Diversity in nutritional and functional quality of sorghum restorer lines collection

SIMONA JAĆIMOVIĆ – BILJANA KIPROVSKI – VLADIMIR SIKORA – LATO PEZO – NEBOJŠA Đ. PANTELIĆ

Summary

This study aimed to examine and compare the main nutritional quality parameters and bioactive compounds of sorghum (*Sorghum bicolor* L. Moench) grain in order to select genotypes suitable for human consumption and predict genotypes with higher antioxidant capacity. Sorghum grains from the restorer lines collection (172 genotypes) were analysed for their nutritional quality (ash, protein, fat and starch), phenolic compounds content (total phenolics, tannins, flavonoids and anthocyanins), as well as for antioxidant activity and colour parameters. The content of the studied items was 1.6–6.6 % for total lipids, 8.7–19.8 % for proteins, 1.2–3.7 % for ash and 57.1–93.3 % for starch. Total phenolics content was 0.6–11.3 $g \cdot kg^{-1}$ (expressed as GAE) in methanolic extracts and 1.1–17.1 $g \cdot kg^{-1}$ (expressed as GAE) in acetone extracts, while the highest antioxidant activity was 90.4 %. There was no correlation between colour and tannin content. The expected and experimentally obtained antioxidant activity confirmed the effectiveness of artificial neural network as a predictive tool. This study showed that sorghum is a valuable material for developing functional food products and it has potential if cultivated in the European climate.

Keywords

coloured grain; nutritive; phenolics; antioxidant; *Sorghum bicolor*

Sorghum (*Sorghum bicolor* L. Moench, Poaceae) is a multipurpose cereal that belongs to the group of the oldest cultivated plants and, by areas and economic importance, it ranks fifth in the world, after rice, wheat, maize and barley. The European production share of sorghum is the lowest in the world among the five most produced cereals [1], regardless of the continual increase in its production areas, as well as the demand for diversification of European agriculture in the transformation of agricultural systems in a changing climate. Primarily, this crop is used as feed and food, because of its grain, which contains 1.3–3.5 % total ash (minerals), 4.4–21.1 % proteins, 2.1–7.6 % lipids, 1.0–3.4 % crude fibre, 57.0–80.6 % total carbohydrates and 55.6–75.2 % starch [2, 3]. Since sorghum is a nutritious and gluten-free grain, it is an attractive raw material in the food sector.

Numerous studies showed that sorghum grain has significant health benefits due to its unique phenolic composition, which provides its seed with white, yellow, red or black colours [4, 5]. Flavonoids, the most abundant phenolic compounds found in sorghum, are responsible for the colour of pericarp (the outer layer of the seed coat) and pigmented testa (the inner layer of the seed coat) [2]. Besides condensed tannins, the main flavonoids reported in sorghum grain are anthocyanins (3-deoxyanthocyanidins, up to 80 %), flavones and flavanones [6]. In contrast to anthocyanins, the lack of a hydroxyl group at the C-3 position leads to increased stability of 3-deoxyanthocyanins at high pH values and temperatures, making them suitable for the food industry as natural food colorants [7]. Sorghum grain contains dominantly orange luteolinidin and yellow apigeninidin and their methoxylated derivatives. Since 3-deoxyanthocyanins are uncommon in higher plants, sor-

Simona Jaćimović, Biljana Kiprovski, Vladimir Sikora, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, 21000 Novi Sad, Serbia.
Lato Pezo, Institute of General and Physical Chemistry, University of Belgrade, Studentski trg 10-12, 11000 Belgrade, Serbia.
Nebojša Đ. Pantelić, Department of Chemistry and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia.

Correspondence author:
Nebojša Đ. Pantelić, e-mail: pantelic@agrif.bg.ac.rs

ghum is considered to be the main source of these compounds for the human population [8].

Tannins are phenolic compounds with an astringent taste and high molecular mass They possess antibacterial properties, allowing them to act against plant pathogens [2]. Tannins content in sorghum grains is a subject of concern because these phytochemical constituents reduce food digestibility [9] due to irreversible denaturation of digestive enzymes and their general ability to cross-link with proteins and polysaccharides, which positions these compounds in a group of antinutritional factors. On the other hand, these properties are regarded as potentially beneficial since the molecular interaction of tannins with starch, proteins and enzymes decreases their digestibility, which further reduces caloric intake and brings benefits to obesity and type 2 diabetes [10], in addition to other health benefits such as anticancer and anti-inflammatory activities.

The place of sorghum grain in crop production is determined by its tolerance to various environmental conditions. Since sorghum is a cereal that originates from the arid regions of Africa, this indicates its great adaptability to extreme conditions. In fact, the plant needs only small quantities of water and is very tolerant to drought and poor soils. Abiotic stresses are location-specific and can occur at any stage of plant growth and development. Water-soluble pigments, 3-deoxyanthocyanidins, play important roles in plant resistance and tolerance to various diseases and environmental stresses [5]. Pinheiro et al. [10] found a notable increase in the total content of 3-deoxyanthocyanidins in tannin-free genotypes when exposed to drought conditions, while it remained unchanged in tannin-containing genotypes. The increase of 3-deoxyanthocyanidins only in tannin-free sorghum genotypes suggests that the presence of tannin in grain reduces the plant's need to produce 3-deoxyanthocyanidins. The selection of varieties tolerant to drought or other abiotic stresses is of great importance in order to achieve high yields in adverse growing conditions.

Breeding for tolerance to stresses and for high nutritional quality is a very important breeding objective. It involves a continuous process of searching for suitable genotypes and using them as donor parents in crop improvement programs. Restorer lines are used to restore the fertility in hybrids and have a central role in breeding programs, so they should be adaptable to various environments [11].

Coloured cereals including coloured sorghum have recently become an attractive raw material for food production. Due to the growing consumers' demand for health-promoting, functional meals, knowledge about variations in the nutritional and functional quality, including the conmtent of bioactive compounds, of different genotypes is essential to support plant breeding programmes to develop high nutritious grain cultivars for human consumption.

Shaped by metabolic processes that occur in plants under the influence of environmental factors and the genetic potential of the plant, the chemical composition of the grain determines its quality. Considering the variability, origin and scope of the analysed collection of restorer lines, it can be considered one of the largest in the South and Southeast Europe. Additionally, there is no such report in the available literature that precedes the number of genotypes processed in the presented research.

Therefore, this study aimed to identify and evaluate the primary bioactive compoounds and nutritional quality characteristics of grains from various sorghum genotypes from various parts of the world, to evaluate their diversity and select the best food-grade genotypes. Furthermore, analysed bioactive compounds and antioxidant activity of sorghum grain extracts were used for the construction of an artificial neural network (ANN) to predict antioxidant activity of sourgum grains.

MATERIALS AND METHODS

Plant material

Seeds of 172 sorghum restorer lines from the collection of Institute of Field and Vegetable Crops Novi Sad, National Institute of the Republic of Serbia (IFVCNS, Novi Sad, Serbia), with high genetic variability were used in this study. Genotypes were standardised as a part of various breeding collections listed in Tab. S1 in supplementary data. Grain samples analysed in this paper were produced in the IFVCNS Department of vegetable and alternative plant species (Bački Petrovac, 45°34'N 19°67'E) in 2020.

The experimental plots consisted of 3 rows 5 m long, spaced 0.7 m between rows and 10 cm between plants in each row. In order to avoid uncontrolled cross fertilisation, 10 panicles were isolated with paper bags before flowering. At the time of technological maturity, the isolated panicles were removed by hand, dried in a dryer and, after threshing, the seeds of the selected panicles were merged and stored in paper bags.

Seeds

Sorghum seed samples were cleaned of im-

purities and foreign matter (foreign impurities of organic and inorganic origin, as well as broken grains) and ground in a basic analytical mill IKA A11 (IKA-Werke, Staufen, Germany) to pass 0.5 mm square holes of a sieve. The ground seeds were stored in vacuum bags in a refrigerator (maximum temperature of +4 °C) until analysis for a maximum of two months.

Nutritional quality determination

Moisture content was determined gravimetrically by drying at 103 °C for 3 h in a universal oven UN55 (Memmert, Büchenbach, Germany).

The ash content was determined gravimetrically by burning samples in a muffle furnace at 550 °C in porcelain vessels, and percent of ash was calculated from the initial grain material.

The starch content was determined polarimetrically (method by Ewers) [12].

The determination of nitrogen content in sorghum seeds was performed by the Kjeldahl method [13].

The content of total proteins was calculated by multiplying the obtained value for nitrogen content by 6.25.

The lipids content was determined by the Soxhlet method [14], using 8 h extraction at 70 °C.

The obtained values for moisture, ash, starch, proteins and lipids content in sorghum seeds were expressed as percent in relation to the dry matter of sorghum seeds. These are standard analyses of sorghum seed quality for the human diet, which are recommended according to the requirements of international food standards Codex Alimentarius CXS 172-1989 [15] and Technical specifications for sorghum grains (CERSOR010) [16]. Accordingly, the limit values for individual parameters are: moisture content – max. 13 %, ash – max. 1.5 %, proteins – min. 7.0 %, lipids – max. 5.0 % and tannin – max. 0.5 % of dry seed matter.

Bioactive compounds

Extraction of phenolic compounds

Extraction of phenolics was performed in two variants, where absolute methanol acidified with 1% hydrochloric acid and 70% aqueous acetone solution were used as extraction agents because these solutions were the most often used in the available literature for extraction of phenolics from sorghum seeds [17–19]. The ratio of plant material subjected to extraction was 1 g of plant material per 10 ml of extraction agent. Ground and sieved samples were weighed (1.0000 ± 0.0001 g) on an analytical balance PI-214.3 (Denver Instrument, Bohemia, New York, USA) the extraction agent (10 ml) was added and the extraction was performed in an ultrasonic bath for 45 min, with the addition of ice to prevent heating of the samples. Afterwards, the extracts were centrifuged (10 min, 1 960 ×*g*) and the obtained supernatants were transferred to separate tubes and used for further work. Extraction of phenolic compounds was performed in three replicates per sample.

Phenolic compounds

The contents of total phenolics, tannins, flavonoids and anthocyanins, as well as the antioxidant activity of the extracts were determined spectrophotometrically using UV-Vis spectrophotometer Lambda Bio 20 (Perkin Elmer, Waltham, Massachusetts, USA).

The determination of total phenolics (*TP*) content in methanolic and acetone extracts (marked as *TPM* and *TPA*, respectively) was performed by the Folin-Ciocalteu method [20]. Results were expressed as grams of gallic acid equivalents (GAE) per kilogram of dry grain weight.

To determine the tannins content in sorghum seeds, in addition to chromatographic methods, butanol-HCl [20], vanillin HCl/H_2SO_4 [18], ferric-ammonium citrate method [21] and precipitation methods [20] could be used. The chosen extraction agents (methanol and acetone) have shown the highest efficiency in extracting tannins by the standard ferri-ammonium citrate method [19]. In this research, total tannins (*TT*) content in methanolic and acetone extracts (marked as *TTM* and *TTA*, respectively) was determined by precipitation by polyvinylpolypyrrolidone (PVPP)/Folin-Ciocalteu method for determination of condensed tannins and by the butanol-HCl method for determination of proanthocyanidins (*ProA*), respectively [20]. Results were expressed as grams of GAE per kilogram of dry grain weight and grams of leucoanthocyanidin standard per kilogram of the dry grain weight, respectively.

Total flavonoids (*TF*) content was determined in methanolic extracts (marked as *TFM*) of sorghum grains [22]. Results were expressed as grams of rutin equivalents per kilogram of dry weight of plant material.

Monomeric anthocyanins (*TA*) contents were determined in methanolic extracts of sorghum grains using the differential method described by Lee et al. [23]. Total monomeric anthocyanin content (marked as *TAM*) was calculated as grams cyanidin-3-O-glucoside equivalents per kilogram of the dry grain weight.

Antioxidant activity

Antioxidant activity (*AA*) of methanolic extracts was determined by the method of Kumar

[24], which is based on the difference in quenching 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by the blank and the working sample. DPPH radical-scavenging activity is expressed as percent of inhibition relative to the blank and as milligrams of ascorbic acid equivalents (AAE) per kilogram of dry weight.

Sorghum grains colour

The sorghum grain colour spaces were determined colorimetrically using Minolta CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan), Light Protection Tube with glass protection plate CR-A33a and expressed as L^*, a^* and b^*. L^* values represent lightness (0 black, 100 white), a^* values represent redness – from green to red (–a* greenness) and b^* values represent yellowness – from blue to yellow (–b* blueness). Before the measurement, the calibration was performed using the white standard. D-65 lighting, a standard viewing angle of 2° and a contact surface diameter of 8 mm were used. All measurements were performed in six replications per sample.

Artificial neural network modelling

A multi-layer perceptron model (MLP) with three layers (input, hidden layer and output) was used for the construction of an artificial neural network (ANN). This was used for the prediction of antioxidant activity (expressed as DPPH value) according to the amount of active compounds in the samples *TPM, TTM, TFM, TAM, TPA, TTA* and *ProA*. The experimental database for ANN modelling was randomly divided into training, cross-validation and testing data (60 %, 20 % and 20 % of experimental data, respectively). The experimentally obtained data were normalised using the min–max normalisation approach to improve the behaviour of ANN. The Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was employed in solving non-linear problems during the network modelling [25]. A series of topologies (approximately 100 000) were tested during the modelling, changing the number of neurons in the hidden layer from 5 to 20 and randomly setting initial weights and biases [26]. As a result, the ANN model could be presented in the matrix form:

$$Y = f_1[W_2 \cdot f_2(W_1 \cdot X + B_1) + B_2] \quad (1)$$

where W_1, B_1, W_2 and B_2, are the coefficients associated with the hidden and the output layers (weights and biases) in the matrix, respectively, while Y is the matrix of the output variables. The transfer functions in the hidden and output layers are f_1 and f_2, respectively, while X is the matrix of input variables.

Global sensitivity analysis

The Yoon's interpretation method was used to determine the relative influence (*RI*) of *TPM, TTM, TFM, TAM, TPA, TTA* and *ProA* content in samples on the antioxidant activity (expressed as DPPH value in percent, according to the ANN model). Yoon's method of global sensitivity was used to calculate the direct influence of the input parameters on the output variables, corresponding to the weighting coefficients within the ANN model [27]:

$$RI_{ij} = \frac{\sum_{k=0}^{n}(w_{ik} \cdot w_{kj})}{\sum_{i=0}^{m}\left[\sum_{k=0}^{n}(w_{ik} \cdot w_{kj})\right]} \cdot 100 \quad (2)$$

where w is weighting factor in the ANN model, i is input variable, j is output variable, k is hidden neuron, n is number of hidden neurons and m is number of inputs.

Statistical analysis

The values of the biochemical parameters were expressed as mean ± standard error of determinations made in duplicates. Principal component analysis (PCA) was performed to classify the genotypes based on the obtained results and to identify patterns among samples and methods used for testing nutritional parameters and bioactive compounds. The results of correlation analysis (CA) and PCA of 172 sorghum grain samples from the collection were presented according to the obtained research variables such as biochemical parameters (nutritional and functional quality) and colour parameters of samples. These data were analysed using StatSoft Statistica 12 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Nutritional quality parameters

A wide diversity among genotypes was recorded for the main nutrients analysed in sorghum grains from the tested collection (the results are presented in Fig. S1–S3 in supplementary data). The oil content in the tested grains of various sorghum genotypes ranged from 1.6 % to 6.6 % of dry matter, and the protein content averaged 19.8 % of dry matter. The results showed that the ash content ranged from 1.2 % to 3.6 %, while the starch content ranged from 57.1 % to 93.3 % of dry matter. The obtained results were similar to those available in the published literature [28, 29], except for the starch content, which was slightly higher than previously reported data.

PCA of the nutritional quality parameters of

samples explained that the first two principal components explained 56.4 % of the total variance in the six parameters (the contents of moisture, dry matter, ash, starch, proteins and lipids). According to the results of PCA, the content of dry matter (which contributed 44.8 % of the total variance, based on correlations) and proteins (7.2 %) exhibited a positive influence on the first principal component (PC1), while moisture content (44.8 %) negatively affected the value of PC1. The starch content (29.2 % of the total variance, based on correlations) showed a positive influence on the second principal component (PC2), while the contents of ash (31.1 %), proteins (19.1 %) and lipids (19.2 %) affected PC2 negatively (Fig. 1). From the obtained data, it could be confirmed that the key parameter for the selection of suitable genotypes for breeding are the protein or starch content, since moisture, protein and starch contents were singled out as the main parameters. This is consistent with the standard for sorghum grains [15, 16].

Total phenolics content and antioxidant activity

Sorghum has a unique phytochemical profile among cereals, which involves anthocyanins and tannins. These phytochemicals are important and give sorghum a higher antioxidant activity than wheat, rice or maize and their high levels make sorghum an interesting cereal for functional foods production. Sorghum could be used for the development of health-promoting food products for people with celiac disease, as a gluten-free ingredient [6]. The major phenolic compounds detected in sorghum were flavonoids (anthocyanins such as 3-deoxyanthocyanidins), flavones and flavanones; tannins (condensed tannins or proanthocyanidins), as well as phenolic acids (vanillic, gallic, cinnamic, protocatechuic, *p*-coumaric, syringic, *p*-hydroxybenzoic, caffeic, ferulic and sinapic acids) [3]. According to the literature, the content of phenolics varies greatly depending on the extraction method and the type of solvent used [30]. Therefore, to achieve efficiency in the measurement of the content of mentioned bioactive components in this study, the sorghum extracts were obtained by extracting the samples with both methanol and acetone as solvents.

The results showed that total phenolics contents (expressed as GAE) in the tested sorghum genotypes ranged from 0.6 $g \cdot kg^{-1}$ to 11.3 $g \cdot kg^{-1}$ in methanolic extracts and from 1.1 $g \cdot kg^{-1}$ to 17.1 $g \cdot kg^{-1}$ in acetone extracts. These results are in agreement with the *TP* contents reported in previously published results [30], where *TP* contents (expressed as GAE) ranged from 2.1 $g \cdot kg^{-1}$ to 8.9 $g \cdot kg^{-1}$ in methanolic extracts. In addition, Rao et al. [4] reported the highest *TP* content of 11.50 $g \cdot kg^{-1}$ (expressed as GAE) in the black pericarp variety of sorghum grain, while the brown pericarp genotype had a content of 3.58 $g \cdot kg^{-1}$.

The level of tannins in sorghum is the most important factor influencing its nutritional value. The amount of tannins does affect the extent of protein digestibility, but sorghum's antioxidant activity is strongly linked to the total content of

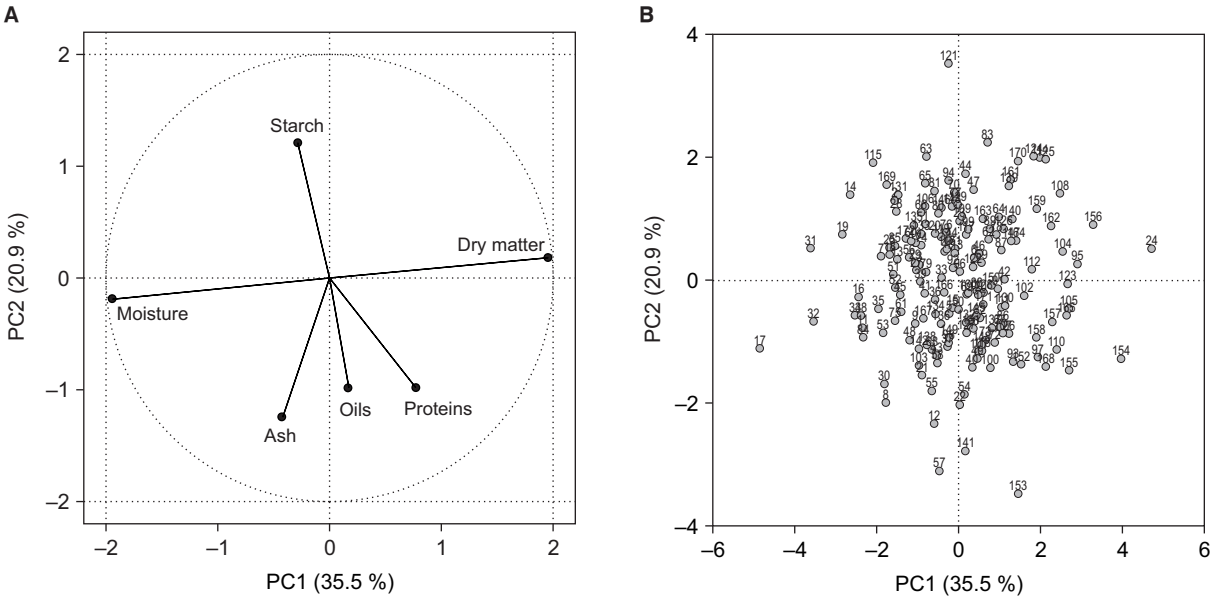

Fig. 1. Principal component analysis of nutritional parameters in sorghum grain.

A – tested nutritional parameters, B – sorghum genotypes tested for nutritional parameters data.

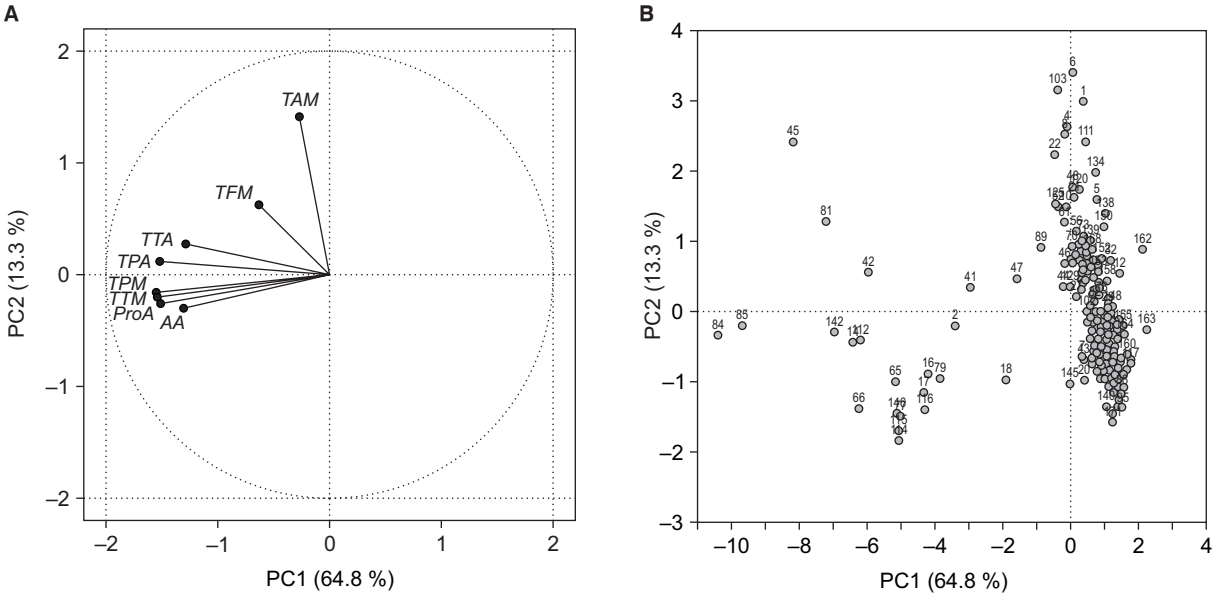

Fig. 2. Principal component analysis of bioactive compounds in sorghum grain.

A – tested bioactive compounds and antioxidant test, B – sorghum genotypes tested for bioactive compounds and antioxidant activity.
TPM – total phenolics in methanolic extracts, *TTM* – total tannins in methanolic extracts, *TFM* – total flavonoids in methanolic extracts, *TAM* – total anthocyanins in methanolic extracts, *TPA* – total phenolics in acetone extracts, *TTA* – total tannins in acetone extracts, *ProA* – proanthocyanidins in acetone extracts, *AA* – antioxidant activity.

condensed tannins [6]. The total tannins content (expressed as GAE) obtained in this study using the precipitation with PVPP ranged from 0.0 g·kg^{-1} to 8.4 g·kg^{-1}, while the results obtained by butanol-HCl assay ranged from 0.0 g·kg^{-1} to 14.3 g·kg^{-1}. Therefore, the tannins levels were in accordance with values reported previously [4, 31].

When compared statistically, results of the same method obtained from two different extraction solvents for total phenolics content showed that *TPA* was in a positive correlation with *TPM* (r = 0.903, $p \leq 0.001$). Results of the butanol-HCl assay (*ProA*, as a content of tannins in acetone extracts) positively correlated with the results of total tannins determined by precipitation of tannins with PVPP from methanolic (*TTM*, r = 0.950, $p \leq 0.001$) and acetone extracts (*TTA*, r = 0.686, $p \leq 0.001$), as well as with *TPM* (r = 0.938, $p \leq 0.001$) and *TPA* (r = 0.881, $p \leq 0.001$).

The antioxidant activity of sorghum grains is linked to the total phenolics content [30, 32], so it can be noticed that it showed a good correlation. However, since there are a couple of different methods and ways of expressing results, which contributes to some deviations between results, antioxidant activity data are hard to compare. This is due to different standard compounds, as well as modifications of protocols for several antioxidant tests. Therefore, correlation analyses was performed with all the obtained data on the phenolics content in the presented research. In this study, the DPPH assay was used to assess the antioxidant activity of the samples by determining their free radical-scavenging activity. At the applied concentration of the samples, the lowest ability to scavenge DPPH free radicals was 25.6 % DPPH neutralisation, while the greatest one was 90.4 %.

The antioxidant activity positively correlated with the contents of *TPM* (r = 0.775, $p \leq 0.001$), *TTM* (r = 0.787, $p \leq 0.001$), *TPA* (r = 0.720, $p \leq 0.001$), *TTA* (r = 0.544, $p \leq 0.001$) and *ProA* (r = 0.783, $p \leq 0.001$). PCA of the phenolic compounds content and antioxidant activity in samples (Fig. 2) explained that the first two principal components summarised 78.1 % of the total variance in eight parameters (*TPM, TTM, TFM, TAM, TPA, TTA, ProA* and *AA*). The contents of *TPM* (18.1 % of the total variance, according to correlations), *TTM* (18.0 %), *TPA* (17.5 %), *TTA* (12.6 %), *ProA* (17.3 %) and *AA* (12.9 %) showed a negative influence on the PC1 coordinate. On the other hand, *TFM* and *TAM* (14.5 % and 74.1 %, respectively) positively influenced PC2 coordinate.

Sorghum grains colour

PCA of the colour coordinates in grain samples (Fig. 3) explained that the first two princi-

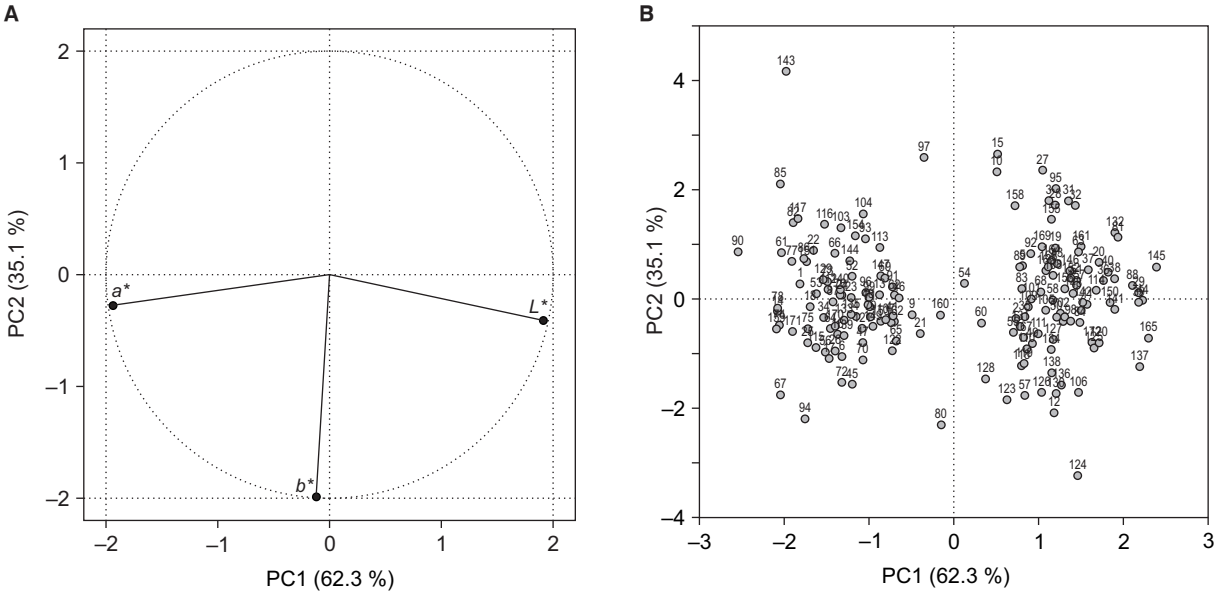

Fig. 3. Principal component analysis of sorghum grain colour.

A – tested grain colour parameters, B – sorghum genotypes tested for grain colour.
*L** – lightness, *a** – redness, *b** – yellowness.

pal components summarised 97.4 % of the total variance in three parameters (*L**, *a** and *b**). The colour coordinate *L** (49.3 % of the total variance, according to correlations) showed a positive influence on PC1 coordinate, while *a** (50.5 %) showed a negative influence on PC1 coordinate. The colour coordinate *b** (94.2 %) showed a positive influence on PC2 coordinate.

Grain colour is influenced by pigments from both pericarp (outer layer of seed coat) and testa (inner layer of seed coat). Genetics behind the colour formation suggests that seed colour is not always in correlation with tannins content [2], which was confirmed by correlation analysis among colour parameters and tannins content (results from all methods applied, determined *r* values were under 0.5) obtained in our research (Tab. 1). Since tannins represent antinutritive compounds, it is important to determine genotypes with low tannins content but higher content of other phenolics, deoxyanthocyanins in the first place, due to antioxidant activity and dietary diversity that such materials provide.

Tab. 1. Correlation among colour parameters and tannins content in sorghum grain.

Colour parameters	*r*		
	TTM	*TTA*	*ProA*
*L**	–0.49	–0.37	–0.47
*a**	0.37	0.30	0.32
*b**	–0.10	–0.04	–0.14

r – correlation coefficient, *TTM* – total tannins in methanolic extracts, *TTA* – total tannins in acetone extracts, *ProA* – proanthocyanidins in acetone extracts, *L** – lightness, *a** – redness, *b** – yellowness.

Artificial neural network model

In developing the model ANN, the output variable (*AA* value) should be determined by the input variables *(TPM, TTM, TFM, TAM, TPA, TTA* and *ProA*), using Eq. 1. The weights and biases defined in Eq. 1 were determined by the ANN model calculation, enabling the model to be accurate enough to predict the output. The ANN model developed for predicting *AA* values showed a good ability to generalise data and predict the output. The optimal model was built with 12 neurons in the hidden layer within the network (with seven inputs and one output), which reached a high r^2 value of 0.969 and a low sum of squares value of 2.403 during the training cycle. The training algorithm was Broyden–Fletcher–Goldfarb–Shanno (BFGS) 5019. The logistic activation function was used in the hidden layer, while the tangent hyperbolics were used in the output layer. The developed ANN model was complex, having 109 weight-bias coefficients.

A scatterplot is one of the most common visualisation techniques to present the comparison between experimental and predicted data

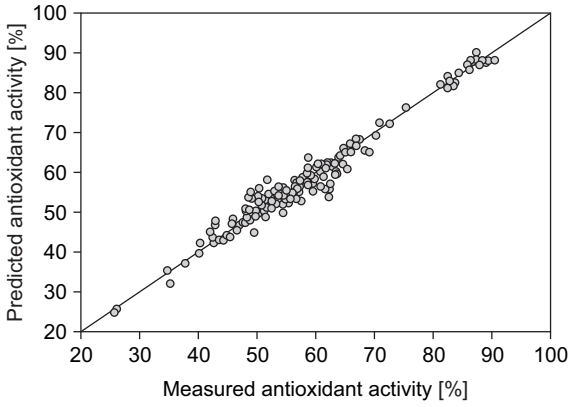

Fig. 4. Experimental and predicted antioxidant activity in sorghum grain.

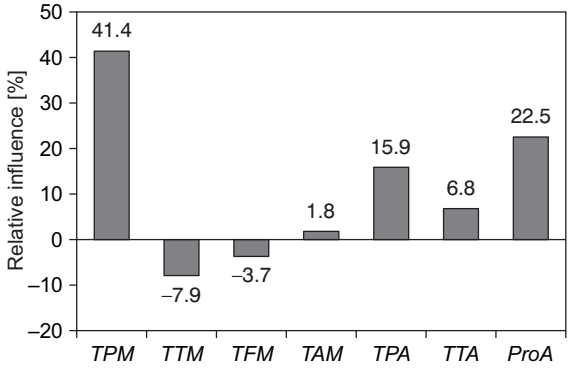

Fig. 5. Relative influence of bioactive compounds content in sorghum grain on antioxidant activity.

TPM – total phenolics in methanolic extracts, *TTM* – total tannins in methanolic extracts, *TFM* – total flavonoids in methanolic extracts, *TAM* – total monomeric anthocyanins in methanolic extracts, *TPA* – total phenolics in acetone extracts, *TTA* – total tannins in acetone extracts, *ProA* – proanthocyanidins in acetone extracts.

to display the behaviour of the developed ANN model [33, 34]. Thus, the data shown in Fig. 4 illustrate the predicted and experimentally gained *AA* values, which largely indicated the good predictive capabilities of the ANN model.

Global sensitivity analysis

In this section, we studied the influence of input variables (*TPM, TTM, TFM, TAM, TPA, TTA* and *ProA*) on antioxidant activity, based on the Yoon's interpretation method of the developed ANN model. The graphical presentation of the ANN model results is presented in Fig. 5. Each input variable's positive or negative influence was expressed as the relative influence compared to the average influence of all variables, which could be realised by the interpretation of Eq. 2. According to Fig. 5, the *AA* value was strongly influenced by the contents of *TPM, ProA* and *TPA*, showing a relative influence of 41.4 %, 22.5 % and 15.9 %, respectively.

CONCLUSIONS

In European agroecological conditions, sorghum's nutritional value has been relatively overlooked compared to other staple crops. However, there is a growing interest in sorghum due to the need for new high-quality food sources aligned with the trend toward healthier lifestyles and natural products. This study aimed to explore the nutritional quality of sorghum within wide sorghum grain collections, emphasizing genetic variation to aid in developing new food-grade varieties. Additionally, it seeks to comprehensively analyse standard biochemical parameters across a wide range of samples. Our study suggested that 16 of the presented genotypes of coloured sorghum grains (Re2, Re31, Re45, Re161, Re168, Re169, Re187, Re192, Re218, Re230, Re248, Re258, Re261, Re262, Re279 and Re284, Tab. S1 in the supplementary data) have a high potential for further application in the food industry, fulfilling standard nutritional requirements and poviding additional properties by the contained bioactive compounds. This screening showed that sorghum has a great potential for growing in European climate conditions, consequently, it is a very suitable plant species to be included in the further improvement of new genotypes, both in terms of nutritional quality of food grade grains as well as adaptability to climatic variations and abiotic pressure.

Acknowledgements

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant numbers 451-03-47/2023-01/200032, 451-03-68/2022-14/200116 and 451-03-68/2022-14/200051).

The authors are grateful to Dr. Dubravka Škrobot and Dr. Jelena Tomić (Senior Research Associates, University of Novi Sad, Institute of Food Technology, Serbia), for providing colorimeter and general support during the implementation of this research.

Supplementary data

Supplementary data related to this article can be found at https://www.vup.sk/download.php?bulID=2243.

REFERENCES

1. Agricultural production statistics 2000–2021. FAOSTAT Analytical Brief 60. Rome : Food and Agriculture Organization of the United Nations FAO, 2022. ISSN: 2709-006X. <https://www.fao.org/3/cc3751en/cc3751en.pdf>
2. Palacios, C. E. – Nagai, A. – Torres, P. – Avelino Rodrigues, J. – Salatino, A.: Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. Food Chemistry, *337*, 2021, article 127970. DOI: 10.1016/j.foodchem.2020.127970.
3. Khalid, W. – Ali, A. – Arshad, M. S. – Afzal, F. – Akram, R. – Siddeeg, A. – Kousar, S. – Rahim, M. A. – Aziz, A. – Maqbool, Z. – Saeed A.: Nutrients and bioactive compounds of *Sorghum bicolor* L. used to prepare functional foods: a review on the efficacy against different chronic disorders. International Journal of Food Properties, *25*, 2022, pp. 1045–1062. DOI: 10.1080/10942912.2022.2071293.
4. Rao, S. – Santhakumar, A. B. – Chinkwo, K. A. – Wu, G. – Johnson, S. K. – Blanchard, C. L.: Characterization of phenolic compounds and antioxidant activity in sorghum grains. Journal of Cereal Science, *84*, 2018, pp. 103–111. DOI: 10.1016/j.jcs.2018.07.013.
5. Xiong, Y. – Zhang, P. – Warner, R. D. – Fang, Z.: Sorghum grain: from genotype, nutrition, and phenolic profile to its health benefits and food applications. Comprehensive Reviews in Food Science and Food Safety, *18*, 2019, pp. 2025–2046. DOI: 10.1111/1541-4337.12506.
6. Istrati, D. I. – Constantin, O. E. – Vizireanu, C. – Rodica, D. – Furdui, B.: Sorghum as source of functional compounds and their importance in human nutrition. The Annals of the University Dunarea de Jos of Galati, Fascicle VI – Food Technology, *43*, 2019, pp. 189–205. DOI: 10.35219/foodtechnology.2019.2.13.
7. Awika, J. M.: Sorghum: Its unique nutritional and health-promoting attributes. In: Taylor, J. R. N. – Awika, J. M. (Eds.): Gluten-free ancient grains. Amsterdam : Elsevier, 2017, pp. 21–54. ISBN: 9780081008669. DOI: 10.1016/B978-0-08-100866-9.00003-0.
8. Davis, H. – Su, X. – Shen, Y. – Xu, J. – Wang, D. – Smith, J. S. – Aramouni, F. – Wang, W.: Phenotypic diversity of colored phytochemicals in sorghum accessions with various pericarp pigments. In: Watson, R. R. (Eds.): Polyphenols in plants. Isolation, purification and extract preparation. 2nd edition. London : Academic Press, Elsevier, 2019, pp. 123–131. ISBN: 9780128137697. DOI: 10.1016/B978-0-12-813768-0.00008-6.
9. Espitia-Hernández, P. – Chávez González, M. L. – Ascacio Valdés, J. A – Dávila-Medina, D. – Flores-Nevada, A. – Silva, T. – Chacón X. R. – Sepúlveda, L.: Sorghum (*Sorghum bicolor* L.) as a potential source of bioactive substances and their biological properties. Critical Reviews in Food Science and Nutrition, *62*, 2020, pp. 2269–2280. DOI: 10.1080/10408398.2020.1852389.
10. Pinheiro, S. S. – Cardoso, L. de M. – Anunciação, P. C. – de Menezes, C. B. – Queiroz, V. A. V. – Martino, H. S. D. – Della Lucia, C. M. – Pinheiro Sant'Ana, H. M.: Water stress increased the flavonoid content in tannin-free sorghum grains. Journal of Food Composition and Analysis, *100*, 2021, article 103892. DOI: 10.1016/j.jfca.2021.103892.
11. Rakshit, S. – Bellundagi, A.: Conventional breeding techniques in sorghum. In: Aruna, C. – Visarada, K. B. R. S. – Venkatesh Bhat, B. – Tonapi, V. A. (Eds.): Breeding sorghum for diverse end uses. Cambridge : Woodhead Publishing, 2018, pp. 77–91. ISBN: 9780081018798. DOI: 10.1016/b978-0-08-101879-8.00005-x.
12. ISO 10520:1997. Native starch – Determination of starch content – Ewers polarimetric method. Geneva : International Organization for Standardization, 1997.
13. ICC Standard No. 105/2. Determination of crude protein in cereals and cereal products for food and for feed. Wien : International Association for Cereal Science and Technology, 1994.
14. Pravilnik o metodama uzimanja uzoraka i vršenja hemijskih i fizičkih analiza belančevinastih proizvoda za prehrambenu industriju. (Rulebook on methods of sampling and performing chemical and physical analyzes of protein products for the food industry.) Službeni list SFRJ, *41*, 1985, pp. 1257–1262. ISSN: 2787-1959. In Serbian.
15. CXS 172-1989. Standard for sorghum grains. Adopted in 1989. Revised in 1995. Amended in 2019. Rome : FAO/WHO Codex Alimentarius Commission, 2019.
16. CERSOR010. Technical specifications for sorghum grains. Adopted in 2020. Revised in 2020. Rome : World Food Programme, 2020.
17. Barros, F. – Dykes, L. – Awika, J. M. – Rooney, L. W.: Accelerated solvent extraction of phenolic compounds from sorghum brans. Journal of Cereal Science, *58*, 2013, pp. 305–312. DOI: 10.1016/j.jcs.2013.05.011.
18. Dykes, L.: Tannin analysis in sorghum grains. In: Zhao, Z. Y. – Dahlberg J. (Eds.): Sorghum. Methods and protocols. New York : Humana Press, 2019, pp. 109–120. ISBN: 978-1-4939-9038-2. DOI: 10.1007/978-1-4939-9039-9_8.
19. Wang, X. – Han, X. – Li, L. – Zheng, X.: Optimization for quantification of sorghum tannins by ferric ammonium citrate assay. Grain and Oil Science and Technology, *3*, 2020, pp. 146–153. DOI: 10.1016/j.gaost.2020.07.001.
20. Makkar, H. P. S.: Quantification of tannins in tree and shrub foliage: a laboratory manual. Berlin : Springer, 2003. ISBN: 978-90-481-6428-8. DOI: 10.1007/978-94-017-0273-7.
21. SRPS ISO 9648:2013. Sorghum – Determination of tannin content. Belgrade : Institute for Standardisation of Serbia, 2013.
22. Pękal, A. – Pyrzynska K.: Evaluation of aluminium complexation reaction for flavonoid content assay. Food Analytical Methods, *7*, 2014, pp. 1776–1782.

DOI: 10.1007/s12161-014-9814-x.
23. Lee, J. – Durst, R. W. – Wrolstad, R. E.: Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC International, *88*, 2005, pp. 1269–1278. DOI: 10.1093/jaoac/88.5.1269.
24. Kumar, P. S.: Assay guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. In: El-Missiry, A. M. (Ed.): Antioxidant enzyme. London : IntechOpen, 2012. DOI: 10.5772/50782.
25. Kollo, T. – Rosen, D.: Advanced multivariate statistics with matrices. Dordrecht : Springer, 2005. ISBN: 978-1-4020-3418-3. DOI: 10.1007/1-4020-3419-9.
26. Ochoa-Martínez, C. I – Ayala-Aponte, A. A.: Prediction of mass transfer kinetics during osmotic dehydration of apples using neural networks. LWT – Food Science and Technology, *40*, 2007, pp. 638–645. DOI: 10.1016/j.lwt.2006.03.013.
27. Yoon, Y. – Swales, G. – Margavio, T. M.: A comparison of discriminant analysis versus artificial neural networks. Journal of the Operational Research Society, *44*, 1993, pp. 51–60. DOI: 10.1057/jors.1993.6.
28. Rocchetti, G. – Giuberti, G. – Busconi, M. – Marocco, A. – Trevisan, M. – Lucini, L.: Pigmented sorghum polyphenols as potential inhibitors of starch digestibility: An *in vitro* study combining starch digestion and untargeted metabolomics. Food Chemistry, *312*, 2020, article 126077. DOI: 10.1016/j.foodchem.2019.126077.
29. Kovtunov, V. V. – Kovtunova, N. A. – Popov, A. S.: The indices of sorghum seed quality in dependence on ecological and geographical origin. IOP Conference Series: Earth and Environmental Science, *843*, 2021, article 012007. DOI: 10.1088/1755-1315/843/1/012007.
30. Dykes, L. – Rooney, L. W. – Waniska, R. D. – Rooney, W. L.: Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 6813–6818. DOI: 10.1021/jf050419e.
31. Kumari, P. K. – Umakanth, A. V. – Bala Narsaiah, T. – Uma, A.: Exploring anthocyanins, antioxidant capacity and α-glucosidase inhibition in bran and flour extracts of selected sorghum genotypes. Food Bioscience, *41*, 2021, article 100979. DOI: 10.1016/j.fbio.2021.100979.
32. Awika, J. M. – Rooney, L. W. – Waniska, R. D.: Anthocyanins from black sorghum and their antioxidant properties. Food Chemistry, *90*, 2005, pp. 293–301. DOI: 10.1016/j.foodchem.2004.03.058.
33. Geladi, P. – Manley, M. – Lestander, T.: Scatter plotting in multivariate data analysis. Journal of Chemometrics, *17*, 2003, pp. 503–511. DOI: 10.1002/cem.814.
34. Keim, D. A. – Hao, M. C. – Dayal, U. – Janetzko, H. – Bak, P.: Generalized scatter plots. Information Visualization, *9*, 2010, pp. 301–311. DOI: 10.1057/ivs.2009.34.

Received 9 August 2023; 1st revised 25 March 2024; accepted 8 April 2024; published online 21 June 2024.