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Colourimetric calibration for estimating grain mixing ratios in wheat samples

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Summary

Traceability of food is crucial for quality and safety in the food industry, particularly in cereal processing. In this study, the CIELAB colour space was applied to investigate cereal mixing and related colour changes. This method enabled the objective evaluation of colour variation and the estimation of grain mixing proportions based on calibrated colourimetric parameters. Wheat samples prepared in different mixing ratios were analysed using a reflectance colourimeter with a D65 light source, with each sample measured in ten repetitions. Based on the experimental results, linear relationships were established, confirming the consistent effect of varying mixing ratios on hue values. The results provided information supporting the delimitation of mixed grain zones and the adjustment of mixing ratios in flat storage conditions. The significance of the study lies in the quantitative evaluation of colour changes caused by grain variety mixing using spectral analysis. The proposed approach is intended as an applied, site-specific method for flat storage monitoring and is applicable under the tested storage, grain type and calibration conditions.

Keywords

colourimetry; calibration; grain mixtures; applied spectroscopy; wheat; CIELAB

Controlled food production, including cereal processing, has an increasingly significant role in quality food production. However, there is little or no traceability of food raw materials. There are numerous methods (microbiological, chemical and optical) for evaluating the quality and the properties of food grains [1]. Optical methods can be categorized into three main groups: spectrophotometric techniques, machine vision and colour measurement. Spectrophotometric techniques primarily involve the visible and infrared spectral ranges. Berman et al. [2] introduced a near-infrared (NIR) hyperspectral method for classifying wheat grains from various Australian varieties. Their study assessed the integrity of the grains and the ability to detect contaminants, achieving an overall classification accuracy of 95 %. Vermeulen et al. [3] discussed in detail the potential of combining NIR hyperspectral imaging with chemometrics for the separation of durum wheat seeds at the stages of storage process. Important criteria for separation include morphology, NIR spectra, protein content and kernel vitreousness. The results showed that 96.5 % and 88.3 % of the samples were correctly classified based on the morphological and spectral profile.

Visible (VIS) and NIR spectroscopy were combined with computer vision in order to distinguish deoxynivalenol-contaminated wheat grains from clean, uncontaminated grains [4]. A dynamic

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sensor system was created that allowed the online recording and analysis of spectral and image data of wheat grains. The resulting data were used to determine the optimal spectral and visual characteristics of healthy wheat grains. Subsequently, using linear discriminant analysis based on spectral and textural features for calibration and validation, an accuracy of 95.1 % and 91.4 % was achieved in distinguishing levels of contamination.

Zayas et al. [5] applied machine vision to identify different wheat varieties and to distinguish wheat kernels from non-wheat components. Digital images were used to analyse the geometry of each kernel. Nine geometrical parameters were defined to distinguish between wheat kernels and other components. Majumdar and Jayas [6] used machine vision to extract twenty-three morphological features from cereal grain images. Based on these features, they developed a discriminant analysis model to classify spring wheat, durum wheat, barley, oats and rye, achieving high classification accuracy for all tested cereal species.

Vithu and Moses [7] analysed the possible usage (benefits, possible deficiencies, and limitations) of machine vision systems in various grain quality assessment systems. They found that machine vision systems, using image processing and analysis techniques, have the potential to replace traditional methods of cereal quality assessment. In particular, manual visual inspection can be challenging even for trained personnel in terms of speed, reliability and accuracy.

Kar et al. [8] introduced a new and innovative system that allows the quick and accurate automatic estimation of food cereal quality. For this purpose, an algorithm was developed to detect and analyse grains scattered on a green A4 sheet of paper based on RGB images. The green colour distinguishes cereal particles (whole, broken, damaged, etc.), contaminations and other grains from the background colour. The algorithm first separates the grains in the image from the background colour and then classifies these selected image parts into different categories. Due to the efficiency and accuracy of the system, manual quality assessment processes were reduced by an order of magnitude.

Ebrahimi et al. [9] developed a vision-based machine approach to assess the purity of wheat. Their model was constructed using four Iranian wheat varieties and eight common weed seeds found in wheat fields. They extracted 52 features related to colour, morphology, and texture from images of the samples. The imperialist competitive algorithm (ICA) and artificial neural networks (ANNs) were used to analyse these features. The ICA-ANN approach achieved overall classification rates of 96.3 % for distinguishing wheat grains from non-wheat seeds, 87.5 % for classifying wheat grain varieties, and 77.2 % for identifying non-wheat kernel types.

Saenz et al. [10] evaluated kernel hardness (including test weight and vitreousness), carotenoid concentration, and colour in thirteen commercial hybrid maize varieties using the HunterLab colour system. They observed significant genotypic differences in kernel hardness, carotenoid concentration, and HunterLab colour parameters. Their findings revealed a relationship between kernel colour and hardness. Notably, grains with similar carotenoid concentrations but differing hardness exhibited variations in yellowness (b^*). However, when whole grains were milled and the flour colour was measured, these genotypic differences in yellowness disappeared, indicating that the vitreous structure of the kernels influences their colour.

Research by Ramirez-Paredes et al. [11] aims to develop algorithms based on machine learning and computer vision to enable the automatic evaluation of malting barley quality. The proposed approach combines the local phase quantization descriptor with colour and shape features, offering superior performance compared to more advanced local descriptors. The support vector machine with radial basis function (SVM-RBF) classifier not only proves suitable for the task but also provides a quick and accurate solution. The study highlights the importance of carefully selected features, high-performance classifiers, and the use of controlled lighting and recording equipment for reliable quality assessment of malting barley samples. Furthermore, colour and texture characteristics play a crucial role in distinguishing barley varieties and identifying damaged samples and contaminants.

Furthermore, the presence of pests and moulds during grain storage significantly impacts food security. This not only impacts grain quality but also poses a potential threat to human health [12]. Mould growth poses a significant risk during grain storage due to contamination by mycotoxins, which are harmful biochemical substances produced by fungi. These mycotoxins not only damage plants but also pose a threat to human and animal health [13]. Improper post-harvest handling accelerates spoilage, reducing the quality of stored grain, as well as its germination capacity and nutritional value [14].

In the following study, our goal is to use the CIELAB system in grain storage to locate mixed batches (formed by mixing grain batches with different quality parameters) within flat storage facilities. The first step of applying this method is to determine the calibration correlations generated from each grain. In addition, we would also like to determine whether it is possible to delineate *Fusarium*-infected batches using colour measurements. Thus, the contaminated batches can be cost-effectively separated from the healthy batches, and the harmful effects can be minimized.

MATERIALS AND METHODS

Location of wheat variety production

Slovakia is located in the heart of Central Europe (16–23° East longitude, 47–50° North latitude) and has an area of 49.035 km^2 [15]. The annual precipitation ranges from 500 mm to 650 mm in the lowlands of the southwestern region, where the back side of the Little Carpathians is located.

The annual precipitation in the experimental parcels was 535 mm in 2021, 472 mm in 2022, and 744 mm in 2023. The lowlands are part of the Pannonian Basin [16]. Salt marshes and steppes along the Danube in southern Slovakia lie at the northernmost edge of the Central European saline soil complex [17]. More than two-thirds of agricultural land is used for growing high-input crops such as winter wheat, maize, rapeseed, barley, sunflower, sugar beet, and other plants [18]. Large agricultural areas are intensely cultivated in the southwestern plains [19].

Wheat varieties were sown in areas with similar saline soil conditions in 2021, 2022, and 2023. Cereal batches of different colours were grown on four different agricultural parcels. Varieties with distinctive colours were used to perform high-precision calibration tests. These included a "conventional" common wheat variety, a yellow wheat variety, and two anthocyanin-rich wheat varieties. Conventional wheat varieties contain only small amounts of anthocyanin compounds [20, 21]. Anthocyanins accumulate in the aleurone layer or seed coat of wheat, giving the grains blue, purple, or red colours [22]. Delphinidin-3-glucoside can be found in anthocyanin-rich wheat varieties [23].

Wheat grain samples

For the experiments conducted in 2021, 2022 and 2023, grain sample preparation was carried out at the facilities of Agrosid (Sanagro Group, Gabčíkovo, Slovakia), where batch-specific calibration and evaluation of wheat grain mixing ratios were also performed. Tab. 1 lists the wheat varieties along with their abbreviations, grain colour, and the corresponding year of use in our experiments. These abbreviations were utilized to individually identify and track different sources of stored wheat. The content of quality parameters in the samples were also determined. The wheat varieties (Tab. 2), distinguished by different grain colours, exhibited diverse internal quality traits. Data received during the examination of content values by NIR are the following. Unfortunately, the content values did not allow us to determine the extent of mixing between the wheat batches. The NIR spectroscopy system was insufficient for objectively identifying the mixing of different wheat batches. While this technology is effective for the rapid analysis of internal quality parameters, accurately identifying the boundary between batches becomes challenging when their content values are very similar. Consequently, it was not possible to clearly establish the point at which two stored grain batches are distinct and unmixed.

Preparation of wheat grain mixtures

A mixture of specified proportions was prepared using grain samples from four different farmers representing the varieties IS-18W1161 (AG), GENIUS (K), BONAVITA II (CS), KARKULKA (MK) and BONAVITA I (MB). Each year, six different mixtures were created from the four tested wheat varieties. Each mixture combined two randomly selected varieties out of

Tab. 1. Description of wheat varieties used in the experiments over the years.

Wheat variety	Abbreviation	Colour of wheat	Year of experiment		
			2021	2022	2023
GENIUS	K	Light brown	X	X	X
KARKULKA	MK	Anthocyanin	X		X
BONAVITA I	MB	Yellow		X	
IS-18W1161	AG	Anthocyanin	X	X	X
BONAVITA II	CS	Yellow	X	X	X

Wheat variety BONAVITA was grown in two different places.

Tab. 2. Internal quality parameters of wheat varieties evaluated by near-infrared spectroscopy over three years.

Wheat variety	Year	Parameter			
		Gluten [%]	Protein [%]	Specific weight [g·l^{-1}]	Falling number [s]
GENIUS	2021	26.5	12.1	775	269
	2022	26.3	12.2	782	287
	2023	27.1	12.4	772	274
KARKULKA	2021	23.4	10.9	754	237
	2022				
	2023	24.0	11.3	766	241
BONAVITA I	2021				
	2022	23.1	10.8	758	224
	2023				
IS-18W1161	2021	28.5	13.0	793	288
	2022	30.0	13.3	807	292
	2023	29.4	13.1	795	304
BONAVITA II	2021	21.3	10.2	748	234
	2022	20.2	10.9	752	221
	2023	19.2	8.7	748	215

the four. In 2023, one mixture was not produced due to the availability of only five mixing combinations among the stored grain samples.

Before analysis, the samples were cleaned under laboratory conditions using the following equipment:

- digital analytical balance RADWAG WPX4500 (Radwag, Radom, Poland),
- calibrated grain sieves (1.0 mm, 2.0 mm, and 3.5 mm long apertures),
- sieve shaker machine for grain grading SWING 160 (MEZOS, Hradec Králové, Czechia).

We measured 100 g of each wheat variety on an analytical balance. The measured samples were graded based on their size (1.0 mm, 2.0 mm and 3.5 mm) and cleaned of foreign seeds and impurities.

The grading and cleaning process was performed using the SWING 160 sieve shaker machine. Each cleaning cycle lasted 5 min at a vibration frequency of 140 min^{-1}. The 1.0 mm and 3.5 mm aperture sieves retained foreign seeds, broken grains, and impurities, respectively. The ratio and quantity of these impurities were recorded. For the laboratory experiments, only clean wheat grains larger than 2.0 mm, free from extraneous matter, were used.

For purified samples, where the mixing ratio of the different wheat varieties varied between 0 % and 100 %, in 10% increments, 200 wheat grains were selected for each mixing ratio for spectral analysis in a special measuring vessel. Consequently, 60 different samples, each with a unique mixing ratio, were analysed annually.

Colour measurement

The separation of grain batches and the change in the heterogeneity of grain piles were carried out using optical methods. The tools used to determine the mixing of anthocyanin and standard winter wheat varieties included the CIELab colour space spectrophotometer MiniScan XE Plus (HunterLab, Reston, Virginia, USA) and an associated software to evaluate data. During our experiments, the type D65 light source (HunterLab) was used. Our CIELAB spectrophotometer used the 45/0° geometry to record the L^*, a^* and b^* colour space data and to determine the value of the total colour difference.

The colour of the samples was measured using a MiniScan XE Plus reflectance colourimeter with a D65 light source. In the CIELAB colour space, L^* measures the luminance from black (0) to white (100). The a^* value indicates hue parameters from red to green, with positive values indicating red and negative values indicating green. The b^* value measures the colouring parameters from blue to yellow, with positive values indicating yellow and negative values indicating blue.

The geometry of the instrument was 45°/0°, in which the incident light emitted by the source (xenon flash lamp) forms an angle of 45° from normal to the sample surface. Light reflected from the same surface arrives at 0° relative to normal. The colour difference dE^* and chroma C^* (saturation of the colour) were calculated according to Eq. 1 and Eq. 2.

$$dE^* = \sqrt{(dL^*)^2 + (da^*)^2 + (db^*)^2} \qquad (1)$$

where dE^* is the perceptible difference between two colours, dL^* represents the difference in lightness, da^* the difference along the green–red axis, and db^* the difference along the blue–yellow axis.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \qquad (2)$$

The colourimeter was connected to a computer using EasyMatch QC software (HunterLab) for automated measurements.

We examined 5 wheat varieties (AG, K, CS, MK, and MB) to determine the effects of different mixing ratios. Each sample was measured ten times, and the average of these measurements was calculated.

Analyses

We conducted a two-step analysis to (1) select the most appropriate colour variable from the nine candidate colour variables and to (2) study whether we can distinguish the homogeneous and heterogeneous parts of the grain mixture of two different wheat cultivars based only on the colour variable selected in step 1. For the first step, correlation analysis was performed. We calculated the absolute value of Pearson's correlation coefficients [24] between the nine candidate colour variables and the ratio of the two cultivars in the 17 grain mixtures. For each colour variable, the mean, maximum and standard deviation of the 17 correlation coefficients were calculated. According to the results of the correlation analysis (detailed in the next section), the hue × chroma ($H \times C^*$) parameter, where H is the colour hue, clearly stood out from the candidate colour variables with the highest mean and maximum, and the lowest standard deviation of the correlation coefficients. Therefore, we used the $H \times C^*$ colour variable for the next step.

In the second step, for each of the 17 grain mixtures, we calibrated a linear model using $H \times C^*$ as the background variable and the ratio of the two cultivars as the response variable. The calibration dataset was used for model calibration. Then we used the calibrated model to predict the ratio of the two cultivars based on $H \times C^*$ values measured for the evaluation dataset. The model's performance was measured by multiple metrics (coefficient of determination (R^2), adjusted coefficient of determination (R^2adj), and root mean square error (*RMSE*) calculated for both the calibration and the evaluation datasets. Moreover, the observed and the predicted cultivar ratios were binarized (i.e., whether the grain mixture is homogeneous or heterogeneous), and the model's confusion matrix was used to calculate additional performance metrics: true positive rate (*TPR*) and precision.

Statistical analyses and visualization were performed in R statistical software (R Foundation for Statistical Computing, Vienna, Austria) and its packages 'caret' [25] and 'ggplot2' [26].

RESULTS AND DISCUSSION

During the 2021 experiments, two different wheat varieties were mixed in varying proportions (10–90 %) across all possible combinations. The resulting mixtures were subjected to spectral analysis, and the measurement results were then examined using statistical methods. Preliminary studies revealed that the best correlation was provided by the $H \times C^*$ index values. We used this index in our comparison and calibration studies. The results of the 2021 experiments (Fig. 1) clearly show that the $H \times C^*$ trend line exhibits a linear relationship across the 10–90 % mixing ratios paired in the spectral analysis. Using mixing data obtained with spectral analysis from the laboratory samples previously illustrated, we could determine the percentage of each stock mixture that was mixed or not with each other. The calibration series determined in the previous year were repeated with grains harvested in 2022. This calibration procedure allows us to minimize the measurement errors, and it ensures that the results are reliable and reproducible. The repetitions allow us to verify the reproducibility of the results obtained each year, as well as the consistency and effectiveness of the developed method.

Compared to the previous experiment in 2021, one wheat variety (KARKULKA) was not available from the producer in 2022; therefore, it was replaced by BONAVITA. The 2022 results significantly confirm the colour differences between the different mixtures and the measurable differences in each mixture (Fig. 2). The linear trend lines show that increasing or decreasing the mixing ratio results in a gradual change in hue. This information may be useful for the localization of stored grain in flat storage facilities and for the more precise adjustment of mixing ratios. The calibration

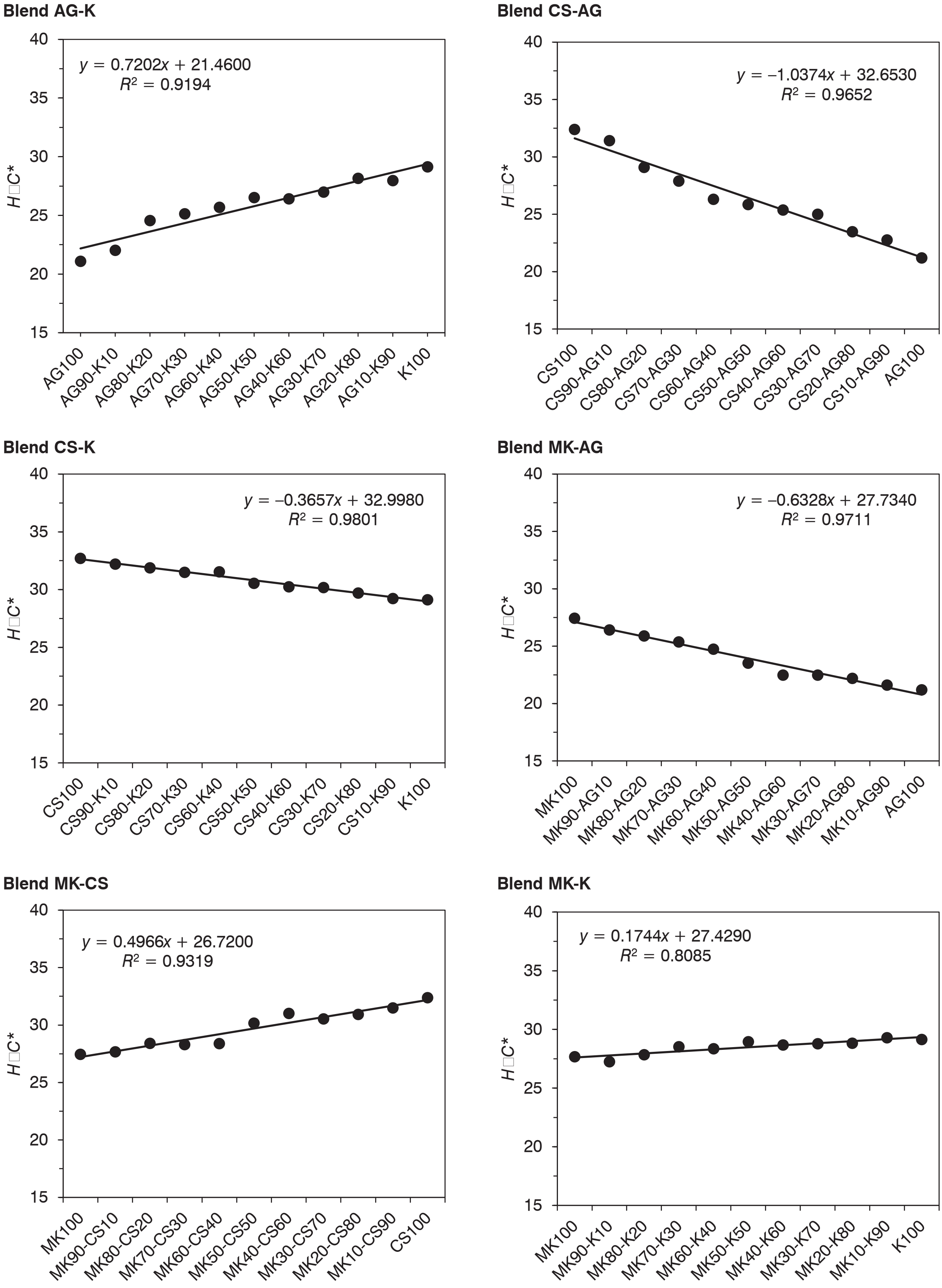

Fig. 1. Relationship between grain mixing ratio and selected colourimetric parameters for wheat mixtures measured in 2021 under calibrated flat storage conditions.

AG – wheat IS-18W1161, K – wheat GENIUS, CS – wheat BONAVITA II, MK – wheat KARKULKA.
The numbers in the sample designation on the x-axis indicate the percentage of each wheat variety in the mixture.

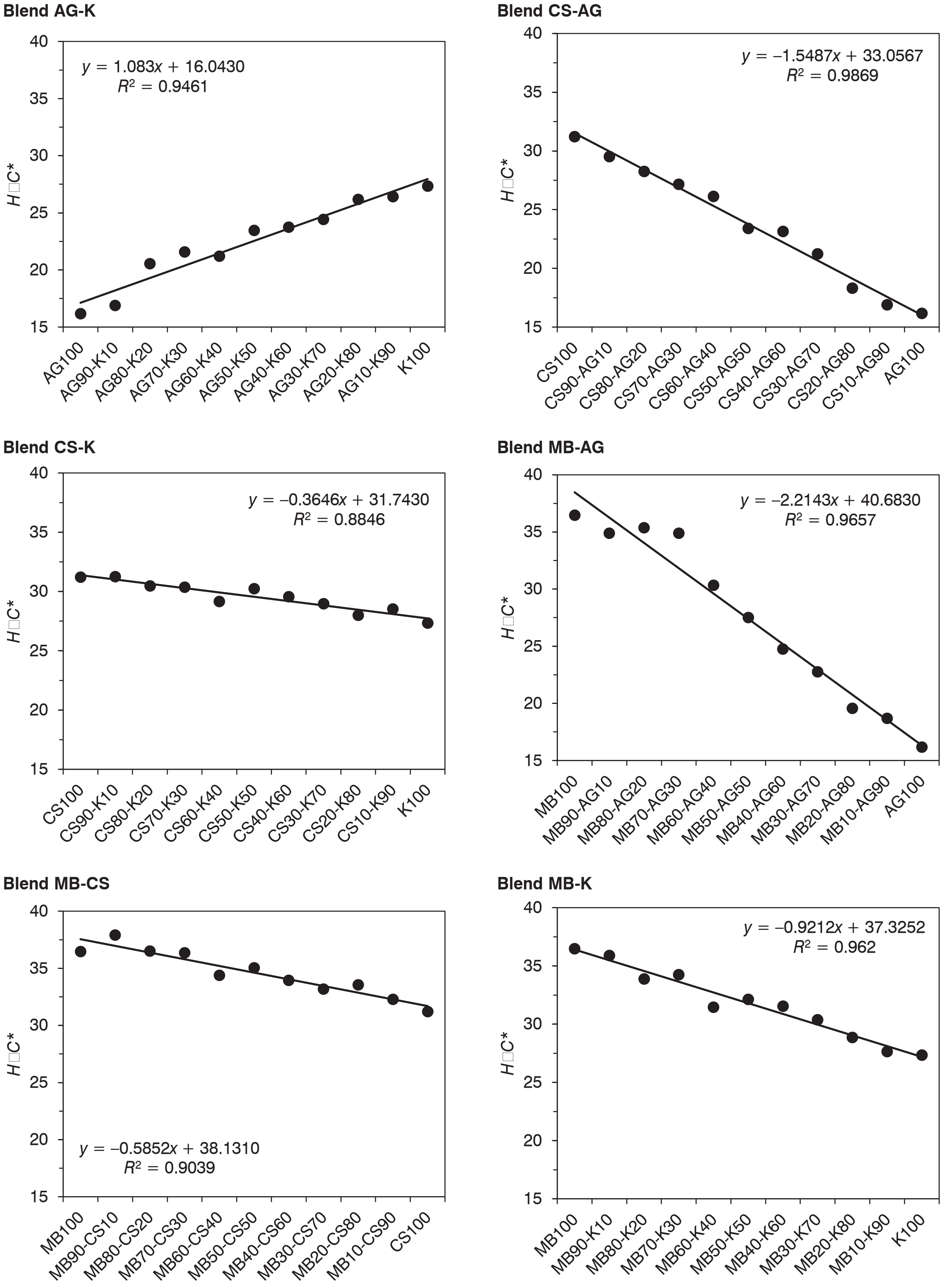

Fig. 2. Relationship between grain mixing ratio and selected colourimetric parameters for wheat mixtures measured in 2022 under calibrated flat storage conditions.

AG – wheat IS-18W1161, K – wheat GENIUS, CS – wheat BONAVITA II, MB – wheat BONAVITA I.
The numbers in the sample designation on the x-axis indicate the percentage of each wheat variety in the mixture.

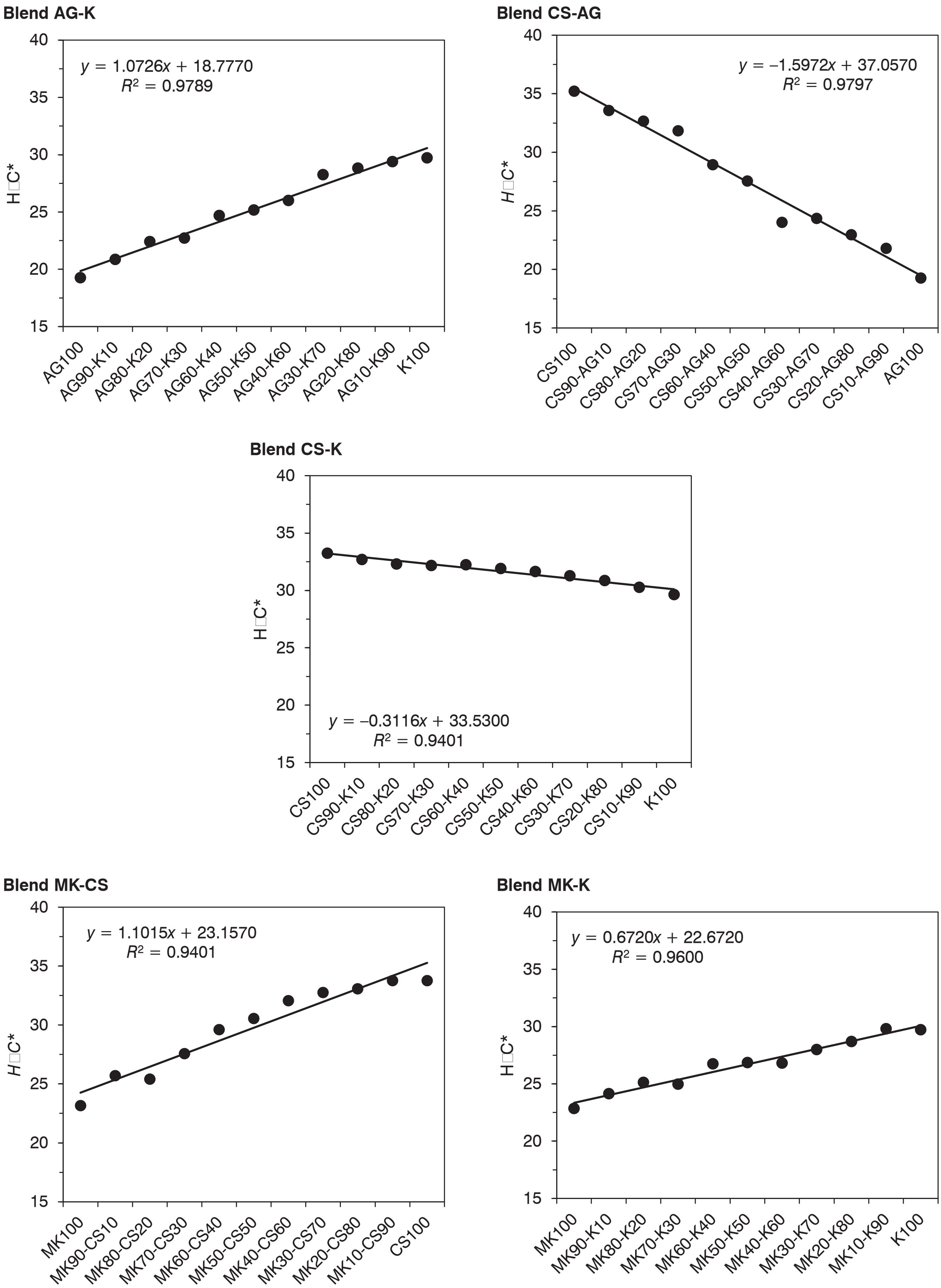

Fig. 3. Relationship between grain mixing ratio and selected colourimetric parameters for wheat mixtures measured in 2023 under calibrated flat storage conditions.

AG – wheat IS-18W1161, K – wheat GENIUS, CS – wheat BONAVITA II, MK – wheat KARKULKA.
The numbers in the sample designation on the x-axis indicate the percentage of each wheat variety in the mixture.

series determined in 2021 and 2022 were repeated in 2023 with the cereals harvested at that time. The goal was to control and optimize the accuracy and reliability of our measuring tools. This calibration process is of crucial importance in minimizing measurement errors, and it ensures that the results are consistent and reproducible. In 2023, for the two mixtures, out of the six different combinations, only five were examined. The data measured with a spectrophotometer, including the colour coordinates (L^*, a^*, b^*) and the parameters of the trend lines (R^2), confirm the differences in colour between various mixtures and the variations within certain mixtures (Fig. 3). The linear nature of the trend lines shows that increasing or decreasing the mixing ratio steadily changes the hue. The high level of R^2 values indicates the reliability of the trend lines and that data measured by a spectrophotometer are a reliable representation of the colour composition of the mixtures. This information can also be beneficial in the aforementioned food production, including quality control and the traceability of grain.

CONCLUSIONS

In this study, we evaluated whether colourimetric measurements obtained using a spectrophotometer can be applied to estimate the proportion of mixing between different wheat varieties. Experimental data collected over three consecutive years provided statistically evaluable results regarding predefined mixing ratios of winter wheat batches. Significant and reproducible differences were observed among the mixtures of the tested wheat varieties, indicating a consistent relationship between mixing ratio and colourimetric response. The results were presented graphically, and calibration line parameters were determined, forming the basis for estimating mixing ratios and delimiting mixed grain zones under calibrated flat storage conditions. The findings demonstrate that the proposed approach can support storage-level monitoring and decision-making in grain handling operations.

From a food safety perspective, the method may complement traceability systems by providing additional information on grain mixing within storage facilities. While regulatory frameworks, such as those established by the European Union, emphasize traceability and quality assurance, the presented approach should be regarded as an applied, site-specific tool that supports compliance efforts under controlled conditions rather than as a standalone traceability solution.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Ladislav Nyári conceived the study, designed the experiments, performed the laboratory work, analysed the data and prepared the original manuscript. Ottó Dóka contributed to the study design, interpretation of the results and critical revision of the manuscript. Ákos Bede-Fazekas contributed to the statistical analysis and interpretation of the data. Gergely Teschner contributed to the study design, methodology and critical revision of the manuscript. Nikolett Baráth contributed to the interpretation of the results and manuscript revision. Attila Kovács supervised the research, contributed to the study design and critically revised the manuscript. All authors read and approved the final manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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