A comparative study of quality and colour characteristics of organically and conventionally produced spelt flours by means of UV-Vis reflectance spectroscopy and multivariate analysis

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Summary

Wholemeal flours prepared from Lajta and ÖKO-10 spelt cultivars grown under organic and conventional agricultural practices in various Slovakian and Hungarian regions were evaluated and statistically compared. Ash, moisture and protein contents, colour and spectral characteristics of CIE $L^*a^*b^*$ colour system calculated from reflectance spectra were monitored. The variation of monitored parameters was the largest for redness (a^*), ash, moisture and protein content, whiteness (*WI*), *Z%* brightness and tinting (*TI*) together with browning indexes (*BI*). All the colour and spectral characteristics were significantly influenced by country or region of origin. Most of the analysed traits showed significant differences ($p < 0.05$) also between spelt cultivars and agricultural practices. Conventional flours were significantly higher in ash and protein content, darker and more saturated by red and brown colour than their organic counterparts. In comparison to Lajta, ÖKO-10 showed higher ($p < 0.05$) ash and protein content as well as higher saturation with yellow. Satisfactory discrimination of flours (80.9–89.2 %) was achieved confirming the significant effect of the spelt cultivar, geographical and agricultural factors on flour colour. In most cases, protein content, a^*, *WI*, *Z%* brightness, yellowness (b^*) and yellowness index (*YI*) were identified as the most important markers for spelt flour differentiation.

Keywords

spelt flour; agricultural practice; geographical origin; colour evaluation; multivariate statistics

There is a growing global demand for products that offer convenience, variety, novelty, health benefits, and are sustainably produced. In the health-promoting food sector, the greatest demand is for organically produced specialty cereals. In general, cereals are a basic component of human and animal nutrition. Flours belong to the most important food ingredients used to produce various bakery products, such as bread, pasta, cakes or biscuits. In addition to common wheat (*Triticum* spp.), which belongs to the most important cultivated crops, attention is focused on the production of ancient wheat species. Their representative is spelt (*Triticum spelta* L.), which was one of the first cultivated crops [1, 2]. The popularity of spelt is related mainly due to the organic farming expansion, its lower soil and nutritional requirements, higher tolerance to diseases and several fungal pathogens [2–4].

Flour production is a complex process. The quality of flour is influenced by various factors, such as chemical composition of the grain (ash, protein, moisture and pigment content), as well by or agricultural and environmental conditions. The requirements for flour are specific to its intended use [5]. Flour colour is an important parameter that can reflect flour quality and milling precision [6].

Traditionally, the flour colour is evaluated visually. However, this method is subjective as colour perception differs among individuals, with surrounding colour and lighting conditions

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also influencing colour judgement. On the other hand, spectral analysis plays dominant role in quantifying flour colour. In general, the colour of an object can be described by several colour system, e.g. by Hunter Lab, Commission Internationale de l'Eclairage's CIE *L*a*b**, CIE *XYZ* or CIE *L*u*v**, which differ in the symmetry of the colour space [7].

Flour colour is usually measured by reflectance spectroscopy using the CIE *L*a*b** colour space. This system classifies colour in three dimensions: *L**, which measures lightness (brightness) and ranges from 0 (black) to 100 (white), *a** indicates redness/greenness (+*a**/–*a**), and *b** indicates yellowness/blueness (+*b**/–*b**). Chroma (*C**) and hue angle (*H**) represent quantitative and qualitative parameters of colour derived from *a** and *b** values. Hue indicates the colour type (an angle of 0° and 360° represent red hue, whilst angles of 90°, 180° and 270° represent yellow, green and blue hues, respectively). *C** determines intensity/saturation of colour [7, 8]. In general, standard illuminant and observer angle selection are necessary for proper colour measurement [7].

Several authors tested various colorimeters and reflectance spectrophotometers for colour evaluation of cereals and flours [5, 9]. Colour values of CIE *L*a*b** and another spectral characteristic (e. g. whiteness – *WI*, yellowness index – *YI*) were previously used for evaluation of flour colour of various spelt cultivars usually grown in Poland, Germany and Serbia (Ceralio, Schwabenkorn, Franckenkorn, Holstenkorn, Schwabenspeltz, Ostro, Baulander Spelz, Rouquin or Oberkulmer) [1, 3], spelt hybrids [10], einkorn wheat [9], durum wheat [9], common wheat [6, 8], rye and barley [8]. As follows from these studies, colour characteristics in CIE *L*a*b** system could potentially be used to describe the colour of flour. However, these parameters have not yet been used to differentiate spelt or other cereals by cultivar, country or agricultural practice.

The aim of the present work was to evaluate the colour and spectral quality (in CIE *L*a*b** colour system) attributes of flours produced from two spelt cultivars (Lajta and ÖKO-10) grown under organic and conventional production practices in various geographical regions of Slovakia and Hungary. The feasibility of treating these colour/spectral characteristics with multivariate analysis was to evaluate the potential of reflectance spectroscopy to differentiate the flours according to selected criteria, i. e. agricultural practices, geographical origin or spelt cultivar.

MATERIALS AND METHODS

Flour samples

A set of 156 wholemeal spelt flours was prepared from two spelt varieties Lajta and ÖKO-10, sown in autumn (October) and grown under organic and conventional production practices in various locations in Hungary and Slovakia. The Hungarian regions were Rábcakapi (47.71° N, 17.28° E; altitude 115 m, average temperature 12.2 °C, average precipitations 504.9 mm, 40 samples), Mosonmagyaróvár (47.87° N, 17.27° E; altitude 119 m, average temperature 12.2 °C, average precipitations 504.9 mm, 43 samples) and Sopron (47.69° N, 16.59° E; altitude 217 m, average temperature 11.8 °C, average precipitations 512.0 mm, 31 samples). The Slovakian regions were Sládkovičovo (48.20° N, 17.64° E; altitude 118 m, average temperature 12.0 °C, average precipitations 534.8 mm, 20 samples) and Lehnice (48.06° N, 17.46° E; altitude 120 m, average temperature 12.1 °C, average precipitations 541.4 mm, 22 samples). The regions are characterized by chernozem soil (also called black soil). Both Lajta and ÖKO-10 are later-ripening spelt cultivars registered in Hungary in 2002 and 1998, respectively [11]. Lajta belongs to the spelt cultivars with white spike colour, while ÖKO-10 has the yellowish-brown spike colour. Both cultivars have good frost and disease resistance and yield 6–7 $t \cdot ha^{-1}$ in favourable areas [12]. The spelt grains were ground on a stone mill to obtain wholemeal flour with particle size of 231 ± 49 μm (mean ± standard error of the mean).

Basic characteristics of flours

Ash and moisture content of flours were determined according to the official AOAC methods 923.03 and 925.10, respectively [13]. Protein content was determined by Kjeldahl method (AOAC method 979.09), using a nitrogen-to-protein conversion factor of 5.7 [13].

Measurement of UV-Vis reflectance spectra

Reflectance spectra were recorded using a UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan) with Large Integrating Sphere Assembly LISR 3100 (Shimadzu) using a 1 cm quartz cuvette QS (Hellma, Müllheim, Germany) in spectral range 200–800 nm with 2 nm intervals and slit width 12 nm. The spectra were acquired in quadruplicate and averaged for use in the data analysis. The reflectance measurements were calibrated using $BaSO_4$ white plate standard with values of perfect reflecting diffuser X = 94.81, Y = 100.00 and Z = 107.34.

Colour evaluation

Colour values and other spectral characteristics in CIE $L^*a^*b^*$ were calculated from reflectance spectra using Panorama 3.1.16 advanced ColorLite (Labcognition, Cologne, Germany) according to the recommendations of the Commission Internationale de L'Éclairage (CIE). The standard Illuminant D65 (representing average daylight) and 10° standard observer (angle of perception of a human observer) and reflectance spectral data from 380–780 nm interval were used in these calculations. Besides basic L^*, a^*, b^*, C^* and H^* values, other spectral characteristics such as yellowness index (*YI*, quantifying product degradation by light, processing, or chemical exposure), whiteness (*WI*, indicating the extent of discoloration during processing) [7], tinting index (*TI*, describing the amount of greenish or reddish tint in the almost white product), *Z%* brightness (measuring brightness of white materials that tend to get yellowish with age or degradation), excitation purity (*EP*, an exactly defined ratio of the distances in the chromaticity diagram indicating how far the given colour is displaced towards the spectrum colour from the achromatic colour) and dominant wavelength (*DW*, defining principal wavelength of the colour) [14] were also evaluated. CIE $L^*a^*b^*$ values were used to calculate the flour colour index (*FCI*, Eq. 1) and the browning index (*BI*, Eq. 2) [7]:

$$FCI = L^* - b^* \quad (1)$$

$$BI = \frac{100 \cdot (X - 0.31)}{0.17} \quad (2)$$

where

$$X = \frac{(a^* + 1.75 \cdot L^*)}{(5.64 \cdot L^* + a^* - 3.012 \cdot b^*)} \quad (3)$$

Statistical analysis

ANOVA and multivariate statistics were used to compare, explore, discriminate and model the experimental data. Multiple comparisons of mean colour values between Slovakian and Hungarian organic and conventional spelt flour samples were performed by Analysis of variance Tukey's honestly significant difference (ANOVA Tukey-HSD) test with the accepted level of significance at $p \leq 0.05$.

Methods of multivariate statistics, which allow the reduction of multidimensional and correlated data to only a few dimensions, were applied to compare and discriminate the colour attributes of wholemeal spelt flours according to production practices, geographical origin and spelt cultivar. Multidimensional pattern recognition techniques principal component analysis (PCA), principal component factoring (PCF), stepwise discriminant and canonical discriminant analysis (CDA) were used in order to define the most appropriate colour and spectral variables, as well as to interpret and visualize the differences between the compared flour types. The convergence criteria of discriminant analysis were chosen for a standardized proximity matrix with maximum number of iterations of 50. The stepwise selection criteria used were tolerance – 0.001, *F* statistic: *F*-to-enter – 3.8416 and *F*-to-remove – 2.7056. Statistical evaluation of the experimental data of flour samples was carried out by Unistat v. 6.0 statistical package (Unistat, London, United Kingdom).

RESULTS AND DISCUSSION

Flour colour affects the colour of the final food products and is, therefore, one of many flour specifications followed by the end user. Determination of lightness (brightness), *WI* and *YI* are the tests used by millers to get the best yield from the milling process [3, 6, 10, 15]. Evaluation of flour colour in CIE $L^*a^*b^*$ system is frequently used. Its main advantages are ease, speed, simple sample preparation, non-destructiveness and the fact that it provides information simultaneously on both brightness (lightness) and yellowness. The main disadvantage of the method in case of flour is that differences in particle size limit the extent to which results can be compared [9]. The ranges of basic characteristics and colour/spectral descriptors determined in wholemeal spelt flours are shown in Tab. 1. An overall examination of the raw data showed that variation evaluated through the coefficient of variation was the largest in the case of a^*, ash, moisture and protein content, *WI*, *Z%* brightness, *TI* and *BI* indexes. Comparison of these parameters between Lajta and ÖKO-10 spelt cultivars is depicted in Fig. 1. As can be seen, higher values of a^*, ash, moisture and protein content, *TI* and *BI* were observed in ÖKO-10. However, largest variations in these parameters were found in flours prepared from Lajta cultivar.

The ash content in the analysed spelt flours varied from 1.0 % to 2.5 % (Tab. 1). This was consistent with the study by Frakolaki et al. [16], who reported the ash content in wholemeal spelt flour to be 2.0 %. In a study by Żuk-Gołaszewska et al. [17], ash content in spelt cultivars grown in Poland was significantly lower (approximately 0.5 %) compared to our results. Ash content in flour is related to the ash content in bran. Its level in flour indi-

Tab. 1. Parameters of quality, colour and spectral characteristics of spelt flours.

Parameter	Mean	Median	Standard deviation	Standard error	Coefficient of variation	Minimal values	Maximal values
Ash [%]	1.6	1.5	0.3	0.0	0.2	1.0	2.5
Moisture [%]	11.0	11.3	2.0	0.2	0.2	3.4	13.1
Total proteins [%]	11.7	11.6	1.9	0.2	0.2	8.2	15.5
Lightness (L^*)	85.71	84.74	3.47	0.28	0.04	78.47	91.81
Redness (a^*)	2.02	2.11	0.65	0.05	0.32	0.81	3.65
Yellowness (b^*)	11.33	11.33	0.85	0.07	0.08	8.75	14.54
Chromaticity (C^*)	11.53	11.46	0.90	0.07	0.08	8.89	14.99
Hue angle (H^*)	80.00	79.40	2.91	0.23	0.04	72.09	85.62
Yellowness index (*YI*)	29.78	29.39	2.26	0.18	0.08	24.38	36.71
Whiteness (*WI*)	18.47	18.98	2.94	0.23	0.16	13.72	24.97
Flour colour index (*FCI*)	74.37	73.72	3.84	0.31	0.05	66.34	80.83
Browning index (*BI*)	15.86	15.54	1.98	0.16	0.12	11.59	21.82
Tinting index (*TI*)	–8.23	–8.29	1.72	0.14	–0.21	–12.83	–5.31
Z% brightness	50.38	48.85	5.84	0.46	0.12	38.94	60.80
Excitation purity (*EP*)	0.14	0.13	0.01	0.00	0.08	0.10	0.18
Dominant wavelength (*DW*)	573.66	573.85	0.87	0.07	0.01	572.01	575.95

Values of analysed spelt flours are presented without respect on farming system, country or region of origin and spelt cultivar.

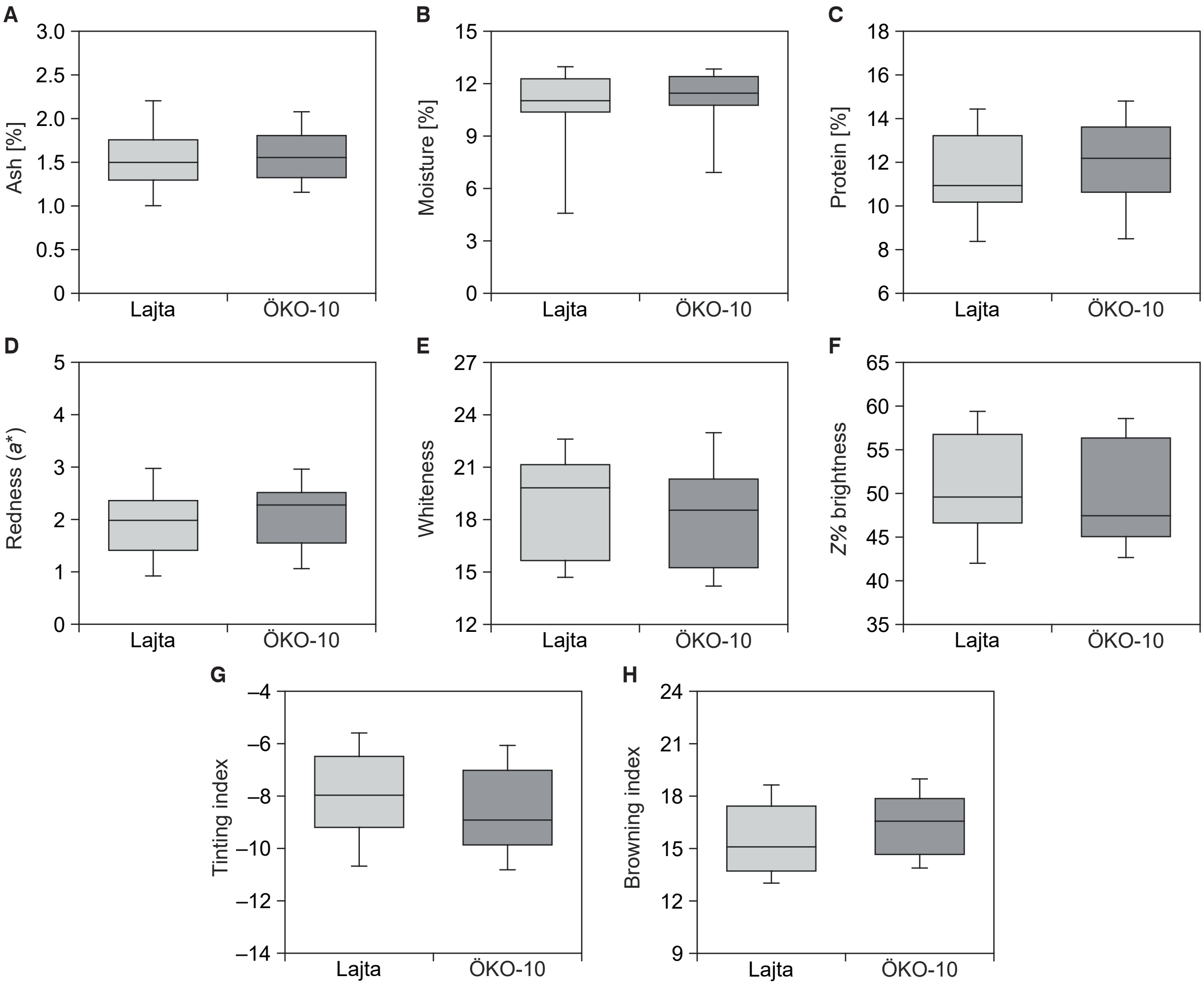

Fig. 1. Variation of quality, colour and spectral characteristics of spelt flours.

A – ash, B – moisture, C – protein, D – redness (a^*), E – whiteness, F – *Z*% brightness, G – tinting index, H – browning index. Spelt flours were prepared from two spelt varieties Lajta and ÖKO-10.

cates milling performance and indirectly reveals the extent of contamination of white or refined flour by bran [18]. It also estimates the degree of bran and germ separation from endosperm during milling, with the bran imparting a darker colour to the final products [19]. A higher ash content is typical for wholemeal flours due to higher levels of the germ, bran and outer endosperm, which may influence the protein content [18].

Protein content varied significantly from 8.2 % up to 15.5 %, which is comparable with previous studies focused on nutritional characterization of spelt flours [16–18]. It is an important parameter for millers and bakers in determining suitability of flours for various uses. Protein content, which affects many processing properties (e. g. water absorption, cohesiveness, dough strength or dough texture), depends on type and class of flour milled, growing conditions of the cereal or fertilizers inputs [1, 18, 20].

The bran content and the milling process influence also the flour lightness/brightness, while the yellowness and redness are related to the presence of pigments, in particular carotenoids, anthocyanins and xanthophylls [15]. The mean values of L^*, a^* and b^* of spelt flours (Tab. 1) were higher than or comparable to those previously reported for spelt [3] and wheat flours [6, 9]. In comparison to our results, Żuk-Gołaszewska et al. [3] reported lighter ($L^* = 92.2–93.4$), less red ($a^* = 0.10–0.25$) and less yellow ($b^* = 7.28–9.89$) spelt flours. These differences could be attributed to the nature of the milling process (flour type), as white flours should be lighter and less yellow than wholemeal flours. This assumption was confirmed by comparing other spectral characteristics (*WI*, *YI* or *Z%* brightness) that could be used to assess food colour. White spelt flours had higher *WI* and *Z%* brightness than wholemeal spelt flours in our study, while an opposite trend was observed for *YI* [3].

A correlation matrix confirmed the previously described relationships linking flour quality parameters with colour characteristics for wheat [19, 21, 22] and triticale flours [23]. Protein content positively correlated with ash ($r = 0.3471$), which is comparable with the value of $r = 0.3560$ reported for flours from Indian wheat varieties [19]. Ash and protein content showed inverse correlation with L^* and *Z%* brightness (r from –0.649 to –0.690). This means that flours with higher ash and protein contents were darker, as indicated by lower L^* values. On the other hand, a^* and b^* values positively correlated with both ash and protein contents (r from 0.5780 to 0.6245). The results suggest that flour colour is controlled by the amount of ash and protein, which are directly influenced by bran contamination, since minerals and pigments are mainly concentrated in the pericarp and bran layers [19, 21]. In addition, flour granulometry also significantly influences colour parameters together with the ash and protein contents. Hidalgo et al. [9] reported that L^* and ash content decreased and while b^* and total carotenoids increased with flour size growth increasing granule size.

Discrimination according to agricultural practice

As all the parameters mentioned above could be influenced by various factors, all the experimental data were processed using statistical methods. ANOVA Tukey-HSD multiple comparison was used to assess the influence of agricultural practice, country or region and spelt cultivar on colour quality of wholemeal spelt flours. Some significant differences were found between organic and conventionally produced flours (Tab. 2), especially in ash ($p = 0.0001$), in protein content ($p = 0.0000$), as well as in colour values of L^* ($p = 0.0060$), a^* ($p = 0.0182$), *FCI* ($p = 0.0001$) and *BI* ($p = 0.0048$). Organically prepared spelt flours were found to be lighter, less reddish and more brownish than their conventional counterparts. On the other hand, conventional flours were generally higher in ash and protein content. These differences could be attributed to the use of intense technology in conventional cultivation, in particular application of fertilizers (higher nitrogen input), which is absent in organic farming [1, 24]. This hypothesis was stated by Ma et al. [25], who reported that nitrogen fertilizers significantly increased a^* and b^* values, while L^* decreased. According to Takač et al. [1], increased protein content in conventional wheat can also be attributed to the timing and doses of nitrogen fertilizers, as well as different nutrient availability in soil or climatic conditions. On the contrary, Mäder et al. [26] reported that nutritional value (protein content, amino acid composition, mineral and trace element content) and baking quality of wheat during a 21-year field trial were not affected by agricultural practices. However, lower addition of plant-available nitrogen, reduced input of other fertilizers and reduced use of herbicides in the organic field plots led to lower wheat yields [26].

Principal component analysis (PCA) was performed in order to test grouping tendency of flour samples and to describe the variability of the experimental characteristics. Many strong correlations were found among monitored colour/spectral descriptors of spelt flours, as most of them are mathematically derived from the basic colour tristimulus values (*X, Y* and *Z*). When applying PCA to detect colour differences between organic and conventional flours, the original data set was transformed into new variables, principal components. As follows from the results obtained, first two principal components explained more than 84.4 % of the whole system variability. The plot of principal components depicted in Fig. 2A shows that spelt flours were partially divided into two clusters in the upper and lower halves of the plot, corresponding to organic and conventional production system, respectively. Descriptors of a^*, b^*, *WI*, *BI* and protein content played a dominant role in principal components construction. Principal component factoring (PCF) with varimax rotation resulted in a plot of factor scores corresponding to PCA data projection (data not presented). These confirmed the importance of protein content together with a^*, b^*, *YI, TI* and *BI*. The plot of factors (Fig. 2B), which shows the importance of each descriptor for the purpose of discrimination, also revealed their mutual positive or inverse correlations. This was consistent with results of Pearson's correlations, as strong positive correlations were found between L^*, *TI, FCI* and *Z%* brightness (r = 0.9165–0.9921), or b^*, *YI* and *BI* (r = 0.8255–0.8643), while *TI* negatively correlated with a^* (r = –0.9578). In contrast to PCA and PCF, stepwise discriminant analysis identified protein and ash content as the key parameters to discriminate organic from conventional spelt flours. Canonical discriminant analysis (CDA) selected *TI, YI,* b^*, *FCI* and *BI* as the most discriminating descriptors, while the percentage of correctly classified samples reached 84.8 %. Although there is little information in the literature on the use of colour or spectral characteristics to discriminate between organic and conventional flours, the classification score obtained suggests that their use is promising. Currently, other analytical

Tab. 2. Statistically significant descriptors of spelt flours selected by ANOVA Tukey–HSD test.

Colour descriptor	Higher value	Difference	Standard error	*q* Stat*	Table *q**	Probability (*p*)
Agricultural practice (organic vs conventional)						
Ash	Conventional	0.3264	0.0788	5.8562	2.8083	0.0001
Protein	Conventional	2.5308	0.3570	10.0264	2.8083	0.0000
Lightness (*L**)	Organic	0.8187	0.2869	4.0355	2.8083	0.0060
Redness (*a**)	Conventional	0.2063	0.0848	3.4403	2.8083	0.0182
Flour colour index (*FCI*)	Organic	4.2489	0.5978	10.8157	2.8083	0.0001
Browning index (*BI*)	Conventional	1.2655	0.4315	4.1473	2.8083	0.0048
Country of origin (Slovakia vs Hungary)						
Protein	Slovakian	0.7521	0.3392	3.1359	2.8083	0.0280
Lightness (*L**)	Hungarian	3.8596	0.5471	9.9775	2.8083	0.0000
Redness (*a**)	Slovakian	0.7869	0.0996	11.1766	2.8083	0.0000
Yellowness (*b**)	Slovakian	0.6877	0.1438	6.7647	2.8083	0.0000
Chromaticity (*C**)	Slovakian	0.8160	0.1488	7.7561	2.8083	0.0000
Hue angle (*H**)	Hungarian	3.1427	0.4628	9.6042	2.8083	0.0000
Yellowness index (*YI*)	Slovakian	2.6582	0.3480	10.8009	2.8083	0.0000
Whiteness (*WI*)	Hungarian	13.1162	1.6574	11.1914	2.8083	0.0000
Flour colour index (*FCI*)	Hungarian	4.5473	0.5906	10.8896	2.8083	0.0000
Browning index (*BI*)	Slovakian	2.4090	0.3015	11.2991	2.8083	0.0000
Tinting index (*TI*)	Hungarian	2.1503	0.2610	11.6497	2.8083	0.0000
Z% brightness	Hungarian	6.6028	0.9141	10.2150	2.8083	0.0000
Excitation purity (*EP*)	Slovakian	0.0142	0.0020	10.2531	2.8043	0.0000
Dominant wavelength (*DW*)	Slovakian	0.9646	0.1357	10.0527	2.8043	0.0000
Spelt cultivar (Lajta vs ÖKO-10)						
Ash	ÖKO-10	0.1602	0.0719	3.1486	2.8083	0.0284
Protein	ÖKO-10	1.7589	0.3348	7.4294	2.8083	0.0000
Yellowness (*b**)	ÖKO-10	0.5484	0.1293	5.9979	2.8083	0.0000
Chromaticity (*C**)	ÖKO-10	0.5642	0.1369	5.8299	2.8083	0.0000
Yellowness index (*YI*)	ÖKO-10	1.1531	0.3499	4.6604	2.8083	0.0012
Whiteness (*WI*)	Lajta	3.9321	1.7117	3.2487	2.8083	0.0229
Browning index (*BI*)	ÖKO-10	0.9389	0.3085	4.3047	2.8083	0.0027

* – a Pairwise test result is significant if its *q* Stat value is greater than Table *q* value.

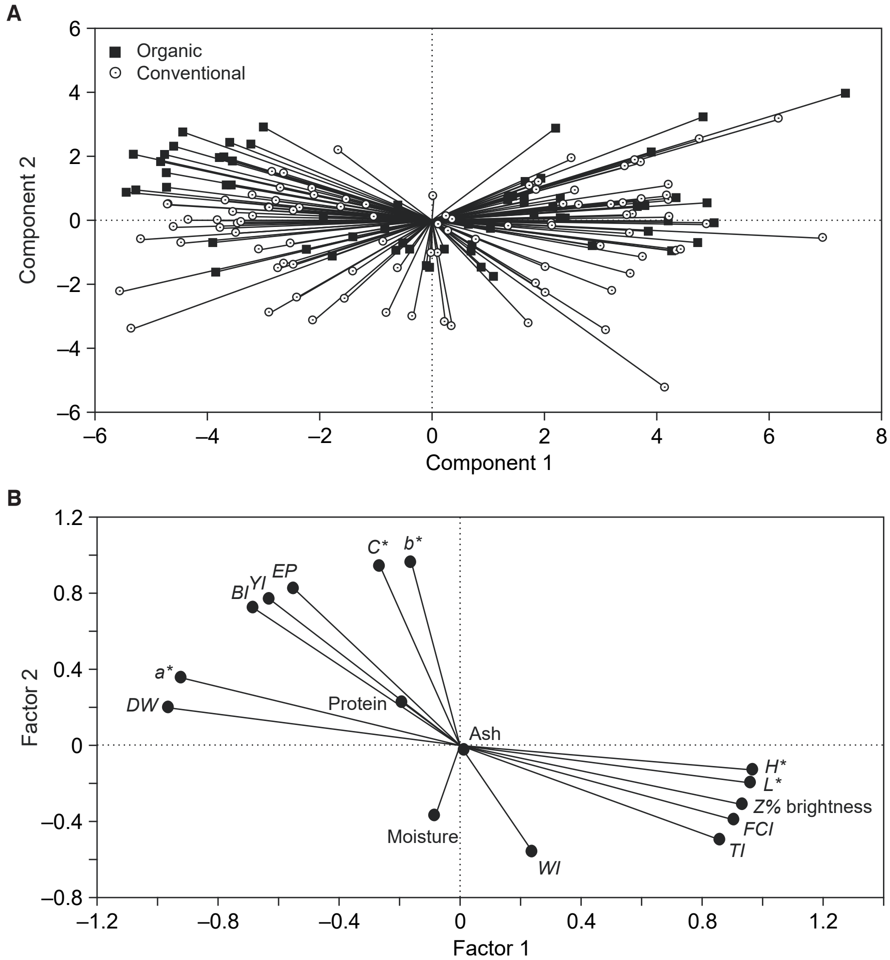

Fig. 2. Principal component analysis and factor analysis of organic and conventional spelt flours.

A – plot of principal components demonstrating differentiation of organic and conventional spelt flours, B – plot of factors (varimax rotation indicating the importance of individual variables for spelt flours discrimination).
*L** – lightness, *a** – redness, *b** – yellowness, *C** – chromaticity, *H** – hue angle, *YI* – yellowness index, *WI* – whiteness, *FCI* – flour colour index, *BI* – browning index, *TI* – tinting index, *EP* – excitation purity, *DW* – dominant wavelength.

techniques have been proposed to discriminate organic flours or grains from their conventional counterparts, e.g. multi-elemental analysis [27, 28], visible-near-infrared (Vis-NIR) spectroscopy [29] or stable isotope analysis [30] with classification accuracy of 80.0–100.0%

Discrimination according to the country or region

Despite the geographical proximity of the growing areas (Slovakia vs Hungary), the same soil type and comparable climatic conditions, ANOVA showed that protein content and all colour/spectral descriptors contributed significantly ($p < 0.05$) to the differentiation of spelt flours by country (Tab. 2). While Slovakian flours were more saturated with yellow (*b**) and red (*a**) colour (and therefore *C*, YI* and *BI* were higher), Hungarian flours were whiter and brighter (higher in *L*, H*, Z%* brightness and *FCI*). The higher protein content and *a** and *b** values in Slovakian samples could be related to the agricultural practices used, as most of these samples were produced under conventional practices with higher nitrogen input. This is in line with differences between organic and conventional flours described above and in previous studies [1, 24]. In addition to production methods that affect plant growth and composition, including levels of secondary bioactive metabolites, environmental factors (temperature, precipitation, location) need to be considered. Although the average temperature in both countries was comparable, the amount of precipitation was slightly higher in Slovakian regions. Moreover, quality and colour characteristics could also be influenced by the elemental and mineral composition of flours, grains and soils in both countries. The latter claim is supported by Zhao et al. [31], who reported that different Chinese regions had

Tab. 3. The most significant descriptors for spelt flours discrimination selected by stepwise discriminant statistics.

Variable	Entered at Step	*F*–to–Enter	Probability	Wilks' lambda
Agricultural practice (organic vs conventional)				
Protein	1	55.9018	0.0000	0.7337
Ash	2	4.4483	0.0366	0.7129
Country of origin (Slovakia vs Hungary)				
Redness (*a**)	1	63.3762	0.0000	0.7111
Ash	2	9.7216	0.0000	0.6941
Yellowness (*b**)	3	8.6296	0.0038	0.6526
Regional differentiation (5 regions: 2 Slovakian + 3 Hungarian)				
Moisture	1	55.1349	0.0000	0.4064
Yellowness index (*YI*)	2	13.8402	0.0000	0.2769
Whiteness (*WI*)	3	8.8883	0.0000	0.2239
Lightness (*L**)	4	9.9151	0.0000	0.1766
Cultivar (Lajta vs ÖKO–10)				
Yellowness index (*YI*)	1	24.6727	0.0000	0.8594
Yellowness (*b**)	2	4.8310	0.0295	0.7549
Protein	3	5.7277	0.0040	0.7017

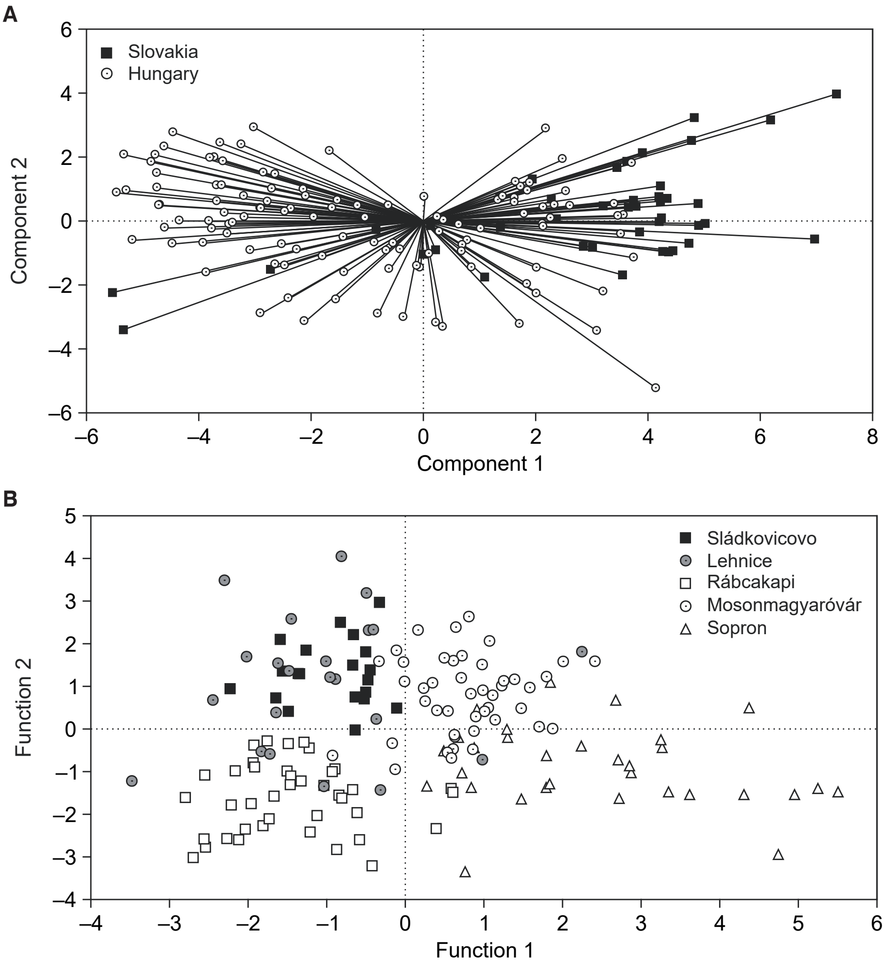

Fig. 3. Discrimination of spelt flours according to the country of origin or region.

A – plot of principal components demonstrating differentiation of spelt flour according to the country of origin, B – canonical discriminant analysis of spelt flours according to the region.

their own elemental characteristics, which significantly influenced the elemental composition of wheat grains and these in turn influenced ash and protein content of grains.

In the regional comparison of spelt flours (Rábcakapi, Mosonmagyaróvár, Sopron, Sládkovičovo, Lehnice; data not shown), significant differences were also found for all colour/spectral descriptors and protein content. In most cases, these were the differences between Slovak and Hungarian samples described above. It could be summarized that flours from Hungarian regions were higher in *L*, H*, Z%* brightness and *FCI*, while flours from Slovakian regions were more saturated with red (*a**) and yellow (*b**). The interregional differences between Hungarian and Slovakian regions were not significant, probably due to the geographical proximity of these regions, mostly comparable average temperature and rainfall values, as well as soil type with similar composition.

The discrimination of samples according to the country of origin (Slovakia vs Hungary) by means of CDA possessed 89.2 % correctness. Regarding the influence of individual descriptors on the discrimination score, *C** and *WI* had dominant role in the construction of discrimination. Results of stepwise discriminant analysis proved a key role of *a*, H** and *b** values in discrimination, as these parameters revealed the highest Wilks' lambda scores (Tab. 3). PCA, as shown in Fig. 3A, did not clearly visualize the differentiation of spelt flour according to country. However, samples from Slovakian regions were in most cases localized in the right part of Fig. 3A. Here, first two principal components explained 84.4 % of the total variance and colour values of *a*, b*, BI, TI, YI* together with protein content were found to be significant descriptors for flour differentiation. For regional differentiation, stepwise discriminant analysis identified *WI* and *YI* values as the most important (Tab. 3). In CDA, which resulted in 82.8 % of correctly classified samples, probably due to a high degree of interregional similarity (Fig. 3B), the parameters *a*, YI, WI, Z%* brightness and *BI* played a dominant role in discrimination.

Traditional agricultural practices and growing conditions such as temperature, rainfall or soil quality are specific characteristics of the growing area and can significantly influence the composition of cereals. Techniques and chemometric methods previously proposed for the geographical traceability of cereals and flour include near-infrared spectroscopy (NIR) [32], non-targeted metabolic fingerprinting [33], stable isotope analysis [34] and multi-element analysis [35] combined with ANOVA, PCA or discriminant analysis. The accuracy of geographic discrimination in these studies was comparable to our results and ranged from 71.0 % to 100.0 %.

Discrimination according to the spelt cultivar

Different varieties of cereals have different uses and nutritional values. For example, winter wheat is best for bakery products, while semolina is better for pasta production. Therefore, discrimination of cereal cultivars is necessary [5].

In a study by Żuk-Gołaszewska et al. [3], white spelts flours (cultivars Ceralio, Schwabenkorn, Franckenkorn, Holstenkorn, Schwabenspeltz, Ostro) differed from each other significantly, especially in *a**, *b** and *YI*. Similarly, Takač et al. [1] reported significant differences in protein content between spelt cultivars Baulander Spelz, Ostro, Rouquin, Schwabenkorn and Oberkulmer grown in Hungary and Serbia [1]. In the case of wholemeal flours from Lajta

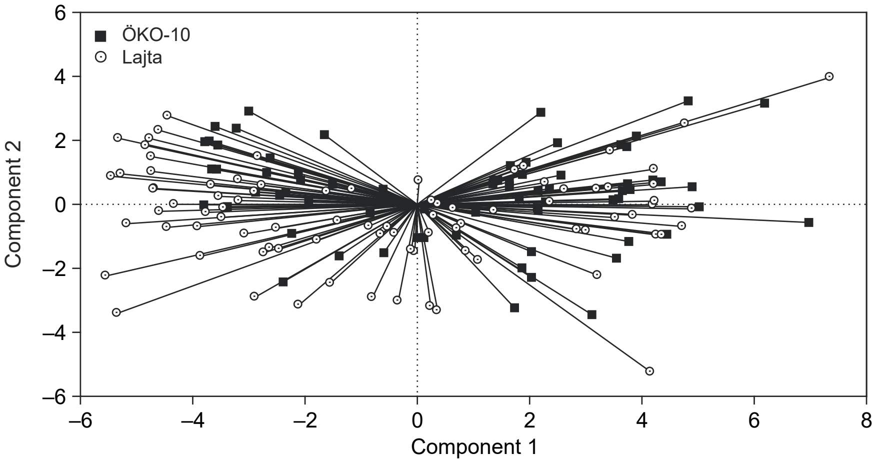

Fig. 4. Plot of principal components demonstrating differentiation of spelt flour according to the spelt cultivar.

and ÖKO-10 cultivars, regardless of the country of origin or the production system, significant differences ($p < 0.05$) were found in ash and protein content, as well as in parameters *b**, *C*, YI* and *WI* (Tab. 2). Cultivar ÖKO-10 was significantly higher ($p < 0.05$) in ash and protein content and saturated more with yellow colour compared to Lajta, which was brighter ($p < 0.05$). These differences can be attributed to the morphological characteristics of both varieties. Lajta is a cultivar with a white grain, whereas ÖKO-10 is a cultivar with a yellowish-brown grain [12]. Based on this fact, it can be assumed that ÖKO-10 flours with higher ash and protein content have a higher bran content and thus a higher concentration of pigments (carotenoids, anthocyanins, xanthophylls).

The plot of principal components (Fig. 4), where all the descriptors are represented by two principal components, shows some similarities between spelt cultivars. The parameters a**, b*, YI, TI, BI* and protein content again showed the highest discrimination efficiency. Results of discriminant analysis also showed a significant variation between cultivars. The colour descriptors *YI* and *b** in stepwise analysis (Tab. 3) and protein content, *a** and *b** values in the CDA were identified as the most discriminating. Classification score for ÖKO-10 and Lajta discrimination reached 80.9 %. This lower classification score could be related to the genetic similarity of both cultivars described above, but also to similar growing conditions and technological processes applied [10, 16, 36]. Although both genetic similarity or variation of some European spelt cultivars were reported [37], data on Lajta and ÖKO-10 cultivars are not available. Slightly higher classification accuracies (80.0–82.0 %) have been reported in previous studies focusing wheat variety (durum, common, einkorn, spelt, emmer) discrimination using NIR spectroscopy [38], Fourier transform near-infrared spectroscopy (FT-NIR) [39] or volatiles profiling [40]. Significantly higher discrimination rates (up to 100.0 %) were obtained using image analysis [41], non-target metabolites analysis [42] or DNA-based methods [43].

CONCLUSIONS

Colour measurement is an important aspect of assessment of flour quality and its potential for use. Therefore, it is necessary to select a suitable instrument and appropriate colour or spectral parameters to objectively assess flour colour. In this study, on Hungarian and Slovakian wholemeal flours of two spelt cultivars (Lajta and ÖKO-10), produced organically and conventionally, colour measurement was performed, measurement results were statistically evaluated and compared on the basis of physico-chemical and colour quality attributes. Redness (*a**), ash, moisture and protein content, *WI, Z%* brightness, *TI* and *BI* indexes were characterized by high coefficient of variation (12–32 %). Variation of remaining colour and spectral characteristics were less than 10 %. All the monitored colour/spectral characteristics as well as ash and protein content were significantly influenced by country or region, while spelt cultivars differed primarily in ash and protein content and colour descriptors related to yellow colour (e. g., *b*, YI* and *BI*). On the other hand, conventionally produced flours were significantly higher in ash and protein content, were darker and more saturated by red and brown colour. Results confirmed that higher ash and protein contents were associated with higher *a** and *b** values and lower *L** values. Categorization of samples according to the agricultural practice, country or region and spelt cultivar reached an accuracy of 84.8 %, 89.2/82.8 % and 80.9 %, respectively. This confirmed the high potential of using colour/spectral characteristics for discrimination of flours. Apart from ash and protein content and basic colour descriptors (*L*, a*, b**), statistical analysis revealed a potential of less commonly used characteristics, namely, *WI* and *YI* of *Z%* brightness, for flours discrimination.

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