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Phenolic nutrient composition and grain morphology of winter spelt wheat (*Triticum aestivum* ssp. *spelta*) cultivated in Poland

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RESEARCH ARTICLE

Abstract

This study analysed the genotypic variation in the physical features of kernels and accumulation of phenolic acids and alkylresorcinols in the grain of 16 spelt wheat genotypes grown under the same environmental conditions in Poland. The average values of 1000-kernel weight, kernel length, width and specific density were 49.9 g, 8.08 mm, 3.32 mm and 1.24 g/cm^3, respectively, with variation between genotypes ranging from 4.03 to 7.70%. The average values of colour attributes hue, saturation and intensity were 35.5°, 32.7% and 58.4%, respectively. The lowest variation of these features was noted for surface hue (1.6%), while the highest was for saturation (5.4%). The content of so-called 'free' phenolic acids ranged from 16.0 to 27.3 mg/kg, with a predominant share of ferulic (ca. 45.8%) and *p*-coumaric acids (circa 18.6%). The total content of phenolic acids was between 206 and 557 mg/kg (345 mg/kg on average). Ferulic acid share was between 86.6 and 91.6% of the total. The average content of alkylresorcinols was 723 mg/kg, and ranged from 561 to 919 mg/kg. The main homologues were heneicosylresorcinol (circa 58%), nonadecylresorcinol (circa 30%), and tricosylresorcinol (circa 6%). The intra-species variation was approximately 3-fold higher for phenolic acids than for alkylresorcinols (37.2 and 13.0%, respectively).

Keywords: phenolic acids, alkylresorcinols, kernel colour, kernel dimensions, thousand kernel weight

1. Introduction

The grain of modern, highly-developed wheat species (*Triticum aestivum*) is considered by a significant number of consumers as a possible cause of obesity and various health problems (De Punder and Pruimboom, 2013; Fardet, 2015). In reaction to these concerns, there has been (especially in highly-developed countries) a growing interest in the cultivation of ancient wheat species, such as diploid einkorn, tetraploid emmer and hexaploid spelt (Longin and Würschum, 2016). Among them, spelt wheat is of the highest industrial importance (Longin and Würschum, 2014). In recent years it has received special market interest (Ziegler *et al.*, 2016). Currently, spelt wheat is cultivated in Europe on an area of over 100,000 ha, primarily in Germany, Austria, Switzerland, Hungary and Poland (Longin and Würschum, 2014; Tyburski and Babalski, 2006). It is preferentially grown in low-input (ecological) or stress environments, although the potential yield of this crop is maximised under high-input conditions (Longin and Würschum, 2014). In 2003, the area of organic spelt crops in Germany was 9,500 ha, while in 2004 it increased to 22,833 ha. Similarly, according to the Agricultural Product Quality Inspection report, in 2004 the area of spelt cultivated in Poland was about 200 ha, and in 2005 it increased to about 400-500 ha (Majewska *et al.*, 2007). In Europe the area under spelt cultivation increased between years 2010 and 2014 from about 18,000 ha to 60,000 ha (Szymańska, 2016). More recent data for Europe production is difficult to find, and even Eurostat summarises the results of bread and spelt wheat cultivation/production as one group of cereal crop (Eurostat, 2016). In the actual 'Common Catalogue of Varieties of Agricultural Plant Species' (EC, 2016) there are 45 registered varieties of *Triticum spelta* L. and four conservation varieties of *Triticum spelta* L. Among them

two are registered in Poland: spring cultivar Wirtas and winter cultivar Rokosz (COBORU, 2016).

Current renaissance of spelt grain is due to the perception that it is 'healthier', more 'natural', or less 'over-bred' than grain of modern wheat (Pruska-Kędzior *et al.*, 2008). The renewed interest in this species is mostly related to its better nutritional quality as a source of valuable protein, lipids and crude fibre (Kohajdová and Karovicova, 2008). Additionally, spelt wheat is assumed to be a richer source of low molecular weight, nutritionally valuable bioactive components and, hence, especially suitable for the production of high value food products with enhanced health benefits (Chen *et al.*, 2015; Lachman *et al.*, 2013; Okarter *et al.*, 2010). Phenolic acids and alkylresorcinols belong to the main pro-healthy components of spelt grain. The health benefits of phenolic acids are associated with their antioxidant activity. They are able to inhibit the oxidation of low-density lipoproteins (LDL) as well as the oxidation of lipid membranes and DNA, what reduce risk of the development of cardiovascular diseases and neoplasms (Andersson *et al.*, 2014). Likewise to phenolic acids, alkylresorcinols are also considered to be compounds which act as antioxidants *in vitro* (Lutharia *et al.*, 2015). Studies on *in vitro* models have shown their inhibitory action on lipoxygenase, copper-induced LDL oxidation, DNA-strand scission, colon cancer cell growth and lipase activity in the adipose tissue cells (Andersson *et al.*, 2014). However, a recent review by Shewry and Hey (2015) showed that a definitive comparison of spelt species with other ancient and modern bread and durum wheat cultivars, especially in relation to bioactive components, is still incomplete. It is hindered, for example, by farming systems that, for ancient wheat species, are generally conducted in organic or traditional low input farming systems, while modern wheat species are usually bred for high input intensive systems (Lu *et al.*, 2015; Shewry and Hey, 2015).

In the present study, an extensive study of sixteen spelt wheat genotypes grown under the same environment conditions in Poland (location, climate and agriculture treatment) was carried out to determine the genotype variation in the content and composition of low molecular weight phenolic compounds deposited in grain: phenolic acids and alkylresorcinols. Additionally, the main morphological features (dimensions, surface colour, 1000-kernel weight and specific density) were analysed to detect variations between genotypes.

2. Materials and methods

Materials

The experiment was conducted in 2015 in Swadzim (Poland) near Poznan (52°26'N, 16°45'E) using sixteen winter spelt (*Triticum aestivum* ssp. *spelta*) wheat genotypes: Schwaben-Speltz; Schwabenkorn, Line 28-46, Line 54-76, Line 55-97, Franckenkorn, Oberkulmer Rothkorn, Badengold, Ceralio, Ostro, Spelt INZ, Rokosz, STH 11, STH 12, Filderstolz and Divimar.

The soil of the experimental fields was classified as luvisol soils, sand texture, shallowly deposited on good rye complex light clay (FAO, 2006). The experiment was established as one factorial in four replications. The forecrop was winter wheat and the plot size for harvest was 15 m^2. In the autumn, the following fertilisers were applied per 1 hectare: 21 kg N, 54 kg P, 84 kg K and 33 kg S. The seeds were planted on 26 September 2014 in the optimum period of agricultural technology. In the spring, after the start of vegetation (25 March 2015) 30 kg N/ha was applied and in the shooting phase (BBCH 34) (5 May 2015) 30 kg N/ha was applied. The plants were protected from weeds using an autumn (28 October 2014) spray of Legato plus 600 SC in a dose of 1.25 l/ha + Granstar ultra SX50SG with a dose of 50 g/ha. The water supply of wheat plants in the vegetation season of 2015 is presented in the form of Sielianinov's hydrothermal coefficient (Figure 1). Hydrothermal Sielianinov K factor (Molga, 1986) was calculated according to the following equation:

$$K = (P \times 10) / (T \times L)$$

where: K = hydrothermal Sielianinov factor, P = total monthly precipitation, T = average temperature of the month and L = number of the days of the month.

Plant growth in the spring proceeded under conditions of insufficient humidity and rain in the first half of April and early May (13.9 and 19.6 mm, respectively). Improved moisture conditions occurred in early June and up to the end of July the water supply was good. A period of drought which occurred in August, favoured plant maturation.

After harvesting and dehulling, grain samples of about 200 g were sifted to separate foreign materials and small and broken kernels using a sieve with 2.2×25 mm meshes. The prepared samples were then dried to circa 14% of moisture and stored at 8±2 °C. Before further analyses, the required amount of grain was equilibrated to room temperature and humidity. Prior to chemical analyses, the grain was carefully ground in a type A10 IKA Labortechnik mill (Staufen, Germany). All chemical analyses were done in triplicate.

Kernel features

The 1000-kernel weight (TKW) was analysed using an LN-S-50 Unitra Cemi (Szczytno, Poland) seed counter in five replicates. The kernel dimensions (length and width) and colour of surface were determined for 100 kernels using the digital image analysis according to Konopka *et al.* (2012). The images were acquired by a high resolution,

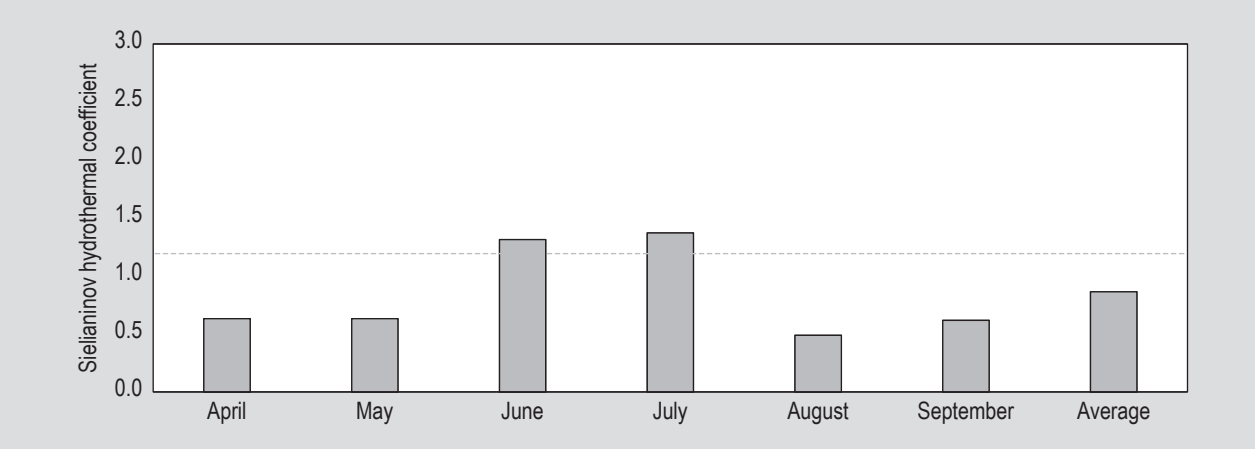

Figure 1. Sielianinov hydrothermal coefficient according to weather conditions from April to September at Experimental Station Swadzim in 2015. Interpretation of Sielianinov's hydrothermal coefficient: K>1.5: moisture for all plants excessively wet; K=1.0-1.5: sufficient moisture; K=0.5-1.0: insufficient moisture; K<0.5: moisture less than the requirement for most of plants – drought. Dashed line indicates optimal conditions for growth.

low-noise Nikon DXM-1200 (Nikon Inc., Melville, NY, USA) charge-coupled device colour camera at a resolution of 1,280×1,024 pixels and analysed with LUCIA G Version 4.8 software (Laboratory Imaging, Prague, Czech Republic). The results of grain surface colour are presented in HSI (H – hue, S – saturation, I – intensity) colour space, where H is expressed in degrees, and S and I in percentage. Kernel specific density (g/cm^3) was calculated as a ratio of single kernel mass and apparent volume using toluene pycnometer method (Markowski *et al.*, 2013).

Phenolic acids

Total phenolic acids were extracted in triplicate with diethyl ether from alkaline hydrolysate of ground grain, while free phenolic acids with 70% methanol from untreated sample according to the method of Konopka *et al.* (2012). Chromatographic analysis was performed by the RP-HPLC technique according to method described by Bojarska *et al.* (2011). An Agilent Technologies (Santa Clara, CA, USA) 1200 series system fitted out with a photodiode detector and with an Agilent Technologies Eclipse XDB-C18 column (150×4.6 mm, 5 µm) at the temperature of 30 °C was used. The mobile phase consisted of water:acetonitrile:formic acid (88:10:2, v/v/v). The isocratic flow rate was equal to 0.8 ml/min. Detection was performed at the wavelength of 260 and 320 nm. Phenolic acids were identified by comparing with retention times and absorption spectra of the reference phenolic acids (Sigma-Aldrich, St. Louis, MO, USA). The content of phenolic acids was determined from the calibration curve of the ferulic acid reference standard (≥99.0% purity) and expressed as mg of ferulic acid equivalent in kg of a sample dry mass (DM). The repeatability for ferulic acid determination (expressed as coefficient of variation) was 2.1 and 3.5% (for free and total form). Limit of quantification (LOQ) was 0.05 mg/kg of sample DM, while linearity of calibration curve was confirmed in range of 1-150 µg/ml.

Alkylresorcinols

The content of alkylresorcinols was determined according to the method described by Sampietro *et al.* (2009). Extraction was carried out with acetone (10 ml/g of a sample) on an ultrasonic bath (InterSonic, Olsztyn, Poland) for 15 min. Subsequently, samples with the solvent were put aside in a dark place at room temperature for 48 h. After that time, the 15-min ultrasonic treatment was repeated, after which the extract was separated from samples in an Eppendorf centrifuge (Hamburg, Germany) at 1000 rpm for 10 min. The extract was evaporated to dryness on a vacuum evaporator (Büchi, type R-210; Büchi Labortechnik, Flawil, Switzerland). The residue was dissolved in 1 ml of methanol and centrifuged on an Eppendorf centrifuge at 1,600 rpm for 10 min. Colour reaction was performed by adding 2 ml of 0.05% Fast Blue RR reagent (4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt) (Sigma-Aldrich) diluted with methanol (1:5) and 10 ml of 10% K_2CO_3 solution to each extract. Absorbance measurements were carried out after 20 min at 480 nm using a UNICAM UV/Vis UV2 spectrophotometer (ATI Unicam, Cambridge, United Kingdom). The content of alkylresorcinols was calculated using a standard curve prepared for olivetol (≥99.0% purity) and expressed in mg/kg of grain DM. The repeatability for olivetol determination (expressed as coefficient of variation) was 2.5%. LOQ was 0.05 mg/kg of sample DM, while linearity of calibration curve was confirmed in range of 1-15 µg/ml. The composition of alkylresorcinols was determined with the use of a GC-MS QP2010 PLUS, manufactured by Shimadzu (Kyoto, Japan). Alkylresorcinols were separated on a ZB-5ms capillary column (30 m ×0.25 mm ×0.25 µm)

(Phenomenex, Torrance, CA, USA), with helium as a carrier gas with a 0.9 ml/min flow rate. The temperatures were as follows: injector 230 °C, column 70 °C increased to 230 °C at 15 °C/min, and to 310 °C at 3 °C/min, and maintained for 10 min, GC-MS interface 240 °C, ion source 220 °C. Electron energy was set as 70 eV. The total ion current (TIC) mode was used for quantification (100-600 *m/z* range). Alkylresorcinols were identified by comparison with the mass spectral library.

Method validation

The standard deviation of y-intercepts (σ) and the slope of regression lines (S) of calibration curves were used to calculate the limit of detection (LOD) (3.3 σ/S) and LOQ (3 LOD) according to validation procedure described by Paithankar (2013). Linearity of calibration curves was confirmed based on analysis of coefficient of determination (R^2>0.99). Repeatability was assessed as 'intra-day', after six determinations on the same day and expressed as coefficient of variations (CV).

Statistical analysis

Statistical analysis of the results was performed using Statistica 12.5 PL software (StatSoft, Kraków, Poland). The variation between genotypes was determined using one-way ANOVA, followed by Tukey test. Principal Component Analysis (PCA) was used for grouping tested genotypes into distinguishable classes. All calculations were performed at P<0.05 significance level.

3. Results and discussion

Selected physical characteristics of spelt kernels/grain

The physical properties of cereal grain are important for machinery, process equipment design, determining the utilisation of grain by the food industry and for standardisation of the market value of grain. In general, large grains, containing large amounts of nutrients, with uniform colour and dimensions, are the most recommended for food processing.

For the tested spelt genotypes, the TKW ranged from 44.6 g (Rokosz) to 58.2 g (Filderstolz) (Table 1). The average value for this species was 49.9 g, with an intra-genotype variation of 7.7%. These values are typical for spelt grain, which is considered to be heavier than bread wheat, with a TKW of circa 45.9 g (Markowski *et al.*, 2013). The average length and width of the kernels ranged from 7.35 mm (STH 11) to 8.86 mm (Schwaben Speltz) and from 3.14 mm (Badengold and Spelt INZ) to 3.58 mm (Line 55-97), respectively. The average values of these dimensions were 8.08 mm and

Table 1. Physical characteristics of 16 genotypes of spelt wheat.[1]

Genotype	Length (mm)	Width (mm)	Hue (°)	Saturation (%)	Intensity (%)	Kernel density $(g/cm)^3$	1000 kernel weight (g)
Schwaben Speltz	8.86±0.75a	3.31±0.21bcd	36.1±1.1ab	30.3±4.2e	59.6±3.0ab	1.20±0.06a	52.7±0.1b
Schwabenkorn	8.65±0.72ab	3.27±0.18bcd	34.5±2.3f	31.1±4.3de	60.0±3.4a	1.19±0.04a	50.3±1.1cde
Line 54-76	7.85±0.61f	3.49±0.24a	36.1±1.1a	35.2±4.4a	54.3±3.1e	1.19±0.03a	48.8±0.3defg
Line 55-97	7.94±0.60ef	3.58±0.27a	34.4±1.2f	31.6±3.9de	59.3±3.3abcd	1.19±0.06a	50.5±0.4bcd
Franckenkorn	8.20±0.65de	3.23±0.27bcde	34.8±1.0ef	31.7±3.8cde	59.8±2.78a	1.23±0.05a	49.0±0.8cdef
Oberkulmer Rotkorn	8.56±0.65abc	3.21±0.23de	35.5±1.0cd	31.5±4.3de	59.6±3.1ab	1.28±0.07a	52.6±0.4b
Badengold	8.24±0.57cde	3.14±0.30e	35.5±0.8bcd	32.8±4.0bcd	59.3±2.7abcd	1.20±0.03a	46.5±0.1hi
Ceralio	8.22±0.70de	3.27±0.29bcd	35.5±1.0cd	31.8±4.3cde	59.4±3.0abc	1.20±0.05a	48.2±0.4fgh
Ostro	8.35±0.78bcd	3.22±0.25cde	35.5±1.0bcd	31.1±4.2de	59.6±3.0ab	1.22±0.05a	49.1±0.1cdef
Spelt INZ	7.70±0.67fg	3.14±0.27e	35.3±1.0cde	31.4±4.3de	60.0±2.9a	1.25±0.04a	45.1±0.5i
Rokosz	7.50±0.81gh	3.21±0.27de	36.2±1.1a	32.8±4.8bcd	58.2±3.6bcd	1.24±0.06a	44.6±1.3i
STH 11	7.35±0.61h	3.34±0.30bc	36.6±1.0a	35.4±5.2a	55.7±3.4e	1.30±0.02a	47.9±0.3fgh
STH 12	7.44±0.65gh	3.35±0.32b	36.2±1.1a	36.0±5.0a	55.1±3.6e	1.25±0.04a	46.7±0.1ghi
Line 28-46	7.68±0.57fg	3.49±0.30a	35.6±0.9bcd	34.8±4.8ab	57.9±3.2cd	1.27±0.03a	51.1±0.4bc
Filderstolz	7.95±0.56ef	3.55±0.27a	35.1±1.3de	33.9±4.1bc	57.8±2.7d	1.38±0.04a	58.2±0.2a
Divimar	8.83±0.70a	3.31±0.23bcd	35.8±1.0abc	32.4±4.2cde	58.7±3.0bcd	1.30±0.05a	57.1±0.4a
Mean	8.08	3.32	35.5	32.7	58.4	1.24	49.9
Standard deviation	0.48	0.14	0.6	1.8	1.8	0.05	3.8
Coefficient of variation (%)	5.94	4.22	1.6	5.4	3.1	4.03	7.7

[1] The results are presented as mean values ± standard deviation (based on triplicate independent analyses (n=3)). Different letters in the same column indicate significant differences (P≤0.05).

3.32 mm, with only 5.9 and 4.2% variation among samples, respectively. These values suggest that especially the length of spelt kernels is significantly higher than that of bread wheat kernels ($P \leq 0.05$). For example, Markowski *et al.* (2013) determined that the length of spelt kernels of the cultivar Oberkulmer Rothkorn (8.08 mm) was approximately 27% higher than that of bread wheat cultivar Korweta (6.42 mm). Kernel mass and volume determine the kernel specific density. The density of the samples used in present study ranged from 1.19 g/cm^3 (Schwabenkorn, Line 54-76 and Line 55-97) to 1.38 g/cm^3 (Filderstolz), with an average value of 1.24 g/cm^3. These results are slightly lower than that of bread wheat grain, determined at circa 1.34 g/cm^3 (Konopka *et al.*, 2015) and may suggest less vitreous (hard) endosperm (Dobraszczyk *et al.*, 2002; Konopka *et al.*, 2015; Samson *et al.*, 2005). The intra-genotype variation of kernel density was relatively low, reaching only 4.0%. Similarly, the surface colour of spelt kernels only slightly differed between samples, with the average values of H, S and I of 35.5°, 32.7% and 58.4%, respectively. The lowest variation in colour attributes was noted for surface hue (1.6%), while the highest was for saturation (5.4%). A comparison of these results to bread wheat cultivars, determined by the same protocol of examination (Konopka *et al.*, 2012, 2015) showed that the hue of bread wheat kernels is slightly shifted to more red values (a decrease in H value by about 7°). In general, lower 'redness' of wheat grain suggests a lower phenolic acid content (Jiang *et al.*, 2011; Konopka *et al.*, 2015).

Phenolic acids

Phenolic acids are strong antioxidants with antiulcer, antidiabetic, cardioprotective, anticancer, anti-inflammatory, neuroprotective, hepatoprotective, anti-aging and antimicrobial activities (Saibabu *et al.*, 2015). Bread wheat grain is a valuable dietary source of these compounds since it contains approximately 326 to 1,171 mg/kg of total phenolic acids (Shewry and Hey, 2015). Spelt genotypes display a similar range of values, for example, from 422 to 1,257 mg/kg determined in six varieties of spelt wheat grown in Poland (Gawlik-Dziki *et al.*, 2012). However, only a minor part of these compounds are easily accessible from the digestive tract, since they are bound to the lignocellulose matrix (Konopka *et al.*, 2014).

The average content of free (unbound) phenolic acids was 20.7 mg/kg, with values ranging from 16.0 mg/kg (Schwaben Speltz) to 27.3 mg/kg (Franckenkorn) (Table 2). In comparison, for bread wheat grain, the free fraction is in the range 3.1-6.7 mg/kg, with an average value of 4.2 mg/kg (Martini *et al.*, 2015). In the free fraction, ferulic and *p*-coumaric acids prevailed, reaching an average share of 45.8 and 18.6%, respectively. Spelt grain

Table 2. Free phenolic acids content of 16 genotypes of spelt wheat (mg/kg).[1]

Genotype	*p*-OH benzoic acid	Vanillic acid	Syringic acid	*p*-coumaric acid	Ferulic acid	Others	Total
Schwaben Speltz	1.05±0.10a	0.692±0.040abc	1.17±0.23ab	2.78±0.31c	6.94±0.05b	3.22±0.23a	16.0±0.2b
Schwabenkorn	1.39±0.12a	0.805±0.034abc	1.40±0.07ab	3.04±0.41abc	8.96±0.04ab	3.32±0.24a	19.0±1.0ab
Line 54-76	1.39±0.41a	0.671±0.239bc	1.93±0.09a	4.99±0.79a	11.4±0.12ab	4.19±0.09a	24.6±2.3ab
Line 55-97	1.42±0.45a	0.890±0.041abc	1.69±0.06ab	4.75±0.46abc	10.4±0.88ab	4.43±0.71a	23.6±3.4ab
Franckenkorn	1.85±0.19a	0.876±0.107abc	1.60±0.22ab	4.84±0.43ab	13.2±0.4a	4.86±0.22a	27.3±1.1a
Oberkulmer Rotkorn	1.64±0.01a	0.968±0.305abc	1.52±0.07ab	4.07±0.41abc	11.9±0.5ab	4.26±0.62a	24.4±1.5ab
Badengold	1.12±0.37a	0.672±0.004bc	1.30±0.12ab	3.60±0.16abc	9.32±1.24ab	3.66±0.53a	19.7±2.5ab
Ceralio	0.929±0.497a	0.597±0.105c	1.39±0.38ab	3.29±1.18abc	8.30±3.23ab	3.06±1.13a	17.6±7.7ab
Ostro	1.39±0.41a	0.762±0.072abc	1.36±0.06ab	3.59±0.52abc	6.67±0.29b	3.91±0.78a	17.7±1.1ab
Spelt INZ	1.17±0.21a	0.942±0.076abc	1.47±0.10ab	2.94±0.04bc	7.65±0.20ab	3.09±0.18a	17.3±1.4ab
Rokosz	0.908±0.220a	0.727±0.139bc	1.12±0.30ab	3.41±0.78abc	7.22±3.07b	3.42±0.39a	16.8±4.8ab
STH 11	0.923±0.013a	0.939±0.068abc	0.96±0.47b	4.72±0.21abc	12.2±0.6ab	3.58±0.33a	23.3±0.8ab
STH 12	0.924±0.125a	1.06±0.012abc	1.09±0.11b	4.97±0.46ab	11.0±1.2ab	3.94±0.35a	23±2.0ab
Line 28-46	0.863±0.156a	0.900±0.073abc	0.95±0.06b	3.67±0.06abc	8.40±0.51ab	3.50±0.18a	18.3±0.9ab
Filderstolz	1.59±0.12a	1.14±0.201ab	1.24±0.02ab	3.37±0.24abc	9.63±0.34ab	5.03±0.49a	22±0.9ab
Divimar	1.46±0.05a	1.27±0.039a	1.12±0.25ab	3.56±0.31abc	9.13±1.06ab	4.12±0.35a	20.7±1.7ab
Mean	1.25	0.870	1.333	3.85	9.53	3.85	20.6
Standard deviation	0.30	0.184	0.268	0.76	2.00	0.60	3.4
Coefficient of variation (%)	24.8	20.5	20.3	19.7	20.9	15.6	16.4

[1] The results are presented as mean values ± standard deviation (based on triplicate independent analyses (n=3)). Different letters in the same column indicate significant differences ($P \leq 0.05$).

also contained free *p*-OH benzoic, vanillic, and syringic acids, but their amounts did not exceed 2 mg/kg of each. The total content of phenolic acids was from 206 mg/kg (Oberkulmer Rotkorn) to 557 mg/kg (Line 54-76), with the average value close to 345 mg/kg (Table 3). Ferulic acid was the main phenolic acid, with a share from 86.6 to 91.6% of the total. Two other hydroxycinnamic acids had an additional important contribution: sinapic acid (circa 4.8%) and *p*-coumaric acid (circa 2.4%). A similar composition of free and bound phenolic acids was previously found in other wheat genotypes (Gawlik-Dziki *et al.*, 2012; Kerienė *et al.*, 2015; Konopka *et al.*, 2012; Yilmaz *et al.*, 2015). In total, free phenolic acids constituted of 3.2 to 11.8% of total phenolic acids, since the majority of phenolic acids exist in the form of esters and conjugates with other grain components (Adom and Liu, 2002; Gani *et al.*, 2012; Konopka *et al.*, 2012; Li *et al.*, 2008). Among phenolic acids, the most bound were sinapic (100% as bound form) and ferulic acids (its free form did not exceed 6%). In contrast, syringic acid existed almost exclusively as a free form.

The total content of phenolic acids in wheat grain is generally related to grain weight (volume) and colour, with the highest accumulation in small and dark kernels (Jiang *et al.*, 2011). In the current study, however, there was no such dependence. For instance, the darkest genotypes with relatively low TKW (STH 11, STH 12 and Line 54-76 – Table 1, Figure 2) differed in total phenolic acids content (233, 351 and 557 mg/kg, respectively, Table 3). Similarly, grain of three cultivars with close, high values of TKW and similar colour (Schwaben Speltz, Filderstolz and Divimar) contained various amounts of phenolic acids: 506, 228 and 273 mg/kg, respectively. The variation determined between genotypes in the current study was lower than previously found and was most likely caused by a lack of environment impact (the same conditions of cultivation). The determined contents were also relatively low, with a significant number of samples that only slightly exceeded 200 mg/kg. It was previously found that for the same genotype, a higher accumulation of phenolic acids is related to stress condition (mainly biotic) during plant growth and grain maturation (Fernandez-Orozco *et al.*, 2010; Sharma *et al.*, 2012). The current results showed that the composition of phenolic acids in spelt grain is similar to that found in bread wheat, but the ratio of unbound/bound fractions is slightly higher (Gawlik-Dziki *et al.*, 2012; Konopka *et al.*, 2014; Wang *et al.*, 2014).

Table 3. Total phenolic acids content of 16 genotypes of spelt wheat grown (mg/kg).[1]

Genotype	*p*-OH benzoic	Vanilic	Syringic	*p*-coumaric	Ferulic	Sinapic	Others	Total
Schwaben Speltz	3.77±0.21bcde	5.06±0.06ab	2.11±0.20ab	15.2±0.1a	455±2b	21.7±0.4abc	3.24±0.08bcde	506±2b
Schwabenkorn	4.53±0.30abc	5.42±0.41a	2.35±0.03a	12.5±0.2abc	370±1c	21.8±1.0abc	3.38±0.17bcd	420±1c
Line 54-76	4.67±0.30ab	5.38±0.68a	1.41±0.08abcd	13.8±0.2ab	501±4a	26.6±1.0a	4.85±0.26a	557±8a
Line 55-97	4.52±0.66abc	5.31±0.37a	1.33±0.34abcd	12.6±0.6abc	456±2b	13.9±0.9de	4.38±0.06ab	498±1b
Franckenkorn	4.63±0.31ab	5.17±0.65ab	2.29±0.09a	10.9±3.6bcd	499±1a	26.9±1.1a	3.98±0.27abc	553±7a
Oberkulmer Rotkorn	2.23±0.07f	3.06±0.29cd	1.06±0.10bcd	5.86±0.86e	185±1g	6.92±1.85f	1.61±0.26fgh	206±4h
Badengold	2.55±0.53ef	3.13±0.63cd	1.44±0.35abcd	6.26±0.35e	194±1fg	14.9±3.3cde	1.72±0.05fgh	224±11gh
Ceralio	2.28±0.31f	2.45±0.38d	0.83±0.37cd	4.94±0.81e	191±1fg	12.7±0.0def	1.38±0.17gh	216±6gh
Ostro	5.32±0.12a	4.75±0.32abc	1.90±0.64abc	7.62±1.47de	317±4d	21.4±1.3abc	2.85±0.69cdef	360±1de
Spelt INZ	4.04±0.23abcd	2.77±0.05d	1.26±0.01abcd	7.66±0.31de	218±5f	13.6±0.9de	1.95±0.05efgh	249±8fg
Rokosz	3.18±0.71cdef	3.00±0.66cd	1.12±0.46bcd	6.39±0.60e	218±10f	14.1±3.8de	2.19±0.30defgh	248±30fg
STH 11	2.32±0.10f	2.63±0.18d	0.80±0.24cd	4.79±1.10e	208±1fg	13.2±1.1def	1.69±0.12fgh	233±2h
STH 12	3.55±0.46bcdef	3.36±0.88bcd	1.53±0.16abcd	7.73±0.09de	311±3d	21.4±0.1abc	2.71±0.05cdefg	351±2e
Line 28-46	2.96±0.30def	3.68±0.40abcd	0.99±0.12cd	8.47±0.59cde	359±6c	16.9±2.5bcd	2.21±0.27defgh	395±10cd
Filderstolz	2.66±0.10def	2.52±0.08d	0.65±0.06d	4.75±0.54e	207±0fg	9.14±0.67ef	1.19±0.11h	228±0gh
Divimar	2.73±0.02def	2.90±0.11d	1.15±0.23bcd	5.51±0.17e	248±1e	11.7±0.3def	1.52±0.13fgh	273±1f
Mean	3.50	3.79	1.39	8.43	284	16.7	2.55	345
Standard deviation	1.02	1.16	0.53	3.46	114	5.9	1.13	129
Coefficient of variation (%)	29.1	30.6	38.1	41	40	35.6	44.3	37.2

[1] The results are presented as mean values ± standard deviation (based on triplicate independent analyses (n=3)). Different letters in the same column indicate significant differences ($P \leq 0.05$).

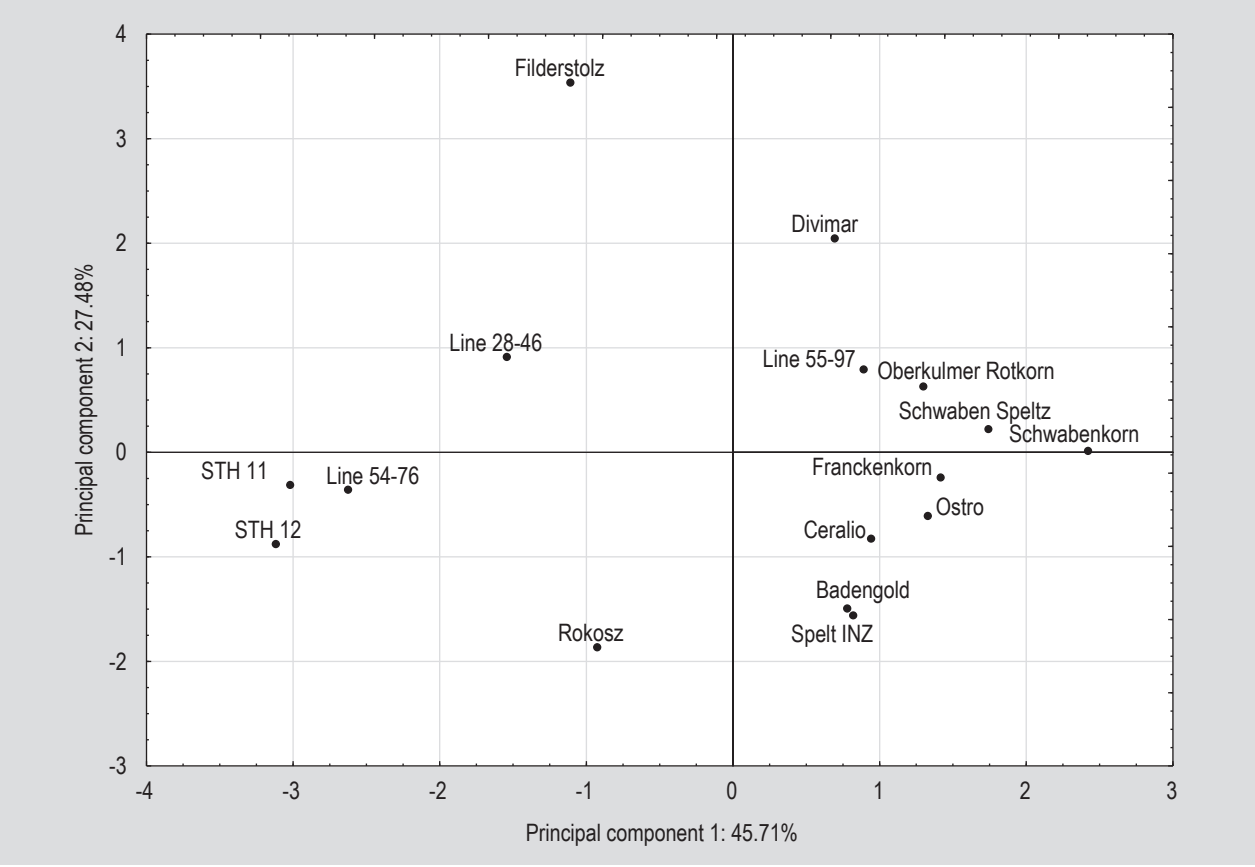

Figure 2. Score plot of the two first principal components after PCA analysis of grain physical features.

Alkylresorcinols

Alkylresorcinols are phenolic lipids, with a resorcinol-type aromatic ring and alkyl chain with up to 27 carbon atoms, mainly in a saturated state (Kozubek and Tyman, 1999; Ross *et al.*, 2003). These compounds have been found in the epicuticular wax layer surrounding the cereal kernel and are accumulated to protect plants against external aggression and predators (Zarnowski and Suzuki, 2004). *In vitro* studies have shown that alkylresorcinols may prevent cells turning cancerous (more details in Ross *et al.*, 2004). Bread wheat grain usually contains between 421 to 677 mg/kg of these compounds (Shewry and Hey, 2015).

The average content of alkylresorcinols in the analysed spelt grain genotypes was close to 723 mg/kg, and ranged from 561 mg/kg (Schwabenkorn) to 919 mg/kg (Schwaben Speltz), with a variation coefficient of 13.0% (Table 4). This was a typical content determined for spelt grain. For example, Ziegler *et al.* (2015) determined that their content ranged from 617 to 817 mg/kg (16 spelt genotypes), while Andersson *et al.* (2008) found it varied between 490-741 mg/kg (5 spelt genotypes). Other studies showed that alkylresorcinol concentrations among different wheat genotypes may vary over a broad range, from 110 to 1,400 mg/kg (Ciccoritti *et al.*, 2014; Kulawinek *et al.*, 2008; Ross *et al.*, 2003). There is considerable variation in the total alkylresorcinol content between wheat species: winter wheat (220-652 mg/kg), spring wheat (254-537 mg/kg), durum wheat (194-531 mg/kg), spelt (490-817 mg/kg), einkorn (545-654 mg/kg) and emmer wheat (531-714 mg/kg) (Andersson *et al.*, 2008; Ziegler *et al.*, 2015). Similarly, Shewry and Ward (2012), summarising the HEALTHGRAIN project, reported the average alkylresorcinol content in bread wheat as approximately 431 mg/kg, with a variation from 220 to 669 mg/kg. The authors also concluded that alkylresorcinol accumulation is predominantly determined by cultivar (circa 60%), while growth environment determines only circa 10% of the variation. In the current study, six cultivars were used (Badengold, Divimar, Franckenkorn, Oberkulmer Rotkorn, Schwabenkorn and Filderstolz), which were previously analysed by Ziegler *et al.* (2015). These cultivars growing in Poland produced grain with very similar content of alkylresorcinols, with differences between 4 to 81 mg/kg. In both studies, Schwabenkorn turned out to be the least abundant genotype, whereas Franckenkorn had the highest amount of alkylresorcinols. Andersson *et al.* (2010) discovered that a warm, dry climate generally results in higher alkylresorcinol contents, whereas high precipitation, especially during plant development and grain-filling, results in lower contents. Figure 1 showed

that hydrothermal conditions for the growth of tested spelt genotypes in 2015 were not suitable for the accumulation of these compounds.

We found that the main homologues, independent of cultivar, were heneicosylresorcinol (C21:0) (circa 58%), nonadecylresorcinol (C19:0) (circa 30%), and tricosylresorcinol (C23:0) (circa 6%) (Table 4). A lower share of C21:0 form (circa 50%), accompanied by a similar share of C19:0 (circa 29%) and an approximately 14% contribution of C23:0 form was stated for spelt grain by Ziegler *et al.* (2015) and Ciccoritti *et al.* (2014). Grain of bread wheat is more abundant in C19:0 homologue (by circa 5%), but slightly less abundant in C21:0 and C23:0 (both by ca. 2%) (Ziegler *et al.* 2015).

Differentiation of spelt genotypes based on grain physical features and phenolic compounds

PCA analysis showed the dispersion of the tested spelt genotypes (Figure 2 and 3). The first two principal components (PC1 and PC2) explained approximately 73% (based on physical features) (Figure 2) and approximately 59% (based on phenolic compounds) (Figure 3) of the determined variance, respectively. For physical features, the most distinguishing cultivar was Filderstolz (with the highest kernel density and TKW) (Figure 2). Additionally, three other genotypes were clearly separated: Divimar (relatively high kernel length and TKW), Line 28-46 (relatively low kernel length, accompanied by medium kernel density and TKW) and Rokosz (relatively low kernel length and TKW). Genotypes Line 54-76, STH 11 and STH 12 created a group with the darkest colour and relatively low TKW, while the rest of the genotypes were highly similar (poorly distinguishable). The first component of PCA for this analysis was mostly affected by colour saturation and intensity of grain surface, while the second component was mostly affected by TKW results. The prepared score plot of the tested genotypes based on determined phenolic compounds was quite different (Figure 3). The most distinguishable was cultivar Franckenkorn (with the highest share of unbound phenolic acids and relatively high content of total phenolic acids and alkylresorcinols). Other genotypes were poorly distinguishable. The highest load in the PC1 was noted for phenolic acids (*p*-OH benzoic, vanillic and ferulic acids) found in total fraction), while in the case of PC2 the highest load was for C17:0 and C19:0 alkyresorcinols.

Table 4. Alkylresorcinols content of 16 genotypes of spelt wheat (mg/kg).[1]

Genotype	C17:0 (Heptadecyl-resorcinol)	C19:0 (Nonadecyl-resorcinol)	C21:0 (Heneicosyl-resorcinol)	C23:0 (Tricosyl-resorcinol)	C25:0 (Pentacosyl-resorcinol)	Total
Schwaben Speltz	20.8±0.3ab	292±1a	553±5a	38.9±0.5d	14.3±3.0bcd	919±8a
Schwabenkorn	15.1±1.4b	164±3g	327±2i	41.6±0.5bcd	12.6±1.7cd	561±4i
Line 54-76	18.6±2.9ab	186±6fg	357±14hi	31.6±1.8d	11.7±0.1d	605±13hi
Line 55-97	21.6±1.8ab	198±6ef	412±6cdefg	36.9±0.4d	14.4±1.8bcd	683±0efg
Franckenkorn	21.0±0.4ab	285±10ab	455±1bc	32.6±2.7d	20.1±0.4ab	814±14ab
Oberkulmer Rotkorn	18.0±0.1ab	212±2def	378±8fgh	31.9±3.0d	13.4±1.2cd	653±10fgh
Badengold	20.0±1.0ab	236±9cd	460±25bc	44.2±7.1bcd	16.1±0.4bcd	776±22c
Ceralio	18.0±3.3ab	189±8fg	416±13cdefg	32.7±0.2d	14.3±1.5bcd	670±23efg
Ostro	20.2±1.6ab	191±3fg	369±15ghi	35.0±0.6d	15.1±0.1bcd	630±16gh
Spelt INZ	16.2±5.5ab	190±14fg	393±6efgh	65.8±15.9a	14.7±0.8bcd	679±12efg
Rokosz	19.9±6.0ab	209±13def	404±16defgh	41.0±0.4cd	20.2±3.1ab	694±20ef
STH 11	25.8±1.2a	269±9ab	420±16cdef	58.9±0.1abc	19.7±1.5ab	794±7ab
STH 12	25.3±1.5a	256±9bc	422±14cdef	59.8±4.9ab	17.5±2.0bcd	781±18ab
Line 28-46	22.2±1.6ab	224±9de	501±12b	64.3±1.5a	18.1±0.6abc	830±3b
Filderstolz	18.8±0.1ab	196±1ef	426±3cde	48.5±0.3abcd	23.7±0.1a	713±4de
Divimar	22.6±0.4ab	229±4cd	450±8cd	41.1±0.8bcd	18.0±0.3abc	761±14cd
Mean	20.3	220	421	44.1	16.5	723
Standard deviation	2.9	38	55	11.9	3.3	94
Coefficient of variation (%)	14.4	17.3	13.2	27	19.8	13

[1] The results are presented as mean values ± standard deviation (based on triplicate independent analyses (n=3)). Different letters in the same column indicate significant differences ($P \leq 0.05$).

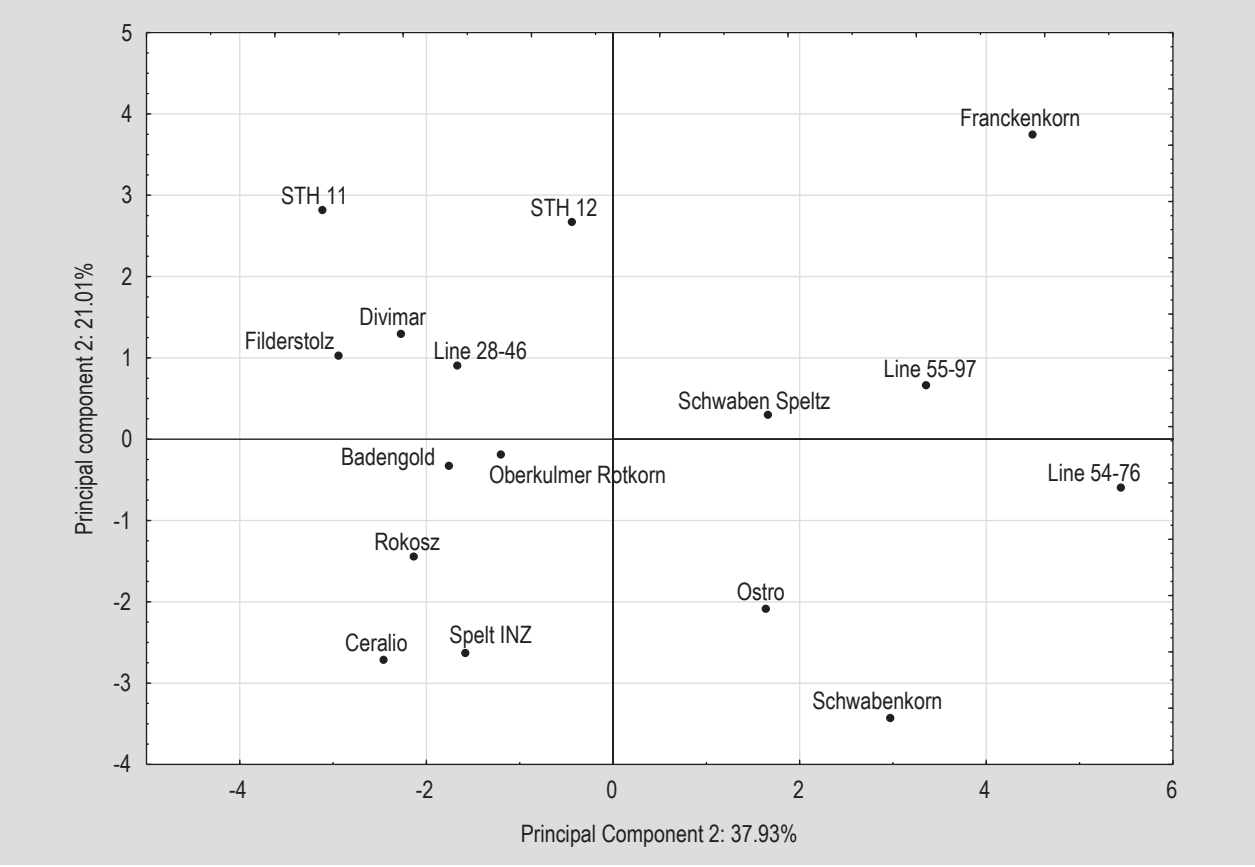

Figure 3. Score plot of the two first principal components after PCA analysis of phenolic compounds.

4. Conclusions

This study provided new and valuable data on the diversity of grain morphology and content and composition of phenolic acids and alkylresorcinols in winter spelt grain cultivated in Poland. The results suggest that genotype variation in spelt grain characteristics cultivated in Poland is relatively narrow, with the highest variation in total phenolic acids. The obtained data could be useful for selecting spelt genotypes with added nutritional values by the highest content of selected phytochemicals. In the context of the ability to accumulate bioactive phenolic compounds, the highest difference was found between Schwaben Speltz and Oberkulmer Rotkorn cvs., which contained 1,425 and 859 mg of total phenolic compounds per kg of grain dry mass, respectively.

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