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RESEARCH ARTICLE

Green grain of spelt (*Triticum aestivum* ssp. *spelta*) harvested at the stage of milk-dough as a rich source of valuable nutrients

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ABSTRACT

The aim of this study was to compare the chemical composition of three spelt wheat cultivars harvested at the milk-dough stage (MDS) and at the fully ripe stage (FRS) in the organic farm in Poland. Green grain of cv. 'Oberkulmer Rotkorn' harvested at MDS showed a significantly higher content of P, K, Ca, Zn, Fe, Na and B, compared to grain harvested at FRS. In unripe grain of cv. 'Rokosz', the content of N, K, Zn, Mn and Na was significantly higher than in ripe grain at FRS. In the case of cv. 'Wirtas', the P, K, Mg, Ca, Zn, Mn and B content determined at MDS was higher than that found in ripe grain at FRS. The content of Lys, Met, Ala and Tyr in green grain of all the cultivars evaluated was higher than that determined at FRS. However, when the grain was harvested at the fully ripe stage, the increase of Arg, His, Leu, Phe, Val, Glu, Pro and Ser was found. The content of fat and α-linolenic fatty acid in green grain of spelt was higher than in ripe grain. The presence of cinnamic and dicarboxylic acids was only found in spelt harvested at MDS. Unripe grain was also characterized by a higher content of hydroxy and phenolic acids.

Keywords: Amino acids; Biologically active compounds; Chemical composition; Fatty acids; Green spelt grain

INTRODUCTION

A clear tendency to increase biological diversity in plant food materials can be seen in recent years. Due to this, one can observe a return to ancient plant species cultivated in the past. One of the oldest cereals used by humans is spelt (*Triticum aestivum* ssp. *spelta* L.) belongs to the wheat subspecies (Sulewska et al., 2008; Andruszczak et al. 2011). In prehistorical times it was grown mainly in the Near East and it probably originates from south-east Asia. For many years spelt was a forgotten cereal, but during the last 20 years its popularity has been growing. The renaissance of spelt wheat is primarily associated with the development of organic farming (Vučković et al., 2013; Kwiatkowski et al., 2014; Biel et al., 2016b). Compared to common wheat, it is characterized by greater resistance to environmental stresses and is perceived as a species that produces stable yields under extensive farming conditions.

Consumers' interest in functional food and food products with the addition of spelt grain continues to increase, even more so as spelt grain is a source of many valuable nutrients and products made from this grain can be a diet component alternative to other cereal grains. Additionally, apart from its nutritional properties, functional food also has health-promoting properties that result from the presence of biologically active substances (Abdel-Aal and Rabalski, 2008; Świeca et al., 2014; Shewry and Hey, 2015; Biel et al., 2016a; Andruszczak 2017). Spelt grain contains on average from 40% to 68% of starch, from 10% to 16% of protein, and from 1% to 5% of fat (Kwiecińska-Poppe et al., 2011; Escarnot et al., 2012). In its fatty acid composition, linoleic acid is predominant, followed by oleic and palmitic acids (Grela 1996). The content of macro- and micronutrients in spelt grain is largely determined by the genetic factor (Kraska et al., 2013; Rachoń et al., 2015; Andruszczak, 2018). Spelt products contain substances

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with antioxidant properties preventing the formation of cancer cells (Gawlik-Dziki et al., 2012; Świeca et al., 2014). Compared to common wheat, it contains more protein, gluten and fat, but less fiber (Kohajdová and Karovičová, 2008; Jablonskytė-Raščė et al., 2013; Kraska et al., 2013).

An important attribute of spelt wheat is the possibility of using its grain harvested at different ripeness stages. Currently, consumers can purchase so-called "green grain" of spelt, which is considered more valuable than grain harvested at full maturity. There is an opinion that spelt grain harvested at the milk-dough stage has all nutrients and at the same time the percentage of starch is not high yet. Such grain dried immediately after harvest and subsequently dehulled is characterized by a high content of protein and mineral salts (Tyburski and Babalski, 2006). The use of unripe grain of spelt wheat as a potential substrate in bakery fits the current trends among consumers towards seeking healthy food with specific health-promoting properties. However, in the available literature, there is no scientific results on the impact of harvesting time on spelt grain chemical composition.

The aim of this study was to compare the quality of grain of three spelt cultivars harvested at the milk-dough stage, the so-called "green grain", and at the fully ripe stage. The contents of macro- and micronutrients, amino acids, fat and fatty acids, and biologically active compounds, such as hydroxy, phenolic, cinnamic and dicarboxylic acids, were compared in spelt grain. It was expected that the chemical composition of "green grain" of spelt would be more beneficial in terms of its nutritional values than grain harvested at the fully ripe stage.

MATERIAL AND METHODS

Spelt wheat sampling

The material for the tests was obtained in the 2016 and 2017 vegetation season at the Model Organic Farm in Chwałowice (Municipality of Iłża, Radom County, Masovian Voivodeship) belonging to the Agricultural Advisory Center in Brwinów, Radom Branch, Poland (51°18' N, 21°30' E). The study evaluated a chemical composition of grain of two winter forms of spelt wheat (cvs. 'Oberkulmer Rotkorn' and 'Rokosz') and one spring cultivar ('Wirtas') harvested at the milk-dough stage (MDS) and at the fully ripe stage (FRS). The grain samples of each cultivar were collected with three replicates. The plots with spelt wheat were situated on brown soil, with an organic carbon content of 14.11 g kg^{-1} and a pH of 6.15 in 1 M KCl. The soil was characterized by the following nutrient content: P – 73.9 mg kg^{-1}; K – 1430 mg kg^{-1}; Mg – 1765 mg kg^{-1}; B – 1.98 mg kg^{-1}; Mn 494 mg kg^{-1}; Cu – 5.67 mg kg^{-1}; Zn – 47 50 mg kg^{-1}; Fe – 1125 mg kg^{-1}. The area of a single plot was 30 m^2.

Spelt ears harvested at the milk-dough stage were freeze dried using an ALPHA 1-4 laboratory freeze dryer consisting of a drying chamber, a cold trap, and a vacuum pump (Rudy et al, 2015. The freeze-drying process was performed at 20°C and with a constant pressure of 52 Pa in the drying chamber. Before freeze drying, the grain was frozen at a temperature of –35°C. The dried ears were threshed in a Wintersteiger LD 180 thresher. An example of picture of freeze-dried spelt grain harvested at the MDS was shown in Fig. 1.

Analytical procedures

The following parameters were determined in spelt grain (both in that harvested at the MDS and FRS): N (mineralization in sulfuric acid and oxygenated water; determination by the Kjeldahl distillation method; titration detection), P (the weighed sample is incinerated with calcium carbonate in an electric muffle furnace at 550°Cand subsequently heated with hydrochloric acid and nitric acid (V), a portion of the acidic solution is mixed with vanadate-molybdate reagent, and the absorbance of the obtained yellow solution is measured using a Shimadzi UV-1800 spectrophotometer at a wavelength of 430 nm), K and Ca (mineralization in sulfuric acid and oxygenated water; determination by flame photometry; Jenway PEP7 photometer), Mg (mineralization in sulfuric acid and oxygenated water; determination by atomic absorption spectrometry; Perkin-Elmer spectrometer), Cu, Zn, Mn, Fe (mineralization in perchloric and nitric acids (1:4); determination by atomic absorption spectrometry; Avanta spectrometer), B (mineralization with $Ca(OH)_2$ in a furnace; determination by spectrophotometric curcumin method; Spekol spectrophotometer). The following characteristics were also determined in spelt grain: fat content (Soxhlet method), fatty acid composition by gas chromatography (a Varian 450-GC gas chromatograph with a FID detector). To control the chromatograph as well as to collect, integrate and calculate results, Galaxie™ Chromatography Data

Fig 1. Picture of spelt grain harvested at MDS after freeze-drying.

System software was used. Autosampler: Varian CP-8400. Injector: 1177 Split/Splitless. Capillary column: Select™ Biodiesel for FAME (Agilent Technologies); L (m) × ID (mm) × OD (mm) – 30 × 0.32 × 0.45. Stationary phase: Select Biodiesel for FAME Fused Silica; Film Thickness 0.25 μm. Detector: flame ionization detector (FID).

Protein acid hydrolysis was performed to determine the amino acid composition without oxidation according to the methodology described by Davis and Thomas (1973). Protein hydrolysis to separate sulfur amino acids was performed according to Schramm et al. (1954).

Amino acids were determined using an AAA 400 amino acid analyzer manufactured by Ingos (Czech Republic, Prague). Amino acids are separated by ion exchange chromatography. A column with the dimensions of 0.37 × 45 cm is packed with ion-exchange resin. Ostion LG ANB is used for hydrolysates. It is a strong cation exchanger with the average grain size of about 12 μm in the form of Na. Column temperature 60°C and 74°C. The analyzer detects amino acids by derivatization with ninhydrin (it is a detection reagent). Identification of amino acids is performed by a photometric detector at a wavelength of 570 nm for all amino acids, and only for proline at 440 nm. To perform separation, the following four buffers were used: 1 – pH 2.6; 2 – pH 3.0; 3 – pH 4.25; 4 – pH 7.9. After amino acid separation, the column was regenerated with 0.2N NaOH.

The chemical composition of spelt grains was analyzed according to the Stocki et al. method (Stocki et al., 2018). Experimental retention indices ($RI_{exp.}$) of analyses were determined, taking into account C_{10}–C_{40} n-alkanes retention times and comparing with literature retention indices ($RI_{lit.}$) from database (Isidorov, 2015).

Statistical calculations

All tests were performed in triplicate. The obtained results were statistically analysed by analysis of variance (two way ANOVA) and least significant differences were calculated using Tukey's confidence half-intervals with an error rate of 5%. ARStat software developed by the Computing Centre of the University of Life Sciences in Lublin was used for the calculations.

RESULTS AND DISCUSSION

The mean concentrations of the mineral nutrients in grain of the different spelt wheat cultivars harvested at the milk-dough stage and at the fully ripe stage are presented in Table 1 and 2. With regard to all the spelt cultivars, a higher content of K and Zn was only determined in grain harvested at MDS compared to FRS. In green grain of cv. 'Rokosz', the N content was significantly higher than in grain obtained at fully ripe stage. In grain of the spring cultivar 'Wirtas', in turn, an opposite relationship was found, while in the case of cv. 'Oberkulmer Rotkorn' grain the differences were within the margin of statistical error. At the same time, the N content in cv. 'Wirtas' grain was higher than that determined for cv. 'Rokosz'. The N content in grain of cvs. 'Oberkulmer Rotkorn' and 'Wirtas' was similar to that found by Andruszczak (2018) in spring

Table 1: Macronutrient content in spelt grain depending on the cultivar and harvesting time

Macronutrient	Oberkulmer Rotkorn		Rokosz		Wirtas	
	MDS	FRS	MDS	FRS	MDS	FRS
	(g kg^{-1})					
N	22.50±0.17^{a}	22.60±0.25^{a}	15.60±0.38^{b}	15.20±0.27^{b}	22.50+0.19^{a}	25.90+0.22^{c}
P	4.41±0.17^{a}	3.86±0.26^{b}	3.43±0.29^{c}	3.48±0.32^{c}	4.62+0.41^{a}	4.36+0.46^{a}
K	4.54±0.24^{a}	3.17±0.21^{b}	5.47±0.32^{c}	3.54±0.24^{b}	6.53+0.55^{d}	3.41+0.28^{b}
Mg	1.26±0.21^{a}	1.37±0.19^{a}	1.04±0.18^{a}	1.07±0.12^{a}	1.37+0.13^{a}	1.17+0.11^{a}
Ca	0.271±0.07^{a}	0.206±0.04^{b}	0.21±0.03^{b}	0.207+0.02^{b}	0.435+0.09^{c}	0.224+0.03^{ab}

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; $^{a-d}$ different superscripts indicate significant differences (p<0.05).

Table 2: Micronutrient content in spelt grain depending on the cultivar and harvesting time

Micronutrient	Oberkulmer Rotkorn		Rokosz		Wirtas	
	MDS	FRS	MDS	FRS	MDS	FRS
	(mg kg^{-1})					
Cu	9.21±0.36^{a}	9.49±0.27^{a}	6.73±0.68^{b}	7.55±0.74^{b}	5.02±0.029^{c}	4.68±0.33^{c}
Zn	31.80±0.82^{a}	26.85±0.45^{b}	20.30±026^{c}	18.05±0.15^{d}	34.50±0.33^{e}	29.75±0.21^{f}
Mn	23.10±0.20^{ae}	22.50±0.53^{a}	29.40±0.18^{b}	25.40±0.55^{c}	34.90±0.76^{d}	24.90±0.63^{c}
Fe	55.15±0.46^{a}	31.35±0.97^{b}	11.65±1.58^{c}	8.68±0.64^{c}	17.50±0.72^{d}	25.25±0.63^{e}
Na	16.10±0.42^{a}	7.30±0.52^{b}	15.65±0.16^{a}	2.93±0.54^{c}	8.98±0.66^{b}	12.65±0.41^{d}
B	3.00±0.15^{a}	2.53±0.17^{b}	1.90±0.26^{c}	1.88±0.22^{c}	2.75±0.24^{ab}	1.94±0.45^{c}

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; $^{a-f}$ different superscripts indicate significant differences (p<0.05).

breeding line of spelt (A12) harvested under conventional farming conditions.

In grain of the cultivars 'Oberkulmer Rotkorn and 'Wirtas' harvested at MDS, a higher content of P, Ca and B was determined than in ripe grain. At the same time, green grain of cv. 'Wirtas' exhibited a higher P content than grain of the other cultivars evaluated. In a study by Rachoń e l. (2015), the P content in ripe grain of the spelt cultivar 'Schwabenkorn' was from 4.53 g kg^{-1} DM to 4.74 g kg^{-1} DM. On the other hand, Kraska et al. (2013) determined the P content, depending on the spelt wheat cultivar, to be from 4.30 g kg^{-1} DM for cv. 'Badengold' to 4.81 g kg^{-1} DM in cv. 'Ostro'.

The grain content of Mg and Cu did not differ significantly between the cultivars. The Mn content in green grain of the spelt wheat cultivars 'Rokosz' and 'Wirtas' was higher than in ripe grain, but at the same time higher than in green grain of cv. 'Oberkulmer Rotkorn'. In grain of the spring breeding lines of spelt, Andruszczak (2018) obtained a similar Mg (1.04 – 1.27 g kg^{-1} DM) and Mn (24.0 – 26.4 mg kg^{-1} DM) content compared to the results in the present study, while Kraska et al. (2013) found a higher Mg content (from 1.46 g kg^{-1} DM to 1.69 g kg^{-1} DM).

In unripe grain of cv. 'Oberkulmer Rotkorn', the Fe content was significantly higher than in grain harvested at FRS, while at the same time it was higher than in all the cultivars evaluated. Moreover, the study by Kraska et al. (2013) did not find such a high Fe content in grain of any of the cultivars evaluated (39.42 mg kg^{-1} DM) as in unripe grain of cv. 'Oberkulmer Rotkorn' (55.15 mg kg^{-1} DM). Rachoń et al. (2015), in turn, also determined a high Fe content in cv. 'Schwabenkorn' grain (from 51.14 to 53.16 mg kg^{-1}), whereas Andruszczak (2018) found it to be 34.1 – 35.0 mg kg^{-1}. The Na content in green grain of cvs. 'Oberkulmer' and 'Rokosz' was higher than in ripe grain.

The content of the essential amino acids His and Phe and of the non-essential amino acids Glu, Pro and Ser was significantly higher in grain harvested at FRS than in green grain for all the spelt wheat cultivars in question (Table 3, Table 4). A similar relationship was found with regard to the grain content of Arg and Ile in the case of the cultivars 'Rokosz' and 'Wirtas', the content of Leu and Cys in the case of cvs. 'Oberkulmer Rotkorn' and 'Wirtas', the content of Thr and Asp in cv. 'Rokosz', as well as for the content of Gly in cvs. 'Rokosz' and 'Wirtas'. In turn, the content of Lys, Met, Ala and Tyr in green grain of all the cultivars evaluated was higher than that determined at the fully ripe stage. A similar relationship

Table 3: Essential amino acid content in spelt grain depending on the cultivar and harvesting time

Essential amino acid	Oberkulmer Rotkorn		Rokosz		Wirtas	
	MDS	FRS	MDS	FRS	MDS	FRS
	(mg g^{-1})					
Arg	6.42±0.22[a]	6.49±0.19[a]	4.83±0.15[b]	5.68±0.24[c]	6.44±0.25[a]	7.88±0.33[d]
His	3.20±0.07[a]	3.42±0.07[b]	2.21±0.06[c]	2.58±0.04[d]	2.94±0.09[e]	3.76±0.11[f]
Ile	4.73±0.17[a]	4.73±0.14[a]	3.17±0.12[b]	3.38±0.21[c]	4.71±0.23[a]	5.25±0.27[d]
Leu	9.52±0.14[a]	9.86±0.11[b]	6.54±0.08[c]	7.00±0.09[c]	9.16±0.16[d]	10.90±0.21[e]
Lys	4.56±0.16[a]	3.71±0.14[b]	3.85±0.10[b]	3.38±0.05[c]	6.40±0.26[d]	4.53±0.14[a]
Phe	6.16±0.14[a]	6.66±0.12[b]	4.01±0.08[c]	4.48±0.06[d]	5.75±0.26[a]	7.74±0.31[e]
Thr	3.89±0.17[a]	3.79±0.13[a]	2.86±0.09[b]	2.96±0.12[b]	4.54±0.33[d]	4.37±0.31[d]
Val	5.95±0.23[a]	5.99±0.26[a]	4.18±0.15[b]	4.49±0.26[b]	6.23±0.19[c]	6.50±0.41[c]
Met	2.99±0.19[a]	2.63±0.16[b]	2.38±0.11[c]	1.87±0.08[d]	3.23±0.17[e]	2.89±0.06[f]

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; [a-f] different superscripts indicate significant differences (p<0.05).

Table 4: Nonessential amino acid content in spelt grain depending on the cultivar and harvesting time

Nonessential amino acid	Oberkulmer Rotkorn		Rokosz		Wirtas	
	MDS	FRS	MDS	FRS	MDS	FRS
	(mg g^{-1})					
Ala	5.38±0.18[a]	4.59±0.14[b]	4.30±0.12[b]	3.86±0.08[d]	6.99±0.26[e]	5.40±0.22[a]
Asp	7.17±0.17[a]	6.81±0.06[b]	5.40±0.09[c]	5.81±0.07[d]	10.10±0.33[e]	8.36±0.28[f]
Glu	36.90±1.36[a]	42.50±1.92[b]	22.90±1.12[c]	27.00±1.34[d]	29.00±1.26[e]	47.60±2.12[f]
Gly	5.18±0.29[a]	5.17±0.33[a]	4.08±0.26[b]	4.31±0.22[b]	5.93±0.52[d]	6.28±0.63[e]
Pro	15.30±0.24[a]	17.40±0.46[b]	8.89±0.22[c]	11.20±0.31[d]	11.30±0.23[d]	19.10±0.32[e]
Ser	6.22±0.07[a]	6.55±0.12[b]	4.28±0.09[c]	4.49±0.06[d]	6.58±0.23[b]	7.70±0.26[e]
Tyr	4.14±0.05[a]	3.40±0.04[b]	3.46±0.06[b]	2.44±0.08[c]	5.84±0.21[d]	4.19±0.07[a]
Cys	3.95±0.12[a]	4.22±0.16[b]	2.78±0.07[c]	2.64±0.07[d]	3.37±0.14[e]	4.71±0.17[f]

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; [a-f] different superscripts indicate significant differences (p<0.05).

Table 5: Fat content and fatty acid composition in spelt grain depending on the cultivar and harvesting time

Fat/Fatty acid	Oberkulmer Rotkorn		Rokosz		Wirtas	
	MDS	FRS	MDS	FRS	MDS	FRS
	(%)					
Fat	2.65±0.56[a]	1.74±0.18[b]	2.29±0.13[c]	1.75±0.15[b]	2.56±0.24[a]	1.82±0.19[b]
C16:0	14.45±1.36[a]	15.21±1.28[a]	14.68±1.15[a]	15.36±1.32[a]	16.36±1.58[a]	16.73±1.49[a]
C18:0	1.13±0.17[a]	1.19±0.22[a]	1.29±0.24[a]	1.38±0.19[a]	0.86±0.13[a]	0.97±0.18[a]
C18:1	27.08±3.68[a]	22.79±1.85[a]	23.44±1.93[a]	19.23±1.52[a]	22.18±2.16[a]	18.97±3.21[a]
C18:2	51.45±2.53[a]	55.47±2.67[a]	53.68±2.28[a]	57.71±3.17[a]	50.06±2.56[a]	57.15±4.12[a]
C18:3n3 (alpha)	3.49±0.26[a]	3.08±0.12[b]	4.63±0.20[c]	3.83±0.33[d]	7.65±0.47[e]	3.60±0.28[ad]
C20:1	1.02±0.12[a]	0.83±0.14[a]	0.83±0.13[a]	0.84±0.11[a]	1.10±0.16[a]	0.88±0.12[a]
Others	1.38±0.23[a]	1.43±0.31[a]	1.45±0.25[a]	1.65±0.15[b]	1.79±0.26[b]	1.70±0.21[b]
SFA	16.37±1.04[a]	17.30±1.25[a]	16.78±1.18[a]	17.75±1.42[a]	18.18±1.58[a]	18.61±1.41[a]
MUFA	28.56±3.63[a]	24.02±1.85[a]	24.71±1.92[a]	20.56±2.46[a]	23.91±2.16[a]	20.44±2.51[a]
PUFA	55.07±4.18[a]	58.63±5.32[a]	58.38±4.98[a]	61.63±5.61[a]	57.83±5.47[a]	60.86±6.02[a]

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; [a-e] different superscripts indicate significant differences ($p<0.05$); SFA-Saturated Fatty Acid; MUFA-Mono Unsaturated Fatty Acid; PUFA-Poli Unsaturated Fatty Acid.

Table 6: Content of compounds in diethyl ether extract from spelt grain depending on the cultivar and harvesting time (%)

Compound	$RI_{exp.}$	$RI_{lit.}$	Oberkulmer Rotkorn		Rokosz		Wirtas	
			MDS	FRS	MDS	FRS	MDS	FRS
Fatty acids			32.34±0.16	30.98±0.15	28.90±0.14	23.41±0.12	30.21±0.15	34.43±0.17
Resorcinol esters, including:			37.49±0.19	64.24±0.32	40.71±0.20	70.32±0.35	18.19±0.09	63.10±0.32
5-Heptadecyl resorcinol	2912	2912	1.25±0.05	0.92±0.05	1.47±0.06	1.19±0.05	0.43±0.02	0.97±0.05
5-Nonadecyl resorcinol	3103	3104	13.50±0.07	17.65±0.09	15.34±0.08	22.85±0.11	5.72±0.03	18.53±0.09
5-Heneicosyl resorcinol	3300	3302	19.52±0.10	39.14±0.20	20.69±0.10	41.43±0.21	10.03±0.05	36.85±0.18
5-Tricosyl resorcinol	3497	3497	2.71±0.08	4.73±0.05	2.87±0.09	4.14±0.04	1.62±0.06	5.18±0.03
5-Pentacosyl resorcinol	3693	3693	0.49±0.02	1.80±0.07	0.35±0.02	0.71±0.04	0.39±0.02	1.57±0.06
Sterols			0.71±0.04	3.40±0.07	3.41±0.07	5.24±0.03	1.40±0.06	1.35±0.05
Hydroxy acids, including:			2.54±0.08	-	2.58±0.08	-	2.69±0.08	-
Lactic acid	1070	1071	1.59±0.06	-	1.79±0.07	-	0.75±0.04	-
Glycolic acid	1084	1084	0.27±0.01	-	0.17±0.01	-	0.49±0.02	-
2-Hydroxy-butanoic acid	1139	1140	-	-	0.16±0.01	-	0.10±0.01	-
3-Hydroxy-propanoic acid	1153	1155	0.52±0.03	-	0.24±0.01	-	1.03±0.04	-
3-Hydroxy-butanoic acid	1170	1170	-	-	-	-	0.16±0.01	-
4-Hydroxy-butanoic acid	1245	1247	0.16±0.01	-	0.23±0.01	-	0.16±0.01	-
Phenolic acids, including:			-	-	-	-	0.26±0.01	-
4-Hydroxy-benzoic acid	1632	1633	-	-	-	-	0.17±0.01	-
Vanillic acid	1775	1776	-	-	-	-	0.09±0.01	-
Cinnamic acids, including:			0.37±0.02	-	0.49±0.02	-	1.26±0.05	-
Cinnamic acid	1547	1549	0.15±0.01	-	-	-	-	-
(E)-p-Coumaric acid	1945	1946	-	-	-	-	0.28±0.01	-
(E)-Ferulic acid	2102	2103	0.22±0.01	-	0.35±0.02	-	0.74±0.04	-
(E)-Caffeic acid	2155	2155	-	-	0.14±0.01	-	0.13±0.01	-
Sinapinic acid	2255	2256	-	-	-	-	0.11±0.01	-
Dicarboxylic acids, including:			6.20±0.03	-	4.83±0.05	-	8.37±0.04	-
Succinic acid	1323	1324	5.34±0.03	-	4.13±0.04	-	6.95±0.03	-
Fumaric acid	1356	1358	0.57±0.03	-	0.37±0.02	-	0.60±0.03	-
Azelaic acid	1807	1808	0.30±0.02	-	0.33±0.02	-	0.72±0.04	-
Sebacic acid	1904	1905	-	-	-	-	0.10±0.01	-
Other compounds, among others:			20.35±0.10	1.38±0.06	19.08±0.0.10	1.03±0.04	37.62±0.19	1.12±0.04
Niacin	1291	1293	0.58±0.03	-	0.27±0.01	-	1.08±0.04	-
Glycerol	1294	1294	13.86±0.07	1.37±0.06	10.55±0.05	1.02±0.04	19.80±0.10	1.12±0.04

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; RI_{exp}-experimental retention indices; RI_{lit}-literature retention indices

Table 7: Content of compounds in hexane extract from spelt grain depending on the cultivar and harvesting time (%)

Compound	$RI_{exp.}$	$RI_{lit.}$	Oberkulmer Rotkorn		Rokosz		Wirtas	
			MDS	FRS	MDS	FRS	MDS	FRS
Fatty acids, among others:			76.58±0.38	72.59±0.36	66.58±0.33	54.60±0.27	86.97±0.43	86.49±0.43
Palmitic acid	2049	2050	16.20±0.08	23.71±0.12	16.48±0.08	13.50±0.07	23.43±0.12	19.64±0.10
Linoleic acid	2212	2214	39.13±0.20	26.83±0.13	33.31±0.17	21.44±0.11	36.64±0.18	39.68±0.20
Oleic acid	2222	2224	17.48±0.09	18.87±0.09	13.94±0.07	13.52±0.07	21.08±0.11	22.08±0.11
Stearic acid	2049	2249	0.59±0.03	1.23±0.05	0.64±0.03	0.69±0.03	0.70±0.04	0.81±0.04
Resorcinol esters			11.81±0.06	13.30±0.07	21.24±0.11	21.64±0.11	1.96±0.08	5.41±0.03
Sterols, among others:			7.37±0.04	7.51±0.04	9.55±0.05	19.12±0.10	5.75±0.03	5.39±0.03
Campesterol	3255	3255	0.57±0.03	1.04±0.04	1.39±0.06	2.82±0.08	1.43±0.06	0.93±0.05
Stigmasterol	3285	3285	0.22±0.01	-	0.31±0.02	0.39±0.02	0.22±0.01	0.13±0.01
β-Sitosterol	3344	3345	2.52±0.08	4.90±0.05	5.78±0.03	10.89±0.05	3.64±0.07	3.18±0.06
Stigmastanol	3353	3353	-	0.85±0.04	0.91±0.05	2.00±0.06	0.20±0.01	0.50±0.03
Avenasterol	3357	3358	0.26±0.01	0.73±0.04	0.54±0.03	1.03±0.04	-	0.26±0.01
Other compounds			4.24±0.04	6.60±0.03	2.63±0.08	4.64±0.05	5.32±0.03	2.71±0.08

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; $RI_{exp.}$-experimental retention indices; $RI_{lit.}$-literature retention indices

Table 8: Content of compounds in methanol extract from spelt grain depending on the cultivar and harvesting time (%)

Compound	$RI_{exp.}$	$RI_{lit.}$	Oberkulmer Rotkorn		Rokosz		Wirtas	
			MDS	FRS	MDS	FRS	MDS	FRS
Carbohydrates, among others:			62.65±0.31	91.18±0.46	70.54±0.35	90.92±0.45	68.30±0.34	90.79±0.45
α-Fructofuranose	1842	1843	2.99±0.09	-	3.08±0.06	-	7.20±0.04	0.07±0.01
β-Fructofuranose	1854	1854	10.18±0.05	1.90±0.08	8.86±0.04	1.43±0.06	15.96±0.08	1.55±0.06
α-D-Glucopyranose	1935	1935	6.66±0.03	1.19±0.05	6.99±0.03	0.71±0.04	11.20±0.06	0.75±0.04
Glucitol	1978	1980	1.00±0.04	0.99±0.05	1.35±0.05	1.01±0.04	0.18±0.01	0.36±0.02
β-D-Glucopyranose	2030	2032	5.46±0.03	1.46±0.06	5.73±0.03	0.87±0.04	7.35±0.04	0.92±0.05
myo-Inositol	2130	2130	3.07±0.06	-	2.54±0.08	-	2.16±0.06	-
Saccharose	2711	2714	16.02±0.08	59.33±0.30	15.31±0.08	65.26±0.33	8.14±0.04	48.29±0.24
Raffinose	3504	3504	0.44±0.02	3.02±0.06	0.45±0.02	3.36±0.07	-	13.11±0.07
Kestose	3715	3517	9.05±0.05	13.82±0.07	13.76±0.07	9.37±0.05	4.90±0.05	19.11±0.10
Amino acids, among others:			16.52±0.08	-	11.10±0.06	0.16±0.01	13.77±0.7	0.60±0.03
Alanine	1110	1110	2.79±0.08	-	1.61±0.06	0.16±0.01	1.55±0.06	0.21±0.01
Valine	1126	1227	1.14±0.05	-	0.68±0.03	-	1.05±0.04	0.20±0.01
Leucine	1282	1284	1.09±0.04	-	0.69±0.03	-	1.06±0.04	-
Proline	1301	1303	1.04±0.04	-	0.71±0.04	-	1.21±0.05	-
Serine	1368	1370	2.28±0.07	-	1.48±0.06	-	1.53±0.06	-
4-Aminobutyric acid	1540	1541	2.36±0.07	-	2.38±0.07	-	2.57±0.08	-
Hydroxy acids, among others:			3.51±0.07	0.40±0.02	3.19±0.06	0.36±0.02	3.28±0.07	0.27±0.01
Malic acid	1512	1512	2.76±0.08	0.40±0.02	2.70±0.08	0.36±0.02	1.97±0.08	0.27±0.01
Quinic acid	1900	1901	0.35±0.02	-	-	-	1.08±0.04	-
Phenolic acids, including:			-	-	-	0.37±0.02	-	0.30±0.02
Gallic acid	1985	1985	-	-	-	0.37±0.02	-	0.30±0.02
Dicarboxylic acids, among others:			1.67±0.07	-	1.86±0.07	-	1.08±0.04	-
Malonic acid	1215	1216	-	-	0.03±0.01	-	0.08±0.01	-
Other compounds, among others:			15.65±0.08	8.42±0.04	13.31±0.07	8.19±0.04	13.57±0.07	8.04±0.04
Phosphoric acid	1289	1290	7.89±0.04	-	6.43±0.03	-	4.00±0.04	0.66±0.03
Glycerol	1294	1294	3.80±0.08	3.61±0.07	3.79±0.08	2.27±0.07	4.59±0.05	4.96±0.05

MDS-grain harvested at the milk-dough stage (green grain); FRS-grain harvested at the fully ripe stage; $RI_{exp.}$-experimental retention indices; $RI_{lit.}$-literature retention indices was determined with respect to the content of Thr and Asp in cvs. 'Oberkulmer Rotkorn' and 'Wirtas' as well as for the content of Cys in cv. 'Rokosz'. The grain content of the essential amino acid Val did not differ significantly. Nevertheless, its content clearly tended to be higher in grain harvested at the fully ripe stage.

Analyzing the amino acid composition, Waga et al. (2002) demonstrated spelt wheat to be characterized by an about 20–60% higher content of nearly all amino acids compared to common wheat grain. In grain of eight winter spelt cultivars, Andruszczak (2017) determined a similar content of amino acids as in grain of the evaluated cultivar 'Oberkulmer Rotkorn', while higher values compared to cv 'Rokosz'. Furthermore, the author reported a lower content of Arg, Lys, Met, Ala, Asp, Gly, Tyr and Cys compared to cultivar 'Wirtas' evaluated in the present experiment.

In grain of the spelt wheat cultivar KR 489-11-15, Dvořáček et al. (2002) found the lysine content to be 4.21 g kg^{-1}, and thus similar to that obtained in this study in ripe grain of cv. 'Wirtas' and in green grain of cv. 'Oberkulmer Rotkorn', but at the same time a clearly lower content than in green grain of cv. 'Wirtas'. In a study by Andruszczak (2017), cv. 'Schwabenkorn' produced the most valuable grain in terms of amino acid content.

The fat content found in green grain of all the spelt wheat cultivars evaluated was higher than in grain harvested at FRS (Table 5). Escarnot et al. (2012) and Andruszczak (2018) reported similar fat content in spelt grain to that found in ripe grain of spelt in the present study. Grausgruber et al. (2004) determined the fat content in spelt flour to be at a level of 2.19%. In ripe grain of the spelt wheat cultivar 'Bauländer', Sulewska et al. (2008) found the fat content to be 2.38%, while in cv. 'Schwabenkor' grain 2.33%; thus, these were values similar to the fat content in green grain found in this study. At the same time, in comparison to the study by Sulewska et al. (2008), in the present study a much lower fat content was obtained in grain harvested at the fully ripe stage. On the other hand, the content of fatty acids C16:0, C18:0, C18:1, C18:2 and C:20:1 did not vary for grain of the spelt wheat cultivars studied. Only the content of acid C18:3 found in green grain of all the spelt wheat cultivars was higher than in ripe grain. When comparing more than 40 spelt wheat genotypes, Escarnot et al. (2012) report a slightly higher grain content of acid C16:0 (16.7 – 18.5% DM), a similar content of C18:0 (0.7 – 1.4% DM) and C18:2 (55.0 –63.2% DM) in comparison with the present study. At the same time, the content of C18:3n3 determined in cv. 'Wirtas' grain at FRS was by far higher than that found by Escarnot et al. (2012).

The content of SFA, MUFA and PUFA did not differ significantly depending on the cultivar and grain harvest stage (Table 5). However, it was found that the content of SFA and PUFA tended to be higher in grain harvested at FRS, while the content of MUFA in green grain of spelt wheat. Filipović et al. (2016) found a higher content of SFA (19.05%) and PUFA (65.05%) in spelt flour, but a distinctly lower content of MUFA (16.08%) compared to grain of the different spelt wheat cultivars evaluated in this study.

Spelt grain harvested at MDS and FRS had a similar content of fatty acids and sterols determined in diethyl ether extract (Table 6). Unripe grain of cvs. 'Oberkulmer Rotkorn' and 'Rokosz', compared to FRS, was characterized by a significantly higher linoleic acid content as well as an almost twice lower content of campesterol, β-sitosterol and avenasterol. Grain of all the spelt wheat cultivars harvested at the fully ripe stage, compared to milk-dough grain, contained a significantly higher amount of resorcinol esters (Table 7) and carbohydrates (Table 8) as well as an almost twenty-fold lower content of amino acids (Table 8). The presence of hydroxy acids such as lactic acid, glycolic acid (Table 7) and quinic acid (Table 8) was found exclusively in milk-dough grain. Malic acid, in turn, occurred in grain harvested both at MDS and FRS stage. The content of cinnamic (Table 7) and dicarboxylic acids (Table 7 and Table 8) was only found in milk-dough spelt. 4-hydroxybenzoic and vanillic acids were identified only in the spelt cultivar 'Wirtas' harvested at the milk-dough stage (Table 7), while gallic acid only in grain of the cultivars 'Rokosz' and 'Wirtas' harvested at the fully ripe stage (Table 8). Moreover, the presence of niacin, phosphoric acid and glycerol was found in the examined samples (Table 7 and Table 8).

CONCLUSIONS

Spelt wheat harvested at the milk-dough stage has proved to be an interesting product that can be used in human nutrition. The macro- and micronutrient content in green grain of the evaluated cultivars was or tended to be higher compared to ripe grain. Only the N content in ripe grain of cv. 'Wirtas' was significantly higher than in grain harvested at the milk-dough stage, while the Fe content in ripe grain tended to be higher. In turn, the content of the essential amino acids Lys and Met and of the non-essential amino acids Ala and Tyr in green grain of all the cultivars evaluated was higher than that determined for grain harvested at the fully ripe stage. Likewise, green grain of the cultivars 'Oberkulmer Rotkorn' and 'Wirtas' was found to have a higher content of Thr and Asp, while cv. 'Rokosz' grain had a higher content of the non-essential amino acid Cys. The fat content in grain harvested at the milk-dough stage of each of the evaluated spelt wheat cultivars was also higher than in grain harvested at the fully ripe stage. Cinnamic and dicarboxylic acids were found to be present only in spelt harvested at the milk-dough stage. The grain material in question also contained larger amounts of hydroxy and phenolic acids. Based on the present study, it has been found that green spelt grain is characterized by a higher content of biologically active substances, which are a factor determining the health-promoting properties of a product. Green spelt grain used as an additive in bakery can largely improve the nutritional value of bakery products made (bread).

REFERENCES

Abdel-Aal, E. S. M. and I. Rabalski. 2008. Effect of baking on nutritional properties of starch in organic spelt whole grain products. Food Chem. 111(1): 150-156.

Andruszczak, S. 2017. Reaction of winter spelt cultivars to reduced tillage system and chemical plant protection. Zemdirbyste Agric. 104(1): 15-22.

Andruszczak, S. 2018. Spelt wheat grain yield and nutritional value response to sowing rate and nitrogen fertilization. J. Anim. Plant Sci. 28(5): 1476-1484.

Andruszczak, S., E. Kwiecińska-Poppe, P. Kraska and E. Pałys. 2011. Yield of winter cultivars of spelt wheat (*Triticum aestivum* ssp. Spelta L.) cultivated under diversified conditions of mineral fertilization and chemical protection. Acta Sci. Pol. Agric. 10(4): 5-14.

Biel, W., A. Jaroszewska, J. Sadkiewicz and P. Bośko. 2016b. Effects of genotype and weed control on the nutrient composition of winter spelt (*Triticum aestivum* ssp. Spelta L.) and common wheat (*Triticum aestivum* ssp. Vulgare). Acta Agric. Scand. B Soil Plant Sci. 66(1): 27-35.

Biel, W., S. Stankowski, A. Jaroszewska, S. Pużyński and P. Bośko. 2016a. The influence of selected agronomic factors on the chemical composition of spelt wheat (*Triticum aestivum* ssp. Spelta L.) grain. J. Integr. Agric. 15(8): 1763-1769.

Davies, M. G. and A. J. Thomas. 1973. An investigation of hydrolytic techniques for the amino acid analysis of foodstuffs. J. Sci. Food Agric. 24: 1525-1540.

Dvořáček, V., V. Čurn and J. Moudrý. 2002. Evaluation of amino acid content and composition in spelt wheat varieties. Cereal Res. Commun. 30(1-2): 187-193.

Escarnot, E., J. M. Jacquemin, R. Agneessens and M. Paquot. 2012. Comparative study of the content and profiles of macronutrients in spelt and wheat, a review. Biotechnol. Agron. Soc. Environ. 16(2): 243-256.

Filipović, J., M. Košutić, V. Filipović and R. Razmovski. 2016. Chemical composition of fatty acids in spelt and flaxseed pasta. J. Process. Energy Agric. 20(3): 140-142.

Gawlik-Dziki, U., M. Świeca and D. Dziki. 2012. Comparison of phenolic acids profile and antioxidant potential of six varieties of spelt (*Triticum spelta* L.). J. Agric. Food Chem. 60(18): 4603-4612.

Grausgruber, H., J. Scheiblauer, R. Schönlechner, P. Ruckenbauer and E. Berghofer. 2004. Variability in chemical composition and biologically active constituents of cereals. In: Vollmann, J., H. Grausgruber and P. Ruckenbauer (Eds.), Genetic Variation for Plant Breeding, EUCARPIA and BOKU, Vienna, Austria, p. 23-26.

Grela, E. 1996. Nutrient composition and content of antinutritional factors in spelt (*Triticum spelta* L.) cultivars. J. Sci. Food Agric. 71: 399-404.

Isidorov, V. 2015. Identification of Biologically and Environmentally Significant Organic Compounds Mass Spectra and Retention Indices Library of Trimethylsilyl Derivatives, PWN, Warszawa, p. 429.

Jablonskytė-Raščė, D., S. Maikštėnienė and A. Mankevičienė. 2013. Evaluation of productivity and quality of common wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.) in relation to nutrition conditions. Zemdirbyste Agric. 100(1): 45-55.

Kohajdová, Z. and J. Karovičová. 2008. Nutritional value and baking applications of spelt wheat. Acta Sci. Pol. Technol. Aliment. 7(3): 5-14.

Kraska, P., S. Andruszczak, E. Kwiecińska-Poppe and E. Pałys. 2013. Effect of chemical crop protection on the content of some elements in grain of spelt wheat (*Triticum aestivum* ssp. Spelta). J. Elementol. 1: 79-90.

Kwiatkowski, C. A., M. Wesołowski, E. Pałys, P. Kraska, M. Haliniarz, A. Nowak, S. Andruszczak and E. Kwiecińska-Poppe. 2014. Pszenica orkisz-ekstensywne zboże powraca na pola. In: Aspekty Proekologicznego Gospodarowania w Agroekosystemach, Perfekta info Press, Lublin, Poland, p. 95-118.

Kwiecińska-Poppe, E., S. Andruszczak, P. Kraska and E. Pałys. 2011. The influence of chemical protection levels on quality of spelt wheat (*Triticum spelta* L.) grain. Prog. Plant Prot. 51(2): 986-989.

Rachoń, L., G. Szumiło, M. Brodowska and A. Woźniak. 2015. Nutritional value and mineral composition of grain of selected wheat species depending on the intensity of a production technology. J. Elementol. 20(3): 705-715.

Rudy, S., D. Dziki, A. Krzykowski, U. Gawlik-Dziki, R. Polak, R. Rózyło and R. Kulig. 2015. Influence of pre-treatments and freeze-drying temperature on the process kinetics and selected physico-chemical properties of cranberries (*Vaccinium macrocarpon* ait.). LWT Food Sci. Technol. 63(1): 497-503.

Schram, E., S. Moore and E. J. Bigwood. 1954. Chromatographic determination of cystine as cysteic acid. Biochem. J. 57(1): 33-37.

Shewry, P. and S. Hey. 2015. Do “ancient” wheat species differ from modern bread wheat in their contents of bioactive components? J. Cereal Sci. 65: 236-243.

Stocki, M., E. Zapora, E. Rój and S. Bakier. 2018. Recovering biologically active compounds from logging residue of birch (*Betula* spp.) with supercritical carbon dioxide. Przem. Chem. 97(5): 1000-1004.

Sulewska, H., W. Koziara, K. Panasiewicz, G. Ptaszyńska and M. Mrozowska. 2008. Chemical composition of grain and protein yield of spelt cultivars depending on selected agrotechnical factors. J. Res. Appl. Agric. Eng. 53(4): 92-95.

Świeca, M., D. Dziki, U. Gawlik-Dziki, R. Różyło, S. Andruszczak, P. Kraska, D. Kowalczyk, E. Pałys and B. Baraniak. 2014. Grinding and nutritional properties of six spelt (*Triticum aestivum* ssp. Spelta L.) cultivars. Cereal Chem. 91(3): 247-254.

Tyburski, J. and M. Babalski. 2006. Uprawa Pszenicy Orkisz. Poradnik dla Rolników, CDR Brwinów, Radom.

Vučković, J., M. Bodroža-Solarov, D. Vujić, A. Bočarov-Stančić and F. Bagi. 2013. The protective effect of hulls on the occurrence of alternaria mycotoxins in spelt wheat. J. Sci. Food Agric. 93(8): 1996-2001.