTIVITY OF RYE GRAINS (*SECALE CEREALE* L.)*

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Abstract. The aim of the study was to check the influence of extrusion on the content of polyphenol and antioxidant/antiradical activity of rye grains. The extrudates prepared from three cultivars of rye were obtained at different process parameters. Total polyphenol, antioxidant and antiradical activity was measured in the samples. Extrusion resulted in a decrease of total polyphenol content (TPC) on average by 40% in rye grains, and the largest loss of endogenic phenolic compounds was observed at the extrusion conditions: 20% moisture of raw material and 120°C. Extrudates obtained at: 14% moisture of raw material and 180°C exhibited either increase of these compounds or no change. It was found that rye extrudates exhibited the highest antiradical activity (measured by the methods with DPPH and ABTS) when raw material contained 14% of moisture and at temperature of the extrusion – 180°C, while the lowest when the parameters were 20% and 120°C. Antioxidant activity in beta-carotene-linoleate model system was high when rye extrusion was performed at 14% and 20% moisture and temperature 180°C. The negative influence of extrusion on the antioxidant activity of rye grains was observed at 20% moisture and 120°C.

Key words: antioxidant/antiradical activity, polyphenols, rye extrudates

INTRODUCTION

Antioxidants are a group of different chemical compounds, where polyphenols plant origin compounds are also included. For many years they were treated as anti-nutritive compounds, but presently the importance of this group has significantly increased, due to the research conducted in the last few years dealing with their health-promoting action on human body. Polyphenols include five groups of compounds: phenolic acids

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(benzoic and cinnamic acid derivatives), flavonoids, tannins and stilbenes [Oszmiański 1995, Rice-Evans et al. 1996, 1997, Bravo 1998, Rosicka-Kaczmarek 2004]. Their action relies on scavenging of free radicals initializing the oxidation process, or chelating the metal ions catalyzing the oxidation process, lowering/stopping the oxidation enzymes activity, and by activation of antioxidants enzymes [Oszmiański 1995, Bravo 1998, Rosicka-Kaczmarek 2004].

One of the perfect source of the biologically active compounds (dietary fibre, vitamins and mineral compounds), including polyphenols (antioxidants), are whole grains of such cereals as: wheat, rye, barley and oat [Rybka et al. 1993, Weidner et al. 1999, 2000, Andreasen et al. 2000, Baublis et al. 2000, Karamać et al. 2002, Peterson et al. 2002, Ötles and Özlem 2006]. For that reason whole cereal grains are recommended in prophylactic of chronic diseases, including CHD, and some types of tumors [Anderson 2003, Vecchia et al. 2003]. Among cereals, special attention should be paid to rye, due to its specific chemical composition, i.e.: high content of soluble dietary fibre, including soluble pentosans, mineral compounds, (Ca, Fe, I, F) [Gąsiorowski 1994], and high level of phenolic acids, counted to polyphenols. In rye grains there were found following phenolic acids: ferulic acid (which level in rye grains is four times higher than in wheat grains) and sinapic acid, caffeic, p-coumaric and vanillic acid [Weidner et al. 1999, 2000, Zieliński et al. 2001, Zieliński 2002].

It should be mentioned, that both polyphenols content and their activity is strongly dependant on species, cultivar, maturity stage (of plant raw material) and it would be changed during technological processing [Nicoli et al. 1999, Weidner et al. 2000, Zieliński et al. 2001, Grajek 2003].

The influence of processing on quantity and activity of antioxidants in cereals were only occasionally investigated, and they were mostly focused on milling process [Baublis et al. 2000, Wołoch and Pisulewski 2003]. And that was the reason to undertake this problem.

The aim of the present research was to evaluate the influence of extrusion on level of polyphenols in rye grains, and to assess the antioxidant (and antiradical) activity of rye extrudates.

MATERIALS AND METHODS

The material were grains of three rye cultivars: 'Amilo' (ZA), 'Rostockie' (ZR) and 'Agrikolo' (ZEA), which were subjected to extrusion process. Extrusion was done in one screw extruder 20 DN Brabender (Duisburg, Germany) under the following conditions: initial moisture was 14% and 20%, and two temperature profiles were applied: 80°C/100°C/120°C and 120°C/160°C/180°C. The screw speed was 190 rpm, die diameter 3 mm, and screw compression 3:1.

In methanol-acetone extracts prepared from the analysed samples the following analyses were performed:

– Total polyphenols content (TPC) by spectrophotometric method [Singleton et al. 1999].
– Antiradical activity by applying method with two synthetic radicals: DPPH [Brand-Williams et al. 1995] and ABTS [Re et al. 1999]. In case of antiradical activity assessed by DPPH method extracts were prepared without HCl.

– Antioxidant activity (AA) in beta-carotene-linoleate model system [Al-Saikhan et al. 1995]. AA was calculated according to Al-Saikhan et al. [1995], beta-carotene oxidation rate ratio (ORR) was calculated according to Oomah et al. [2005].

RESULTS AND DISCUSSION

Total polyphenols content (TPC) calculated as catechin in grains of three rye cultivars (Amilo, Rostockie and Agrikolo) was: 1.86; 2.14 and 2.11 mg catechin/g d.m. respectively and was presented in Table 1. It is difficult to compare these data with other data from literature, due to different methods of extraction, determination and results calculations applied by other authors. Zieliński and Troszyńska [2000] gave the polyphenols level in rye grains 0.65-0.94 mg catechin/g, and Karamać et al. [2002] – 7.9 mg catechin/g of extract. The important factor is the way in which the extraction was performed and its time [Baraniak et al. 2002]. Zieliński and Troszyńska [2000] applying the extraction in buffer (PBS) obtained TPC at level 0.94 mg catechin/g of rye grain, and in 80% methanol – 0.65 mg catechin/g of rye grain. But Zieliński et al. [1998] determined total polyphenols content in rye grains at level 1.4 mg catechin/g of rye, by extraction of plant material in phosphate buffer. In this work two-stages extraction methanol-acetone was used, and for that reason the results were higher than obtained by Zieliński and Troszyńska [2000] and Zieliński et al. [1998].

Table 1. Total polyphenol content in grains of three rye cultivars and rye extrudates, mg (±) catechin·g^{-1} d.m.
Tabela 1. Zawartość polifenoli ogółem w ziarniakach trzech odmian żyta oraz ekstrudatach żytnich, mg (±) katechiny·g^{-1} s.m.

Extrusion parameters Parametry procesu ekstruzji	Rye grain cultivar – Odmiany ziarna żyta		
	Amilo (ZA)	Rostockie (ZR)	Agrikolo (ZEA)
Raw material Surowiec	1.86 d	2.14 c	2.11 c
14°C/120°C	1.01 b	1.63 b	1.52 b
14°C/180°C	1.98 e	2.20 c	2.14 c
20°C/120°C	0.73 a	0.92 a	0.89 a
20°C/180°C	1.28 c	1.63 b	1.41 b

Different letters denote mean values that statistically differ from one another (Duncan's test, at p = 0.05 ±) within one cultivar.

Różne litery oznaczają wartości średnie różniące się statystycznie istotnie między sobą (test Duncana, przy poziomie istotności p = 0,05 ±) w obrębie jednej odmiany.

The effect of grains extrusion of three rye cultivars decreased total polyphenols content by about 40%, with exception for extrudates prepared at the following conditions: 14% initial moisture and temperature 180°C. In this case total polyphenols content was about 6% higher (Table 1). In all the analysed rye extrudates the highest 60% decrease of polyphenols content was denoted following parameters of hydrothermal process: 20% initial moisture and temperature 120°C (Table 1). Similar results were obtained by Zieliński and Troszyńska [2000] extracting the samples with 80% methanol, because TPC in rye extrudates was decreased by about 40% at extrusion temperature 120°C, and at 200°C an increase was observed in content of these compounds by about 11% in comparison to raw material. But in researches of Viscidi et al. [2004] concerning changes in TPC in plant material it was observed, that extrusion process caused losses in the endogenous polyphenols content (up to 46%). Moreover, it was observed, that lower extrusion temperature (120°C) caused greater loss of endogenous polyphenols in rye grains than temperature 180°C, and lower level of initial moisture (14%) favoured higher polyphenols retention (Table 1).

An increase of polyphenols compounds in plant material after extrusion (14%/ 180°C) in comparison to raw material (rye grains) could be explained by the release of these compounds from cell walls. Reverse opinion was presented by Camire and Dougherty [1998] and Viscidi et al. [2004], because they revealed that extrusion process decreased the level of natural antioxidants in plant material, that was also confirmed by the results of this work, where other extrusion parameters were applied (14%/120°C, 20%/120°C, 20%/180°C; Table 1).

Antiradical activity of rye grains before and after extrusion was measured by means of two synthetic, free radicals: DPPH and ABTS (radical cation), and results were presented in Table 2.

Among all the analysed rye cultivars the highest antiradical activity, measured with means of the mentioned above radicals, had 'Agrikolo', the lowest 'Amilo' (Table 2). It is difficult to compare the obtained values of antiradical effects with other authors' results due to differences in methodology and results calculations. Zieliński and Troszyńska [2000] measured TEAC at 2-4 µmol Tx/g of rye grains applying another method.

It was discovered (antiradical activity measured by means of DPPH and ABTS) that among rye extrudates, the highest ability to scavenging free radicals had extracts from extrudates obtained at the following parameters: 14% initial moisture and 180°C, in comparison to raw material (Table 2). In case of extrudate from 'Amilo' cultivar obtained at extrusion parameters 14% initial moisture and 180°C an increase was observed in antiradical activity by about 8.6% in DPPH method and 26% in ABTS method in respect to raw material. Extrudate from 'Rostockie' cultivar made at the mentioned above parameters was characterized by 5% (DPPH) and 13% (ABTS) higher antiradical activity in comparison to unprocessed grains. Similar increase of antiradical potential (2.2% – DPPH and 3% – ABTS) was observed in extrudate from 'Agrikolo' obtained at 14% and 180°C in comparison to non-extruded material (Table 2). It could be explained by higher total polyphenols content only in case of extrudates from Amilo cv., in contradiction to remaining cultivars, where polyphenols content was constant, identical as in the raw material (Table 1). Such high antiradical activity of extrudates (from Rostockie cv. and Agrikolo cv.) could be caused by antioxidants composition in this material, because there were differences in activity of phenolic compounds [Pekarinen et al. 1999, Samotyja et al. 2002]. The lowest ability to scavenging of free radicals exhibited extracts prepared from extrudates made at 20% and 120°C (Table 2), that partially resulted from low total polyphenols content (Table 1) and possibility of creation at

Table 2. Antiradical activity (measured by methods DPPH and ABTS) of rye grains before and after extrusion
Tabela 2. Aktywność przeciwrodnikowa (oznaczona metodami z DPPH i ABTS) ziarniaków żyta przed i po ekstruzji

Sample Nazwa próbki	TEAC – Trolox Equivalent Antioxidant Capacity			
	$mgTx \cdot g^{-1}$ d.m. $mgTx \cdot g^{-1}$ s.m.	$mMTx \cdot kg^{-1}$ d.m. $mMTx \cdot kg^{-1}$ s.m.	$mgTx \cdot g^{-1}$ d.m. $mgTx \cdot g^{-1}$ s.m.	$mMTx \cdot kg^{-1}$ d.m. $mMTx \cdot kg^{-1}$ s.m.
	DPPH		ABTS	
ZA	1.42	5.70 b	3.56	14.21 c
ZA 14/120	1.45	5.81 bc	3.02	12.07 b
ZA 14/180	1.54	6.19 c	4.49	17.92 d
ZA 20/120	1.38	5.54 a	2.20	8.81 a
ZA 20/180	1.52	6.09 c	3.49	13.95 c
ZR	1.44	5.78 b	4.21	16.81 c
ZR 14/120	1.45	5.81 b	3.65	14.57 b
ZR 14/180	1.51	6.04 c	4.74	18.93 d
ZR 20/120	1.37	5.50 a	2.78	11.10 a
ZR 20/180	1.43	5.70 b	3.66	14.63 b
ZEA	1.46	5.85 b	4.47	17.86 c
ZEA 14/120	1.47	5.89 c	3.47	13.86 b
ZEA 14/180	1.49	5.98 c	4.60	18.37 cd
ZEA 20/120	1.19	4.76 a	2.57	10.28 a
ZEA 20/180	1.18	4.71 a	3.48	13.90 b

ZA14/120 – rye extrudate, cultivar Amilo obtained from 14% moisture raw material at 120°C; other abbreviations the same, ZA – rye grain of Amilo cultivar, ZR – rye grain of Rostockie cultivar, ZEA – rye grain of Agrikolo cultivar.

Different letters denote mean values that statistically differ from one another (Duncan's test, at $p = 0.05 \pm$) within one cultivar.

ZA14/120 – ekstrudat z żyta odmiany 'Amilo', otrzymany przy wilgotności materiału wyjściowego 14% w temperaturze120°C, analogicznie pozostałe skróty, ZA – żyto odmiany 'Amilo', ZR – żyto odmiany 'Rostockie', ZEA – żyto odmiany 'Agrikolo'.

Różne litery oznaczają wartości średnie różniące się statystycznie istotnie między sobą (test Duncana, przy poziomie istotności $p = 0,05 \pm$) w obrębie jednej odmiany.

low temperature of 120°C compounds with oxidation supporting character [Nicoli et al. 1999]. Parameters of extrusion process: 20% initial moisture and 120°C influenced the decrease of antiradical activity of rye extrudates in range 3-19% (DPPH method) and 34-42% (ABTS method). The greatest increase of antiradical activity (measured by ABTS method) of rye bran extrudates obtained at 14% initial moisture and 180°C and the greatest loss at 20%/120°C, in comparison to raw material, were observed by Gumul and Korus [2006].

Rye extrudates obtained at remaining hydrothermal process parameters: 20%/180°C and 14%/120°C exhibited higher (in case DPPH) or lower (in case ABTS) antiradical activity in comparison to raw material, that resulted from selective action of radicals.

As a result of extrusion of rye grains about 7% decrease was observed of antioxidant activity in the beta-carotene-linoleate model system, when 20%/120°C in contrast to the remaining parameters of the process, when about 6% increase (in comparison to raw material) in activity was observed (Table 3). The highest increase of antioxidant activity was measured in extrudates obtained at 14%/180°C (Table 3). An increase in antioxidant activity of rye extrudates at mentioned above parameters was 15% (Amilo cv.), 9% (Rostockie cv.) and 1% (Agrikolo cv.) in comparison to unprocessed material (Table 3). High antioxidant potential of 'Amilo' extrudates obtained at 14%/180°C resulted from high total polyphenols content in extrudates of this type (Tables 1 and 3). High antioxidant activity was also marked in extrudates from all rye cultivars prepared at 20%/180°C (Table 3). This increase of antioxidant activity could be probably bound to individual polyphenols composition in this material. Moreover, contribution to the antioxidant capacity can possess other compounds such as: aromas, proteins or tocopherols, which possess antioxidant properties [Nicoli et al. 1999]. But low antioxidant activity of extrudates obtained at the following parameters: 20%/120°C (Table 3) could be connected with a low total polyphenols content in this product (Table 1), because according to other authors [Goupy et al. 1999, Wołoch and Pisulewski 2003] there was a close correlation between antioxidant activity and total polyphenols content. Attention should be paid to reverse tendency, which was observed in case of beta-carotene decomposition (ORR) in this analytical method (Table 3). The highest values of ORR were detected in all extrudates (20%/120°C) that were products with the lowest antioxidant activity measured in the beta-carotene-linoleate model system, and the lowest values of ORR were detected in extrudates 14%/180°C, where the highest antioxidant activity was determined (Table 3).

Table 3. Antioxidant activity (AA) and beta-carotene oxidation rate ratio (ORR) of rye grains before and after extrusion
Tabela 3. Aktywność przeciwutleniająca (AA) i współczynnik utlenienia beta-karotenu (ORR) ziarniaków żyta przed i po ekstruzji

Extrusion parameters Parametry procesu ekstruzji	Rye grain cultivar – Odmiany ziarna żyta					
	Amilo (ZA)		Rostockie (ZR)		Agrikolo (ZEA)	
	AA %	ORR	AA %	ORR	AA %	ORR
Raw material Surowiec	44.36 b	0.556 c	49.95 b	0.500 b	49.11 b	0.508 bc
14°C/120°C	45.16 bc	0.548 bc	47.70 a	0.515 c	50.02 bc	0.499 b
14°C/180°C	50.79 cd	0.492 a	54.44 cd	0.455 a	49.52 bc	0.504 b
20°C/120°C	43.20 a	0.570 d	47.90 a	0.521 c	41.00 a	0.590 c
20°C/180°C	50.00 c	0.501 b	53.00 c	0.469 ab	55.73 c	0.442 a

Different letters denote mean values that statistically differ from one another (Duncan's test, at p = 0.05 ±) within one cultivar.

Różne litery oznaczają wartości średnie różniące się statystycznie istotnie między sobą (test Duncana, przy poziomie istotności p = 0,05 ±) w obrębie jednej odmiany.

Antioxidant activity of rye extrudates prepared using following parameters: 14% initial moisture and temperature 180°C and also 20% and 120°C measured in the system: beta-carotene-linoleate is consent with antiradical activity (measured by DPPH and ABTS methods). But there were some discrepancies between antiradical and antioxidant activity observed in some extrudates prepared using other parameters 14%/120°C and 20%/180°C.

CONCLUSIONS

1. Extrusion caused lowering of total polyphenols content (TPC) at the average 40% in comparison to rye grains, and the highest losses of endogenous polyphenols were denoted when extrusion was performed at material with 20% initial moisture content and temperature of 120°C. The increase in content of these compounds or their constant value were measured in rye extrudates prepared at 14%/180°C.

2. It was also discovered, that in case of rye extrudates lower temperature of extrusion (120°C) was the cause of greater endogenous polyphenols loss, than at temperature of 180°C. Lower initial moisture level (14%) favoured the retention of higher amount of polyphenols compounds.

3. It was stated, that rye grains extrudates exhibited the highest antiradical activity (measured by DPPH and ABTS methods) when prepared at 14% initial moisture and temperature of 180°C, and the lowest at 20%/120°C, in comparison to raw material.

4. High antioxidant activity, measured in beta-carotene-linoleate model system, was detected in rye extrudates prepared at 14% and 20% initial moisture content and temperature of 180°C. The destructive influence on antioxidant activity had the following parameters: 20% initial moisture content and temperature 120°C.

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WPŁYW EKSTRUZJI NA ZAWARTOŚĆ POLIFENOLI ORAZ AKTYWNOŚĆ PRZECIWRODNIKOWĄ I PRZECIWUTLENIAJĄCĄ ZIAREN ŻYTA (*SECALE CEREALE* L.)

Streszczenie. Celem badań było określenie wpływu ekstruzji ziaren żyta na zawartość polifenoli i aktywność przeciwrodnikową i przeciwutleniającą ekstrudatów żytnich. Materiałem do badań były ekstrudaty sporządzone z ziaren trzech odmian żyta, przy różnych parametrach procesu ekstruzji. W próbkach oznaczono ogólną zawartość polifenoli oraz określono aktywność przeciwrodnikową i przeciwutleniającą ekstrudatów żytnich. Ekstruzja obniżyła ilość polifenoli ogółem (TPC) średnio o 40% w ziarnach żyta, przy czym największe straty endogennych związków fenolowych odnotowano przy parametrach procesu ekstruzji: 20% wilgotności materiału wyjściowego i temperatura 120°C. Wzrost zawartości tych związków lub jej stałą wartość wykazały ekstrudaty otrzymane przy parametrach: 14%/180°C. Stwierdzono, że w odniesieniu do surowca ekstrudaty z ziaren żyta charakteryzowały się największą aktywnością przeciwrodnikową (określoną metodą z DPPH i ABTS) przy parametrach procesu ekstruzji: 14% wilgotności materiału wyjściowego i temperatura 180°C, a najmniejszą osiągnięto, stosując parametry: 20% wilgotności i temperaturę 120°C. W wypadku oznaczenia aktywności przeciwutleniającej w układzie beta-karoten – kwas linolenowy dużą aktywność ekstrudatów z żyta wyznaczono przy parametrach: 14 i 20% wilgotności surowca i temperatura 180°C. Destrukcyjny wpływ na aktywność przeciwutleniającą ekstrudatów miały parametry: 20% wilgotności materiału wyjściowego i temperatura 120°C.

Słowa kluczowe: aktywność przeciwutleniająca, aktywność przeciwrodnikowa, ekstrudaty żytnie, polifenole

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