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RELATIONS BETWEEN FERULIC ACID CONTENT IN WHEAT COAT, AND MILLING QUALITY AND COLOUR OF GRAIN

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ABSTRACT

Relations between the milling quality and colour of wheat grain, and the content of ferulic acid were studied. It was found that the content of ferulic acid in the bran of the analysed varieties of wheat is a statistically significant feature of a variety which depends on grain size. Next, the colour of wheat grain differentiates winter and spring varieties according to their technologic quality. The analysis of the correlation between the content of ferulic acid in the bran and the colour of wheat grain indicates relations between the colour specified as the average value of grey level occurrence and the content of ferulic acid. The higher the content of ferulic acid in the bran, the lower number of grey level determines the colour of wheat grain surface, i.e. the higher content of ferulic acid, the darker the colour of grain surface. The milling quality produced from the grain of the analysed varieties of wheat is correlated with the colour of grain surface expressed as the average and maximum values of grey level occurrence.

Key words: wheat, varieties, seed coat, ferulic acid, colour, milling quality

INTRODUCTION

Ferulic acid (4-hydroxy-3-methoxycinnamic) is the main phenolic acid which appears in the cell walls of monocotyledons [12]. The presence of many dehydrodimers of ferulic acid was established in the pentosan fraction of wheat, for example 5-5' diferulic acid which is present in wheat grain in the largest quantity [2].

Hatcher and Kruger [4] noticed the presence of ferulic acid in wheat grain at the level from 27.48 to 33.76 mg/100 g, depending on variety. The research of Lempereur et al. [9] confirmed high variety dependence for the changes in the content of ferulic acid in durum wheat grain which varied from 69.3 to 244.3 mg/100 g d.m. On the other hand, Weidner et al. [20] studied the presence of ferulic acid in three fractions: free phenolic acids, soluble esters and soluble glycosides. The content of ferulic acid in whole wheat grains determined by these authors amounted to the following levels, depending on variety, 0.19-0.20 mg/100 g; 1.35-2.49 mg/100 g and 0.13-0.35 mg/100 g respectively.

Moreover, Lempereur et al. [9] revealed that the content of ferulic acid depends on the size of wheat grain. Varieties of small grain were characterised by higher content of arabinoxylans and ferulic acid compared to varieties of large grain.

Pussayanawin and Wetzel [13]suggested that ferulic acid can provide an indication of the degree of purity of flour (bran contamination) which is useful for determining the milling quality for wheat grain. Symons and Dexter [19] established the fluorescence of aleuron layer cells of wheat grain in order to determine the purity of flour. Kelfkens [6] used the determination of the ferulic acid content to establish whether it provides better opportunity to evaluate the presence of coat parts in flour than the content of ash in flour.

From the above-mentioned research it follows that the content of ash in flour can be replaced with the determination of ferulic acid. The HPLC method is recommended for the determination of the content of ferulic acid [13]. It would be interesting to find answers to the following question: is it possible to simplify the analysis process which exploits the relations between the colour of wheat grain coat and the content of ferulic acid as an index of milling quality. The following experimental assumptions were made to run the experiment: wheat grain of uniform variety of a specified size, milling at constant conditions, the determination of the content of ferulic acid in the bran with the HPLC method, the determination of grain surface colour using digital image analysis (DIA).

MATERIALS AND METHODS

MATERIAL

Wheat grain of spring and winter varieties which originated from 1999-2000 crops from Plant Breeding Centre in Wrócikowo (Warmińsko-Mazurskie Voivodeship) were analysed. The preliminary preparation of grain samples was the screening and collecting the overtails from sieves: 3.2 x 25mm fraction F_1, 2.8 x 25 mm fraction F_2, 2.5 x 25mm fraction F_3, 2.2 x 25mm fraction F_4. The predominant fractions in sample of crops in 1999 F_3 and F > 1.7 x 25 mm and fraction F_2 of crops in 2000 were used to analyse the milling quality and grain colour.

METHODS

For each variety and chosen fraction of wheat grain seven millings were done with a laboratory mill Brabender Quadrumat Junior applying the same procedure for grain preparation. Before milling the moisture content of grain was adjusted to 14,5% using 24 hours tempering after addition of proper amount of water. The average value of milling quality was calculated of two repeatings. Moisture content was determined in accordance to country standard method PN-91/A 74010. The content of protein was determined with Kjeldahl method using Kjel-Foss Automatic 16210 of AISN Foss Electric, Denmark. Protein multiplier of 5.70 was applied.

The determination of total free phenolic compounds in bran was done in accordance to Ribereau-Gayon [14] using extraction of phenolic compounds with methanol, evaporation of methanol, the addition of Folin-Denis reagent and sodium carbonate and then measurement the absorbance at the wavelength of 720 nm against the reference sample. The results were expressed as catechin equivalent in mg/100 g with a reference curve plotted for D-catechin. The content of ferulic acid in the bran was determined by Pussayanawin and Wetzel method [13] releasing ferulic acid from the wheat bran with acid and enzymatic hydrolysis and then with the separation with the HPLC method. The following separation conditions were applied: Hewlett Packard liquid chromatograph; column - length: 100 mm, internal diameter: 4.6 mm; mobile phase: solution of 12% methanol in sodium citrate buffer; stationary phase: silica gel of 5 micrometer granulation which was chemically modified with bond aliphatic hydrocarbons with 18 molecules of carbon in a chain; flow rate: 1cm^3/min.; fluorescence detector of excitation wavelength λ = 312 nm and emissivity wavelength λ = 418 nm. Qualitative interpretation of the obtained chromatographic splits was carried out on the basis of the retention time for the peak of ferulic acid in the standard sample (reference) to the retention time for ferulic acid in the analysed sample. Quantitative interpretation was done on the basis of the comparison of the height of peaks of ferulic acid in the standard sample and the analysed sample determined through integrating them. The reference sample was the standard ferulic acid (Sigma, St. Louis, MO, USA).

The determination of wheat grain surface colour was carried out using DIA method. The measurement was performed as following (system I) [3]:

1. System calibration based on the standard,
2. Placement of objects on the shadeless bench groove down,
3. Light projection onto the object from the bottom:

- the achieved picture was introduced to the VFG card memory and saved as 512 x 512 pixel matrix of the grey levels width between 0 and 255,
- decimal – to – binary conversion of picture for 116 ± 2 grey levels,
- segmentation and localisation of objects and the measurement of the basic geometrical features.

4. Light projection onto objects from the top - from four symmetrical white light sources:

- the achieved picture was introduced to the VFG card memory,
- the information introduction concerning localisation and dimensions of the analysed objects,
- scanning of the optical density of the individual objects, surface and determining the maximal (max.) and minimal (min.) grey level, the difference of the levels (max. –min.) and the average grey level.

The results were presented as the percentage occurrence of max., min. and the average value of grey level [3]. Measurement of the colour of wheat grain surface with LUCIA system (system II) was equipped with:

- colour camera CCD with 512 x 512 pixel resolution,
- card for computer analysis of image MATROX,
- digital monitor,
- Lucia v.4.11 software [Laboratory Imaging].

To determine the colour of wheat grain surface the analysed grain was placed on a black plate with a furrow downward. They were lit evenly with white light (optical fibre). The grain was analysed individually. The average brightness of red (R), green (G) and blue (B) colours was determined – such values were determined automatically after selecting an appropriate option of the program.

The findings were presented as the frequency of the average value of grey levels corresponding to the brightness of red (R), green (G) and blue (B) colours.

The data were analysed statistically with statistical software packages “STATISTICA PL” and “WINSTAT” applying the analysis of variance and regression, correlation and tests of significance for dependent and independent samples [11, 18].

RESULTS AND DISCUSSION

The milling quality

The statistical analysis of significance of the differences between flour yield and wheat variety for the grain fraction > 1.7 mm x 25 mm showed that the grain of Zyta and Elena varieties was characterised by significantly higher flour yield than the grain of Henika and Torka varieties (Table 1). Zyta and Elena varieties did not differ significantly from each other but differed significantly from Henika and Torka varieties. Lack of statistically significant differences in flour yield between the grain of Elena and Zyta varieties was also confirmed by the results of statistical analysis for the grain fraction 2.5 < F_3 < 2.8 mm x 25 mm. On the other hand, flour yield differed significantly between winter and spring varieties within this fraction. Flour yield of fraction 2.8 mm < F_2 < 3.2 mm x 25 mm of winter and spring varieties differed in a statistically significant way between the analysed varieties. The grain of Almari and Nawra varieties makes an exception as it did not differ significantly as far as flour yield is concerned (Table 1).

PRINT

Table 1. Results of statistical analysis. Milling quality - variety

Wheat variety		Quality grade (*)	Fraction	Average value [%]
Zyta	winter	A	$F > 1.7 \times 25$ mm	78.0^{a}
Elena	winter	C		78.2^{a}
Torka	spring	A		77.3^{c}
Henika	spring	B		74.5^{b}
Zyta	winter	A	$2.5 < F_3 < 2.8 \times 25$ mm	78.1^{a}
Elena	winter	C		77.9^{a}
Torka	spring	A		78.6^{c}
Henika	spring	B		76.2^{b}
Begra	winter	A	$2.8 < F_2 < 3.2 \times 25$ mm	75.2^{d}
Zyta	winter	A		72.7^{b}
Almari	winter	C		75.9^{c}
Elena	winter	C		79.1^{a}
Ismena	spring	A(E)		71.9^{g}
Nawra	spring	A(E)		75.8^{c}
Jasna	spring	A		71.5^{f}
Torka	spring	A		74.4^{e}
Mean values followed by the same letter are not significantly different at p = 0.05				

(*) – grade of technological value in accordance with multiple evaluation method applied in the evaluation of varieties in Poland: E – elite wheat, A – quality wheat, B – bread wheat, C – other wheat (feed). The brackets indicate proximity of other group [5].

In the case of all the analysed fractions of wheat grain it was found that the average value of flour yield was higher in the case of the grain of winter varieties compared to spring varieties. Such relation is confirmed according polish varieties by the research of Sitkowski [17]. Spring wheat is characterised by lower size, higher vitreosity and higher ash content of grain compared to winter wheat. These features are the main cause for worse results obtained from milling spring wheat [16].

The content of total free phenolic compounds and ferulic acid in wheat bran

The content of total phenolic compounds in the fraction of grain > 1.7 mm x 25 mm differed significantly between Zyta and Henika varieties but it did not differ between Elena and Torka varieties although these two varieties differed from both Zyta and Henika varieties (Table 2). In the bran of the grain fraction 2.5mm < F_3 < 2.8 mm x 25 mm the content of total phenolic compounds differed significantly among Elena, Torka and Henika varieties but no statistically significant differences were found between Zyta and Torka varieties.

Table 2. Statistical analysis of total phenolic compounds content in the bran depending on variety and grain size

Wheat variety		Quality grade (*)	Fraction	Average value [mg/100g]
Zyta	winter	A	$F > 1.7 \times 25$ mm	121.86^{b}
Elena	winter	C		102.79^{a}
Torka	spring	A		109.86^{a}
Henika	spring	B		73.23^{c}
Zyta	winter	A	$2.5 < F_3 < 2.8 \times 25$ mm	89.56^{b}
Elena	winter	C		128.36^{a}
Torka	spring	A		91.55^{b}
Henika	spring	B		100.39^{c}

Table 2 cont.

Begra	winter	A	2.8 < F_2 < 3.2 x 25 mm	100.44^{d}
Zyta	winter	A		54.38^{b}
Almari	winter	C		115.65^{c}
Elena	winter	C		121.89^{a}
Ismena	spring	A(E)		81.12^{f}
Nawra	spring	A(E)		$77.95^{e,f}$
Jasna	spring	A		76.06^{e}
Torka	spring	A		75.48^{e}
Mean values followed by the same letter are not significantly different at p = 0.05				

(*) – grade of technological value in accordance with multiple evaluation method applied in the evaluation of varieties in Poland: E – elite wheat, A – quality wheat, B – bread wheat, C – other wheat (feed). The brackets indicate proximity of other group [5].

The comparison of the content of total phenolic compounds in the bran of the grain of 2.8 < F_2 < 3.2 x 25 mm fraction of winter and spring varieties results in the following relations: the differences among Begra, Zyta, Almari, Elena, Ismena and Jasna varieties are statistically significant although the differences among spring varieties Ismena, Nawra, Jasna and Torka are statistically insignificant.

The analysis of significance differences with two variables assumed, winter and spring varieties and the content of total phenolic compounds, confirms a suggestion about the significance of the sample preparation applied in the experiment (grain size) for the determination of statistically significant differences among varieties. It can be claimed that varieties of varied quality grade, e.g. Elena (C grade) and Zyta (A grade) differ significantly regardless of grain size as far as the content of total phenolic compounds is concerned. The differences in the content of total phenolic compounds depending on wheat variety are confirmed by the research of Zieliński and Kozłowska [21].

Assuming constant grain size and variables, the content of ferulic acid and variety, the following relations were found. In the bran of the grain fraction > 1.7 mm x 25 mm the content of ferulic acid differed in a statistically significant way among Zyta, Elena, Torka and Henika varieties (Table 3).

Table 3. Statistical analysis of ferulic acid content in the bran depending on variety and grain size

Wheat variety		Quality grade (*)	Fraction	Average value		
				mg/100g	mg/100g dry matter	mg/100g non-protein dry matter
Zyta	winter	A	F > 1.7 x 25mm	28.40^{b}	36.41^{b}	39.77^{b}
Elena	winter	C		26.02^{a}	29.62^{a}	35.35^{a}
Torka	spring	A		37.91^{d}	42.81^{d}	50.96^{d}
Henika	spring	B		36.08^{c}	40.46^{c}	48.99^{c}
Zyta	winter	A	2.5 < F_3 < 2.8 x 25mm	28.47^{a}	32.66^{a}	39.37^{b}
Elena	winter	C		28.70^{a}	32.06^{a}	38.03^{a}
Torka	spring	A		37.80^{c}	42.44^{c}	50.42^{d}
Henika	spring	B		31.17^{b}	34.78^{b}	42.00^{c}
Begra	winter	A	2.8 < F_2 < 3.2 x 25mm	56.63^{c}	65.25^{c}	78.52^{b}
Zyta	winter	A		43.31^{b}	$49.80^{b,e}$	61.60^{a}
Almari	winter	C		43.92^{b}	50.59^{b}	62.51^{a}
Elena	winter	C		45.50^{a}	52.29^{a}	61.83^{a}
Ismena	spring	A(E)		37.56^{e}	43.44^{f}	54.51^{d}
Nawra	spring	A(E)		34.72^{f}	39.99^{g}	50.96^{e}
Jasna	spring	A		42.15^{d}	49.02^{e}	62.52^{a}
Torka	spring	A		41.37^{d}	47.94^{d}	59.02^{c}
Mean values followed by the same letter are not significantly different at p=0.05						

(*) – grade of technological value in accordance with multiple evaluation method applied in the evaluation of varieties in Poland: E – elite wheat, A – quality wheat, B – bread wheat, C – other wheat (feed). The brackets indicate proximity of other group [5].

The content of ferulic acid in the bran of the grain of this size can be presented in the following order: Torka > Henika > Zyta > Elena. In the bran obtained from the grain fraction 2.5mm < F_3 < 2.8 mm x 25 mm the content of ferulic acid in the same varieties, but expressed in mg/100g of proteinfree dry matter, differed in a statistically significant way among the varieties. The content of ferulic acid in fraction F_3 expressed in mg/100 g of dry matter or mg/100 g of proteinfree dry matter can be arranged in the following order: Torka > Henika > Zyta > Elena (Table 3). In the bran obtained from fraction of grain 2.8 mm < F_2 < 3.2 mm x 25 mm the content of ferulic acid expressed in µg/g of dry matter differed in a statistically significant way among Begra, Almari, Elena, Ismena, Nawra, Jasna and Torka varieties. On the other hand, evaluating the content of ferulic acid into mg/100g proteinfree dry matter in the bran from Zyta, Almari and Elena varieties did not show significant differences (Table 3). The content of ferulic acid as variety feature of wheat grain is documented by numerous research. Hatcher and Kruger [4] revealed variation in the content of ferulic acid in wheat grain and flour, depending on the milling quality, of five varieties of Canadian wheat. The relation between variation in the content of ferulic acid depending on wheat variety was also confirmed by other experiments [15, 9, 20, 1].

The results of statistical analysis of the content of ferulic acid in the bran indicates the significance of equalisation of sample. It also follows from the experiments that the bigger the grain is, the smaller significance of the variety feature is. The relation of the different content of ferulic acid in wheat grains depending on their size is confirmed by the research of Lempereur et al. [9].

Characteristics of the colour of wheat grain surface

The colour of wheat grain measured as the frequency of grey level values from 0 to 255 expressed as the min., max. and average values had many common characteristics among the analysed varieties (Table 4).

Table 4. Statistical analysis in the colour of wheat grain (measurement of grey levels 0-255)

Wheat variety	Fraction	Number of the analysed items	Average value of grey levels frequency in the range X_{min}, X_{max} and $X_{śr}$		
			X_{min} (average value.)	$X_{śr.}$ (average value.)	$X_{max.}$ (average value.)
Zyta Elena Torka Henika	2.5 < F_3 < 2.8 x 25mm	100	107.2^{b} 102.3^{a,d} 101.4^{a} 103.6^{c,d}	145.3^{b,c} 149.4^{a} 143.7^{c} 146.8^{b}	188.2^{b} 200.5^{a} 196.1^{c} 197.3^{a,c}
Begra Zyta Almari Elena Ismena Nawra Jasna Torka	2.8 < F_2 < 3.2 x 25mm	320	98.5^{b} 100.7^{a} 100.8^{a} 94.4^{e} 93.3^{e} 96.5^{c,d} 97.7^{b,c} 95.8^{d,e}	132.2^{c} 136.7^{b} 133.2^{c} 141.1^{a} 126.9^{d} 129.6^{e} 129.1^{e} 126.9^{d}	184.1^{c} 186.6^{b} 186.6^{b} 196.6^{a} 181.0^{e} 184.7^{b,c} 183.3^{c} 178.8^{d}
Mean values followed by the same letter are not significantly different at p=0.05					

The differences among winter varieties, Zyta and Elena, fraction 2.5 mm < F_3 < 2.8 mm x 25 mm were statistically significant regardless of the analysed values of grey levels. On the other hand, the colour of grain of spring varieties, Torka and Henika, of the same fraction differed in a statistically significant way for min. and average value of grey levels (Table 4). While comparing all the varieties fraction 2.5 mm < F_3 < 2.8 mm x 25 mm it should be stated that only the colour of Elena variety differed significantly from the other varieties at the average value of grey level.

The analysis of the colour of fraction 2.8 mm < F_2 < 3.2 mm x 25 mm it can be stated that the frequency of average value of grey level for Elena variety differs in a statistically significant way from all the analysed varieties (Table 4). Similar relations were revealed for Zyta variety. There is a similarity between the grain colour of Begra and Almari, Ismena and Torka, and Nawra and Jasna varieties. The average max. value of grey level confirmed statistically significant difference in the colour of the grain of Elena variety but also spring varieties Ismena and Torka (Table 4).

The min. and max. average values or the average value of grey level in the range from 0 to 255 confirm that the experimental assumptions were right. Statistically significant differences in the variety evaluation of the grain colour expressed with the grey levels from 0 to 255 depend on the size of the analysed surface and the method of presenting results applied for the evaluation – the frequency of min., max. or average value of grey level.

The colour of the F_2 and F_3 grain fraction of Elena and Zyta varieties expressed as the frequency of the average value of grey level differed from the other analysed varieties. These varieties, classified in different quality grades (A and C), are differed in a statistically significant way by colour (Table 4).

The analysis of three components (RGB) of the colour the grain of the analysed winter and spring varieties revealed many similarities and differences (Table 5).

Table 5. Statistical analysis of significance of the differences in the colour of wheat grain (system LUCIA), $2.8 < F_2 < 3.2$ x 25 mm fraction

Variety	Number of the analysed items	Average level of the brightness of colour ($X_{śr}$)		
		Red (R)	Green (G)	Blue (B)
Begra	200	177.0^{b}	142.8^{b}	76.8^{a}
Zyta		186.3^{a}	147.4^{a}	74.2^{b}
Almari		$176.4^{b,d}$	143.0^{b}	$77.^{a,e}$
Elena		184.6^{a}	147.6^{a}	$77.3^{a,e}$
Ismena		174.8^{d}	142.3^{b}	78.7^{e}
Nawra		203.4^{e}	160.5^{d}	$77.8^{a,e}$
Jasna		192.2^{c}	160.3^{d}	92.1^{d}
Torka		193.5^{c}	162.6^{c}	87.8^{c}
Mean values followed by the same letter are not significantly different at p=0.05				

There were statistically insignificant differences in the brightness of red, green or blue colours in the surface among many of the analysed varieties. Only the colour of the grain of Nawra variety differed from the colour of the grain of other varieties in the brightness of red colour and Torka variety in the brightness of green or blue colours.

The analysis of correlation among the analysed discriminants

Correlation between the content of ferulic acid and total phenolic compounds, and the flour yield and the colour of the grain of the analysed wheat varieties

The analysis of correlation coefficients indicated significant relations between the content of ferulic acid and the milling quality determined on the basis of the flour yield (milling yield) in the grain of two wheat varieties fraction > 1.7 mm x 25 mm (Table 6).

Table 6. Correlation between the content of ferulic acid, total phenolic compounds, flour yield and the grain colour of the analysed wheat varieties

Fraction	Variety	Discriminant	Ferulic acid			Total phenols mg/100g
			mg/100g	mg/100g d.m.	mg/100g non-protein d. m.	
			Value of correlation coefficient			
F > 1.7 x 25 mm	Zyta	Flour yield [%]	**-0.8410***	**-0.8717***	**-0.8230***	-0.0550
	Elena		**0.5776***	**0.5810***	**0.5894***	**0.6497***
	Torka		-0.0925	-0.1271	-0.0888	**0.6278***
	Henika		0.2943	0.2741	0.2734	0.4531
2.5 < F_3 < 2.8 x 25 mm	Zyta	Flour yield [%]	-0.4526	-0.4311	-0.4805	0.4680
	Elena		-0.2359	-0.2850	-0.2344	**-0.6886***
	Torka		-0.1908	-0.1499	-0.1683	-0.0230
	Henika		-0.5242	-0.5261	-0.5076	-0.0505
	total	Average grey level	-0.7046	-0.7579	-0.7838	0.9253
2.8 < F_2 < 3.2 x 25 mm	Begra	Flour yield [%]	-0.2623	-0.2523	-0.2202	-0.1840
	Zyta		0.1283	0.1366	0.0884	0.2209
	Almari		0.1037	0.0706	0.0979	-0.2497
	Elena		-0.2251	-0.2102	-0.2456	0.2759
	Ismena		-0.1936	-0.2439	-0.2580	-0.2889
	Nawra		0.4488	0.4842	0.4914	-0.4332
	Jasna		0.3533	0.3454	0.4042	0.3937
	Torka		**0.7412***	**0.7392***	**0.7226***	0.3170
	Colour					
	total	Average grey level	0.3567	0.3378	0.2503	0.4038
		Discriminant R**	-0.5220	-0.5191	-0.4988	-0.4394
		Discriminant G**	-0.4672	-0.4548	-0.4170	-0.4334
		Discriminant B**	-0.1863	-0.1619	-0.0947	-0.2279
total		Average grey level	-0.5280	-0.5609	**-0.6468***	0.5072

***- statistically significant correlation**
****- frequency of grey levels corresponding to the brightness of colour R (red), G (green), B (blue)**

The content of ferulic acid in the grain of such a size was correlated in a statistically significant way with flour yield in the case of two winter wheat varieties - Zyta and Elena. The varieties differ between each other in respect of quality grade and direction of correlation between flour yield and the amount of ferulic acid. In the case of grain of Zyta variety negative correlation was found but in Elena variety positive correlation was found between the content of ferulic acid and flour extract obtained from the grain of these varieties. Positive correlation between ferulic acid and flour yield was also found in grain fraction 2.8 mm < F_2 < 3.2 mm x 25 mm of Torka variety (Table 6). Flour yield is a significant discriminant for the milling quality of grain. Kelfkens [6] checked the possibility to use an index of the content of ferulic acid for predicting the milling quality of wheat. On the basis of 29 analysed varieties of wheat he found a wide range of variance in the content of ferulic acid in the grain endosperm of the analysed varieties from which the author concludes that the content of ferulic acid cannot provide an absolute measurement for the share of coat compounds in flour. On the other hand, the correlations between the content of ferulic acid and flour yield, which were determined in this experiment, suggest necessity to analyse the content of ferulic acid with respect to its relation with flour extract and the size or colour of grain surface, which requires further research.

The content of total phenolic compounds was correlated with flour extract in the case of two wheat varieties - Elena and Torka. The grain of Elena variety showed different directions of correlation depending to grain size. The grain of fraction > 1.7 mm x 25 mm was characterised by positive correlation but the grain of the same variety of fraction 2.5 mm < F_3 < 2.8 mm x 25 mm revealed negative correlation between the content of total phenols and flour yield (Table 6).

The analysis of the relation between the colour and the content of ferulic acid showed that in wheat varieties (no division into fractions) there is a relation between the colour determined with the value of the average grey level ($X_{śr}$) and the content of ferulic acid in proteinfree dry matter of grain. The higher content of ferulic acid characterised wheat grain, the lower level of grey characterised its colour (Table 6). References lack data regarding the relation between the content of ferulic acid and the colour of wheat grain. However, there is data available concerning correlations between the content of total phenolic compounds and the colour of seed coat for leguminous plants [8].

Correlations between flour yield and the grain colour of the analysed wheat varieties

Statistically significant correlations between flour yield and the colour of wheat grain were determined only in the case of determining the colour of grain with 0-255 grey levels. The flour yield obtained from the grain of fraction $2.8 < F_2 < 3.2$ x 25 mm was correlated positively with the colour expressed with the frequency of the max. grey level (X_{max}). The analysis of the studied wheat varieties without division into fractions confirmed positive correlation between flour yield and the colour of grain expressed as the max. value of grey level and revealed that the amount of flour obtained from wheat grain also depends on their colour determined on the basis of the average value of the grey level($X_{śr}$) (Table 7).

Table 7. Correlation between flour yield and the grain colour of the analysed wheat varieties

Fraction	Discriminant **		Flour yield [%] Value of correlation coefficient
$2.5 < F_3 < 2.8$ x 25 mm	System I	$X_{śr}$	-0.3853
		X_{min}	-0.0997
		X_{max}	-0.2572
$2.8 < F_2 < 3.2$ x 25 mm	System I	$X_{śr}$	0.6238
		X_{min}	-0.1098
		X_{max}	**0.7252***
	System II	X_R	0.4542
		X_G	0.2987
		X_B	-0.1445
Total (no allowance for fractions)	System I	$X_{śr}$	**0.7348***
		X_{min}	0.4102
		X_{max}	**0.7357***

*** - statistically significant correlation;**
**** - $X_{śr}$ – average value of grey level; X_{min} – min. value of grey level; X_{max} – max. value of grey level;**
System I – grey levels 0-255;
System II – LUCIA (R,G,B).

If determination of ferulic acid in cereal grain with the HPLC method has got a quite wide range of references, determining relations or connection to the colour of grain and the milling quality requires further research. Technique and accuracy of the determination of the colour is of a large significance to using DIA. From the research of Majumdar and Jayas [10] it follows that the algorithm for the grain colour should allow for the texture of grain specified as combination of the brightness of red (R), green (G) and blue (B) compounds or selected grey levels for the determination of colour. The findings of this experiment can provide grounds for further consideration and improvement the measurement of wheat grain colour, particularly when variety differences are slight or varieties are isogeneic.

CONCLUSIONS

1. The content of ferulic acid in the bran of the analysed wheat varieties is a variety feature which depends on grain size. The highest content of ferulic acid characterises the bran obtained from the fraction of wheat grain of 2.8 mm $< F_2 <$ 3.2 mm x 25 mm . The statistical analysis of significance of the differences indicates significance of the distribution of grain size analysis for a grain sample for variety evaluation of the content of ferulic acid.
2. The colour of wheat grain for Elena variety differs from the majority of other analysed wheat varieties in the scope of the frequency of min., average and max. value of grey level. The results of Duncan test indicate that the colour of grain , winter and spring varieties, of the same quality grade differs in a statistically significant way. The statistical analysis of significance of the differences in the average value of brightness of red and green colours indicates significant differences between Begra and Elena varieties and in red, green and blue colours between Elena and Jasna and Torka varieties.

3. The analysis of correlation between ferulic acid content and the colour of wheat grain shows relations between the colour determined as the average value of grey level ($X_{śr}$) and the amount of ferulic acid in the bran expressed in mg/100 g of proteinfree dry matter (r = - 0.6468). The higher the content of ferulic acid in the bran, the lower grey levels determine the colour of wheat grain .
4. The flour yield obtained of the analysed wheat varieties grain is correlated with the colour of grain expressed as the average value of grey level(r = 0.7348) and as the max. value of the grey level (r = 0.7357).

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