

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

**EVALUATION OF BIOACTIVE COMPOUNDS OF
SORGHUM FOR PRODUCT DEVELOPMENT**

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DEBRECEN, 2024

1 BACKGROUND AND OBJECTIVES OF THE DOCTORAL THESIS

One of the biggest crises of our time is the accelerated warming caused by industrialisation and the ongoing changes in climate. According to NASA data, global average temperatures have risen by about 1°C since the late 19th century, and meteorological data show that the period 2015-2022 was the warmest period on record globally in the last 100 years. And these changes will fundamentally determine the potential and costs of the modern intensive agricultural sector and indirectly affect the food supply of the population. This has led to the need to intensify the production of crops that are more tolerant of extreme weather conditions and can also be used for food purposes. In the last decade, an increasing number of national and international scientific publications have been devoted to the agronomic, biochemical, human physiological and food applications of millets (sorghum, millet, teff), as the species and varieties of these crops, including sorghum, have excellent drought tolerance and resistance to extreme environmental factors. In Hungary, significant progress has been made in sorghum and millet breeding and agronomy over the last 70 years (Assefa et al., 2010; Hadebe et al., 2017; Teuling, 2018; Orimoloye, 2022)

In addition, food intolerances and allergies are a growing problem worldwide, affecting a significant proportion of the population (1-10%). Among these diseases, celiac disease and other types of gluten intolerance are also prominent, with a worldwide prevalence of 1-2% and still increasing. This has led to the need to develop products that meet the needs of this specific consumer sector, which requires gluten-free, bioactive compound-rich and nutritious raw materials such as sorghum. Sorghum has been an important source of nutrients for centuries and today provides food for more than 500 million people, mainly in developing regions. It is only recently that its true value, beneficial physiological properties and potential uses have been discovered by western consumers and its use is now becoming increasingly widespread. These positive physiological effects have been reported in several literature, mainly in the form of inhibition of inflammatory processes, neutralization of free radicals, antibacterial and antitumor effects (Awika, 2011, 2017; Basli et al., 2017; Duodu and Awika, 2019). Therefore, our research aimed to investigate the effects of nutrient supplementation and environmental factors on the amount and antioxidant properties of bioactive compounds in red and white cultivars under domestic cultivation, and to determine the exact amount and extraction efficiency of these bioactive compounds in different solvents for human and forage cultivars. Further objective was also to develop and test the quality of a high added fibre sorghum-based gluten-free bakery product.

2 MATERIAL AND METHOD

2.1 MEASUREMENTS OF ANTIOXIDANT CAPACITY *IN VITRO*

2.1.1. Sample preparation and moisture content determination

During my doctoral research, 3 red (Zádor, Alföldi1, ES Foehn) and 3 white (Albita, Albanus, Farmsugro 180) grain sorghum varieties were tested, provided by the Karcag Research Institute of the Hungarian University of Agricultural and Life Sciences. The experiment was set up in 3 years interval during the period of 2019-2021. The experimental area is located at the border of Hortobágy and Nagykunság, it is a flat and even terrain, with meadow chernozem soil type. According to the tests, the area has a soil with acidic chemistry, clay-loam physical structure, high humus content (2.81-4%), good mineral content in 2019 and 2020, and medium in 2021. The weather data over the 3 years were significantly different. The average annual sunshine hours were 249, 230, and 244 hours per year. In 2019 high spring precipitation resulted in a shift in sowing to June, while during the summer growing season, precipitation fell below the 50-year average. In 2020, both precipitation (17.8 mm, against a multi-year average of 54 mm) and temperature (14.6 °C, against an annual average of 16.3 °C) at the beginning of the growing season were lower than in previous years, while in 2021, the winter period was high and the summer period was relatively dry and hot. The study years were further characterized by the Palfai Drought Index (PAI) to assess the exposure of the experimental area to drought. Among the experimental years, the PAI values for 2019 and 2020 were ~5 and 3, respectively, while for 2021 it was ~6 °C.100 mm⁻¹, indicating that 2019 and 2021 were mild drought years (PAI = 4-6 °C.100 mm⁻¹), while 2020 was drought-free (PAI <4 °C.100 mm⁻¹) (Kovács et al., 2022). The sorghum grains received were manually cleaned and then hulled using a laboratory SATAKE stone huller (SATAKE, TM05C, Thailand) at a maximum power of 50 s. After hulling, the bran fraction was separated from the hulled kernel fraction and stored in sealable plastic bags at -20 °C until further analysis. The hulled grains were then ground into flour on a laboratory roller (Metefém FQC 109) and stored in sealable plastic bags at -20 °C. From the samples, 1-1 g were weighed for determination of dry matter content, which was carried out gravimetrically in a drying oven at 105 °C for 3 h until constant weight was reached.

2.1.2. Extraction of antioxidant compounds from sorghum

Samples were extracted according to measurement protocols. Three types of extraction were used to investigate the effects of nutrient supplementation and environmental factors during the laboratory work:

1. 0.5 g of the samples were weighed on an analytical balance into 15 ml volume centrifuge tubes, then 5 ml of an 80:20 mixture of methanol-distilled water was added, homogenized on a vortex for 30 s and extracted in an ultrasonic water bath at 25 °C for 20 min. The resulting extract was 80-20% methanol-water extract.
2. 0.5 g per sample was weighed into 15 ml centrifuge tubes, 5 ml methanol was added and after vortexing for 30 seconds, these samples were also extracted in an ultrasonic water bath at 25 °C for 20 minutes. This became the methanol extract.
3. In the third extraction method, 5 ml of distilled water was added to 0.5 g of bran and extracted in a water bath after vortexing for 20 min. This became the aqueous extract.

At the end of each of the three extractions, the samples were centrifuged (Frontier 5000 Series, Ohaus Europe, Nänikon, Switzerland) at a maximum speed of 3600 x g for 10 min and after centrifugation the supernatant was used for further work. For spectrophotometric measurements, a SpectroStar nano spectrophotometer (BMG Labtech, Ortenberg, Germany) microplate reader was used.

In addition, the amount of antioxidants and polyphenol polyphenols present in the bound form was determined using the previously described methanol-water extraction as a first step in preparation. After this step, the residue was dried overnight at 40 °C in a drying oven (Metefém). For the preparation of bound polyphenol polyphenolic compounds, the previously extracted residue was used. The extraction was performed according to the method of Wang et al. (2019). Preparation was repeated three times per sample and per extraction and stored at -20 °C until analysis.

The flavonoid profile of sorghum and their extraction efficiency were investigated separately, whereby the sample preparation procedure was modified as follows.

1 red variety (Zádor) and 1 white variety (Albita) from the 2019 harvest were used for the tests. For the sample preparation, the bran fraction already separated at the hulling stage was used.

For the extraction, acetone was chosen as the solvent based on the literature as a universal solvent, and the following acetone - water extraction mixtures were prepared for the efficiency test: 50 - 50 (AC1), 60 - 40 (AC2), 70 - 30 (AC3), 80 - 20 (AC4), and 90 - 10 (AC5). Of the samples, 2 g were weighed on an analytical balance in 3 replicates in 50 ml centrifuge tubes, 20 ml of extractant was added, and the solution was extracted using an ultrasonic water bath for 30 min and centrifuged. The supernatant was then decanted and the extraction was repeated 3 more times. After extraction, the supernatants were weighed and homogenised, and 20 ml volumes were transferred to 25 ml centrifuge tubes and stored at -20 °C until MS/MS analysis. The remaining sample was evaporated dry on a rotary evaporator and stored at -20 °C. The weight of the dry evaporated extracts was counted and the yield efficiency was determined for each acetone-water solvent.

2.1.3. Measurement of antioxidant activities of sorghum

Polyphenol, flavonoid and condensed content determination

The total polyphenol content was determined by the Folin-Ciocalteu method, based on the description of Singleton and Rossi, modified by Nemes et al. (2018) (Nemes et al., 2018), while the flavonoid content was measured by the commonly used aluminium chloride method, based on the method of Zhishen et al. (1999) with slight modification (Zhishen et al, 1999). For the determination of condensed tannin content, the vanillin hydrochloric acid method was used according to the modified protocol of Price et al. (1978) (Price et al., 1978). For the measurement, the previously prepared methanol-water (80:20 v/v%) and methanol extracts were used.

Determination of antioxidant capacity

Three methods for measuring antioxidant capacity were used: the Trolox equivalent antioxidant capacity (TEAC) measurement method based on ABTS radical neutralization, the DPPH measurement method based on DPPH radical neutralization, and the ferric reducing ability-based antioxidant capacity (FRAP) measurement method based on the modified protocols of Zhu et al. (2009), Blois et al. (1958), and Benzie et al. (1996) with slight modifications (Blois, 1958; Benzie and Strain, 1996; Nemes et al., 2018). Methanol extracts were used for the TEAC and DPPH methods, while aqueous extracts were used for the FRAP measurement method.

2.2 DETERMINATION OF THE FLAVONOID PROFILE OF SORGHUM USING HPLC AND MS/MS SYSTEM

2.2.1. Sample preparation and measurement on HPLC-MS/MS

One red (Zádor) and one white (Albita) cultivar were selected for the flavonoid profile analysis and the previously prepared acetone extracts were used for the determination. The measurement was performed using a UHPLC-ESI-ORBITRAP tandem mass spectrometer. The mobile phases used for the separation were acid methanol (A) and water (B). The separation was performed by eluent gradient. The results obtained were evaluated using Thermo Trace Finder 2.1 software and an in-house database.

2.2.2. Sample preparation and measurement un HPLC-PDA SYSTEM

HPLC-DAD was used for the quantification of the flavonoid profile. The measurement was based on the results of the previously performed MS/MS measurement. Procyanidin-type tannins were prepared from the previously prepared AC3 extract. The samples were purified on Sephadex LH-20 dextran column. Separation was performed by RP-HPLC equipped with a PDA detector using the following mobile phases: solvent A methanol, solvent B 3% formic acid solution. Separation was performed by gradient detection at 280, 340, 350 nm wavelengths.

Further determination of flavonoid type compounds was performed from fresh sample preparation by hydrochloric acid methanol extraction. The samples were extracted with 100 ml HCl-methanol for 2 h. The resulting extract was centrifuged at 7500 x g for 5 min. After dry evaporation, the extracts were stored at -20°C and dissolved in 8 ml HCl-methanol before measurement. A portion of the sample was hydrolysed in a water bath at 80°C for 2 hours. Samples were analysed on an RP-UHPLC-DAD system on a Hypersil ODS silica column stationary phase using methanol (Solvent A) and 3% formic acid (Solvent B) mobile phase. 10 µl of the samples were injected at an eluent gradient with a flow rate of 1 ml.min⁻¹. The components were detected on a PDA detector in the wavelength range 200-600 nm, including 280, 340 and 350 nm. Accurate identification and quantification of the compounds was performed using external standards (naringenin, apigenin, luteolin, Merck) based on retention time, spectra and integrated area.

2.3 DETERMINATION OF THE COMPOSITION OF VITAMIN CONTENT OF SORGHUM

2.3.1. Preparation and determination of Vitamin E

Determination and preparation of tocopherol distribution was carried out on an RP-UPLC-PDA system based on Bíró's method with minor modifications (Bíró et al., 2015). The stationary phase was a Hypersil ODS silica column, while the mobile phase was a 63:35:5 mixture of acetonitrile-methanol-DKM. Detection was performed using a PDA detector at 295 nm.

2.3.2. Preparation and determination of Vitamin B

Four vitamin B forms specific for sorghum were determined, namely thiamine (B1), niacin (B3), pyridoxine (B6), and riboflavin (B2). The method of Nemes (2015) was used for the measurement with minor modifications. Determination of B vitamins was carried out using an HPLC system (Waters 2695 Separation Module) equipped with a two-channel UV/VIS detector (Waters, 2487). The separated components were detected by UV/VIS detection at 254 nm wavelength.

2.4 DEVELOPMENT OF SORGHUM-BASED GLUTEN-FREE PRODUCTS, AND MEASUREMENT OF RHEOLOGICAL AND ORGANOLEPTIC PROPERTIES

2.4.1. Materials and physical treatment of flours

Commercially available raw materials (sorghum flour, potato starch, sunflower lecithin, yeast, psyllium seed husk flour) were used for the product development. In the framework of the product development, sorghum-based gluten-free breads were developed, and wheat, millet and oat flour were also purchased from the local supermarket (Debrecen) as reference material for further testing. Thermal treatment was used to modify the physical properties of sorghum flour, based on the work of Marston et al. (2016), with minor modifications (Marston et al., 2016).

2.4.2. Product development process

The development of the sorghum-based gluten-free breads was carried out at the MÉK Food Innovation Centre of the University of Debrecen (Debrecen, Hungary). The basic recipe used for the development was: sorghum flour (140 g), potato starch (60 g), sunflower lecithin (4 g), salt (2 g), granulated sugar (2 g), low-fat milk powder (2 g), dried yeast (4 g) and drinking water. This recipe was modified by enriching it with psyllium seed husks, with 5, 7 and 10 %

substitution. The products were prepared in two replicates. The dough was prepared using a food processor (KitchenAid Artisan Mixer, KitchenAid Hungary, Budapest, Hungary) with a mixing head attachment, and 330-400 ml of water was added, depending on the composition. The water was added until a malleable structure was obtained. The dough was kneaded for 90 seconds, then halved by weight and shaped before rising. Rising was carried out in a rising chamber (F-608, FM INDUSTRIAL Lucena, Spain) for 30-40 min at 35 °C until a height of 4,5 cm was reached. Baking was performed in a convection oven (RXB 610 SMART, FM INDUSTRIAL Lucena, Spain) at 220 °C, 95% humidity, 10 min, 220 °C, 50% humidity, 25 min, and 180 °C, 30% humidity, 20 min. After baking, the breads were refrigerated for 1 h until room temperature was reached before further testing.

2.5 DETERMINATION OF FUNCTIONAL PROPERTIES OF SORGHUM FLOUR AND QUALITY ASSESMENT OF FIISHED PRODUCT

The research investigated the basic functional properties of treated and untreated sorghum flour, as well as the organoleptic parameters and rheological properties of the final product. The water and oil absorption capacity (WAC,OAC) was determined according to the modified method of Elkhalfa and Bernhardt (2010) (Elkhalfa and Bernhardt, 2010).The WAC and OAC values were expressed as a percentage of wet weight and initial weight. The swell ability was determined based on Singh's method (Singh et al., 2017). The percentage mass ratio obtained was used as the swell ability value.

The quality parameters of the finished products were tested after 1 h cooling period. The volume of the loaves was determined using rapeseed displacement method, while the specific volume of the loaves was determined as the ratio of the volume of the loaves determined in the previous study to the mass of the loaves. The aspect ratio of the test loaves was also determined as the ratio of the height to the width of the loaves.

Stock analysis of the finished products was carried out using a TA-XT2 (Stable Micro System, Godalming, United Kingdom) type stock analyser. For the tests, slices of bread of equal thickness (25-30 mm) were cut and the two slices in the middle were measured. The hardness and elasticity of the breads were determined using the following setup: cylindrical head 10 mm in diameter, equipped with a 5 kg load cell, pre- and post-test speed 1 mm.s⁻¹ and 10 mm.s⁻¹, test speed 0.50 mm. s⁻¹, maximum depth 5 mm.

The finished products were also subjected to an organoleptic test involving 20 tasters. The tasters also completed a preliminary questionnaire to assess age, gender distribution, food allergies, intolerances and eating habits. An 8-step hedonic scale was used for scoring, with tasting scoring products on taste, colour, smell, texture, appearance and average impression.

2.6 STATISTICAL ANALYSIS

In the course of the research, 6 sorghum varieties were tested. A completely randomized design with 4 replications was used to study the effect of nutrient supplementation, environmental factors and variety on the bioactive profile. The data obtained were analysed by means of three- and two-factor analysis of variance, and correlation analysis between the individual factors was also performed. In addition, the flavonoid and vitamin composition of two typical varieties (Zádor - red; Albita - white) and the functional properties of sorghum flour and sorghum-based products were analysed using two-way analysis of variance with post-hoc tests. To evaluate the results of the extraction efficiency test, a paired t-test was used to compare the two types of extraction per variety. For statistical analysis, SPSS statistical software (version 24) was used, while GraphPad Prism 8 and Microsoft Excel (Microsoft Office Professional Plus 2018) were used to generate graphs and tables.

3 RESULTS AND DISCUSSION

3.1 EFFECT OF NUTRIENT SUPPLEMENTATION ON BIOACTIVE COMPOUNDS OF SORGHUM INFLUENCED BY ENVIRONMENTAL FACTORS

We investigated the changes in the bioactive profile of 6 sorghum cultivars in response to nutrient supplementation and changing environmental factors, taking into account the antioxidant properties of the cultivars. ANOVA revealed that year of harvest significantly ($p < 0.001$) determined the bioactive properties of the varieties, while nitrogen supplementation showed significance (0.01) only for total polyphenol content and FRAP antioxidant value. Variety had a significant effect for all measured parameters, which was an expected result since seed colour is a fundamental indicator of the amount, or possibly type, of bioactive compounds present.

The varieties differed significantly in terms of antioxidant activity and bioactive profile. The polyphenol content of the 6 cultivars was investigated and the highest polyphenol content was found in the bran of the red grain cultivar Alföld1 for the 3 years (945-1802 mg.100 g⁻¹ GAE), which also had a higher condensed tannin content than the other cultivars (7.9-47.4 mg.g⁻¹). In the third year, a significant jump (1800 mg.100 g⁻¹ GAE and 47.4 mg.g⁻¹) was observed due to extreme environmental conditions. By variety, only some varieties (Zádor, Albita, Farmsugro 180) showed a detectable effect of nitrogen supplementation on polyphenol content, while for tannin content the effect of nitrogen supplementation was not significant. It has also been shown that grain colour does not always indicate high polyphenol content or antioxidant effects. The red-grain variety ES Foehn was found to contain similar concentrations of polyphenolic compounds (139-200 mg.100 g⁻¹ GAE) as the white-grain varieties, and its tannin content was significantly lower than the other red varieties.

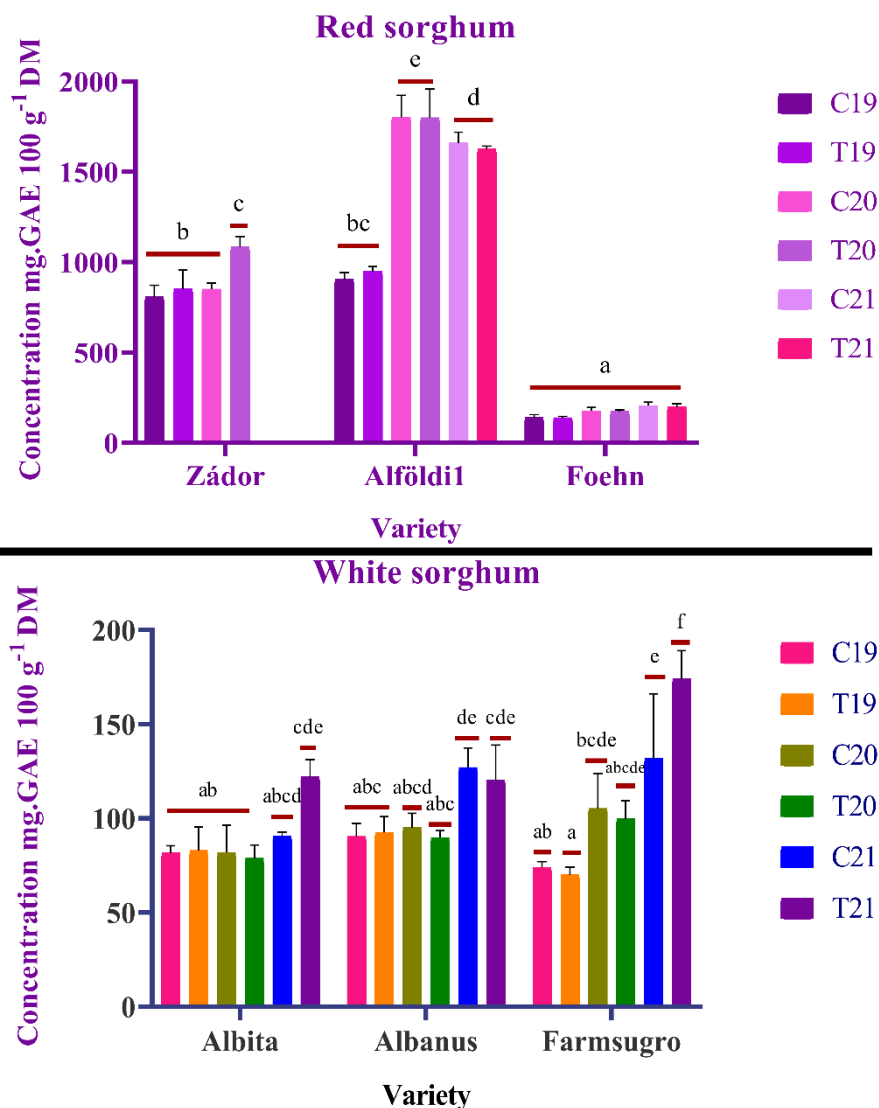


Figure 1. Total polyphenol content of the 6 sorghum varieties tested. *Notes: T= Treated samples, C= Control samples. Means with the same letter are not statistically different from each other. Values are expressed in dry matter.*

Antioxidant capacity values showed similar trends with total polyphenol content. Among the three methods used, TEAC and DPPH gave exceptionally high results, which were highest for the cultivar Alföldi1 with 45.6 ± 17.7 and $281.7 \pm 17.5 \mu\text{mol.TE g}^{-1}$ concentrations for TEAC and DPPH, respectively. The highest antioxidant value was observed in the Alföldi1 sample in 2021 ($281.7 \mu\text{mol.TE g}^{-1}$), which also confirmed the effect of environmental factors in the third year of the study. In contrast, the FRAP method, which is more suitable for measuring water-soluble antioxidants, yielded significantly lower values ($5\text{-}20 \mu\text{mol.AAE g}^{-1}$), suggesting that the amount of water-soluble iron-reducing antioxidants in the varieties studied is negligible. Nutrient supplementation did not consistently influence the antioxidant effect, with a significant

difference ($p < 0.01$) only for the Albanus variety. However, there was a significant difference ($p < 0.01$) between different years for all cultivars except Zádor and Albita genotypes.

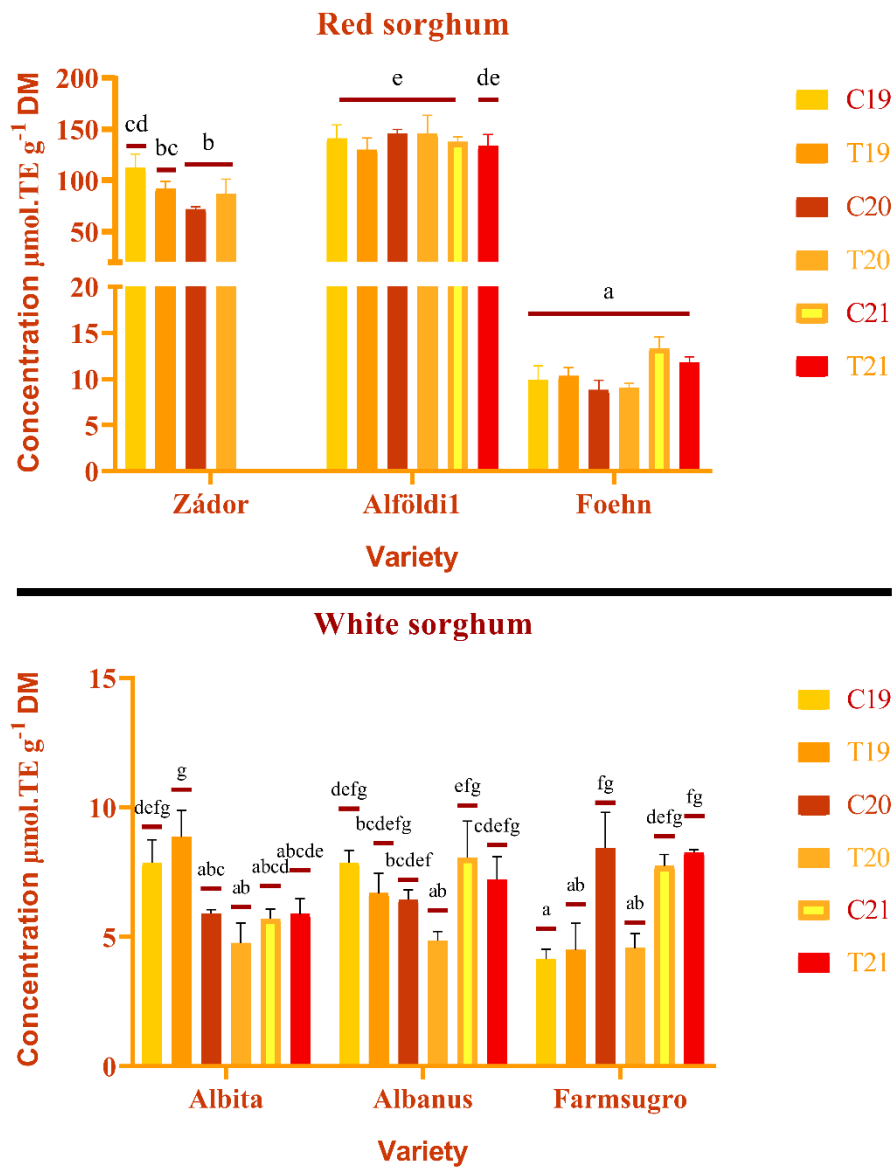


Figure 2. Antioxidant capacity of the 6 sorghum varieties tested, as measured by the TEAC method. Notes: T= Treated samples, C= Control samples. Means with the same letter are not statistically different from each other. Values are expressed in dry matter.

The amount and composition of polyphenols and tannins interact with the antioxidant capacities measured. Correlation analysis was performed to investigate this. The measured TEAC and DPPH values are nearly identical, both radicals being able to provide both proton and electron transfer. The similarity was confirmed by the scattering curves and the correlation coefficient (0.925). Furthermore, correlation analysis revealed a strong (0.798) interaction between total

polyphenol and condensed tannin content and variation, thus these compounds constitute a significant fraction of sorghum polyphenols. Furthermore, total polyphenol content was strongly correlated with TEAC and DPPH antioxidant capacity values, of which the correlation coefficient of TEAC (0.963) was slightly higher than that of DPPH (0.947), which may be due to differences in the structural properties of the free radicals used in the two methods. In all cases, significant differences were observed between the values of the different harvest year. While TEAC showed a stronger interaction for total polyphenol content, when considering the evolution of condensed tannin content, the results of the DPPH method correlated more strongly (0.916) with TEAC values (0.738). Thus, a significant proportion of the antioxidant capacity measured by the DPPH method may be due to the tannin content of sorghum.

3.2 FREE AND BOUND POLYPHENOL CONTENT AND THEIR VARIABILITY IN RED SORGHUM VARIETIES AFFECTED BY EXTRACTION METHOD

Two polyphenol-rich cultivars (Alföldi1, Zádor) were analysed for their free and bound polyphenol and flavonoid content. The extraction with two different solvents was also compared. Similar to the previous results, Alföldi1 had the highest total polyphenol value with 2667 ± 42.9 mg.GAE 100 g^{-1} free extractable total polyphenol content, while the amount of polyphenols in bound form reached a maximum of 104.1 ± 11.4 mg.GAE 100 g^{-1} when extracted with 70% acetone. Moreover, the extracted flavonoid content was also significant with a maximum value of 1574.9 ± 4.12 mg. CE 100 g^{-1} for variety Alföldi1. The amount of flavonoids in bound form was low for both varieties. The extraction method used significantly determined the amount of recoverable compounds. Aqueous mixtures of methanol (80:20 v/v%) and acetone (70:30 v/v%) were compared. The results showed that in most cases, extraction with acetone gave significantly higher ($p < 0.05$ and 0.01) extraction yields for both total polyphenols and flavonoids content. For components in bound form, only the polyphenol content differed between the two extractions. Condensed tannins were present only in the free fraction and there was no significant difference between the results of the two extractions.

Changes in the antioxidant activities of the extracts were also investigated by extraction. No quantitative differences in polyphenolic compounds were observed for antioxidant capacity. No statistically significant difference was detected between the antioxidant values of the extracts of the two extractions, which may indicate compositional differences. The highest antioxidant value was shown by the Alföldi1 variety for antioxidant potential as determined by TEAC method with $241\text{-}269 \text{ } \mu\text{mol.TE g}^{-1}$ compared to $151\text{-}165 \text{ } \mu\text{mol.TE g}^{-1}$ for DPPH. This contrasts with previous measurements, where DPPH values were typically higher. The antioxidant

activity of polyphenols and flavonoids present in bound form had extremely low values irrespective of species and extraction. For the DPPH method, there was a statistically significant ($p < 0.05$ and 0.01) difference between the two extractions.

3.3 EVALUATION OF EXTRACTABILITY, AMOUNT AND ANTIOXIDANT EFFECTS OF CONDENSED TANNINS IN RED AND WHITE SORGHUM VARIETIES

Based on previous literature data, an aqueous solutions of acetone in different proportions were used to measure the condensed tannin content. The extraction and antioxidant capacity of each extract were determined. In terms of yield, the AC3 (70:30 v/v%) extraction proved to be the most effective, which was most striking for the yield obtained with the red (Zádor) variety with high tannin content, while this yield was not present for the white (Albita) variety with very low tannin content.

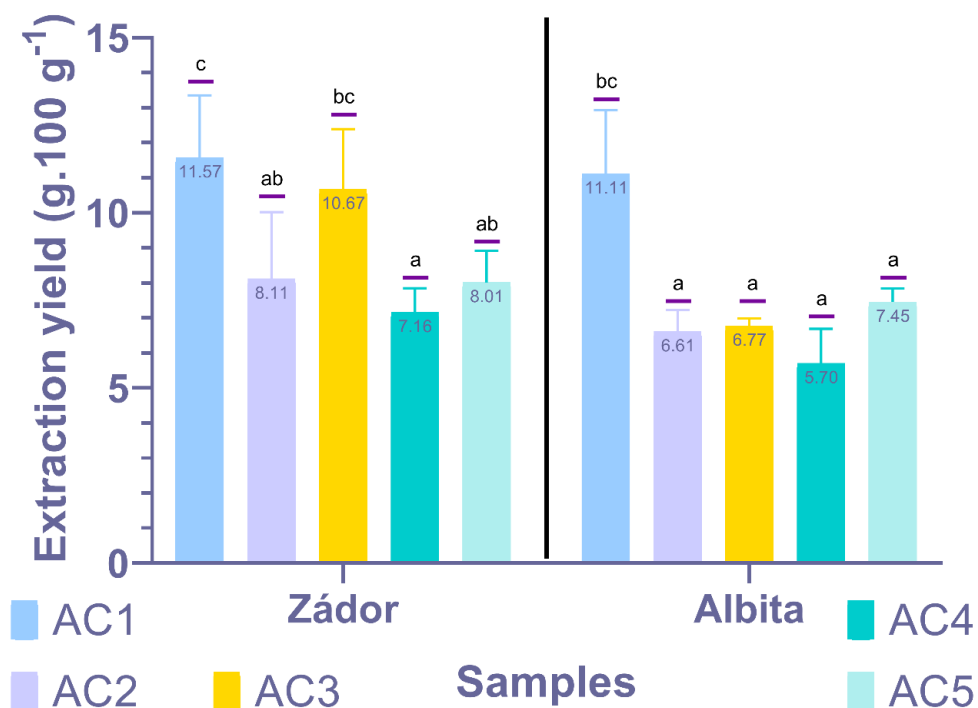


Figure 3.: Extraction yield of different acetone-water ratios for red (Zádor) and white (Albita) sorghum. *Legend: AC1 = 50:50 acetone-water v/v%, AC5 = 90:10 acetone-water v/v%.*

The antioxidant properties of each extract were also investigated using DPPH, TEAC and FRAP. As expected, a significant difference was observed between the antioxidant properties of the two tested varieties, as the white variety typically has a very low tannin content, which

is one of the main antioxidants in sorghum. Among the three methods, TEAC was found to be the most efficient method, with the maximum antioxidant capacity value measured reaching a maximum of $182.6 \pm 14.9 \mu\text{mol.TE g}^{-1} \text{ DM}$ for AC5 extract. This is presumably due to the multiple antioxidant binding capacity of tannins. AC3 extraction showed lower concentration with $118.5 \pm 14.4 \mu\text{mol.TE g}^{-1} \text{ DM}$, however, in case of DPPH method, AC1 and AC3 extraction showed the highest antioxidant activity with 72.7 ± 1.59 and $67.8 \pm 5.52 \mu\text{mol. TE g}^{-1} \text{ DM}$ values. Here, a correlation was observed between the size limiting property of the free radicals used in the method and the degree of polarization and size of the tannins in the extract. The results of the TEAC and DPPH methods suggest that AC3 is presumably richer in shorter-chain, smaller tannin oligomers, whereas longer polymers are predominant in the AC5 extract. However, this requires further investigation.

No trend was observed in the values of the third FRAP method, with the more polar AC1 extracts typically showing higher values, with the highest water-soluble antioxidant content being found in Zádor AC1 extract with $38.9 \pm 3.49 \mu\text{mol.AAE g}^{-1} \text{ DW}$.

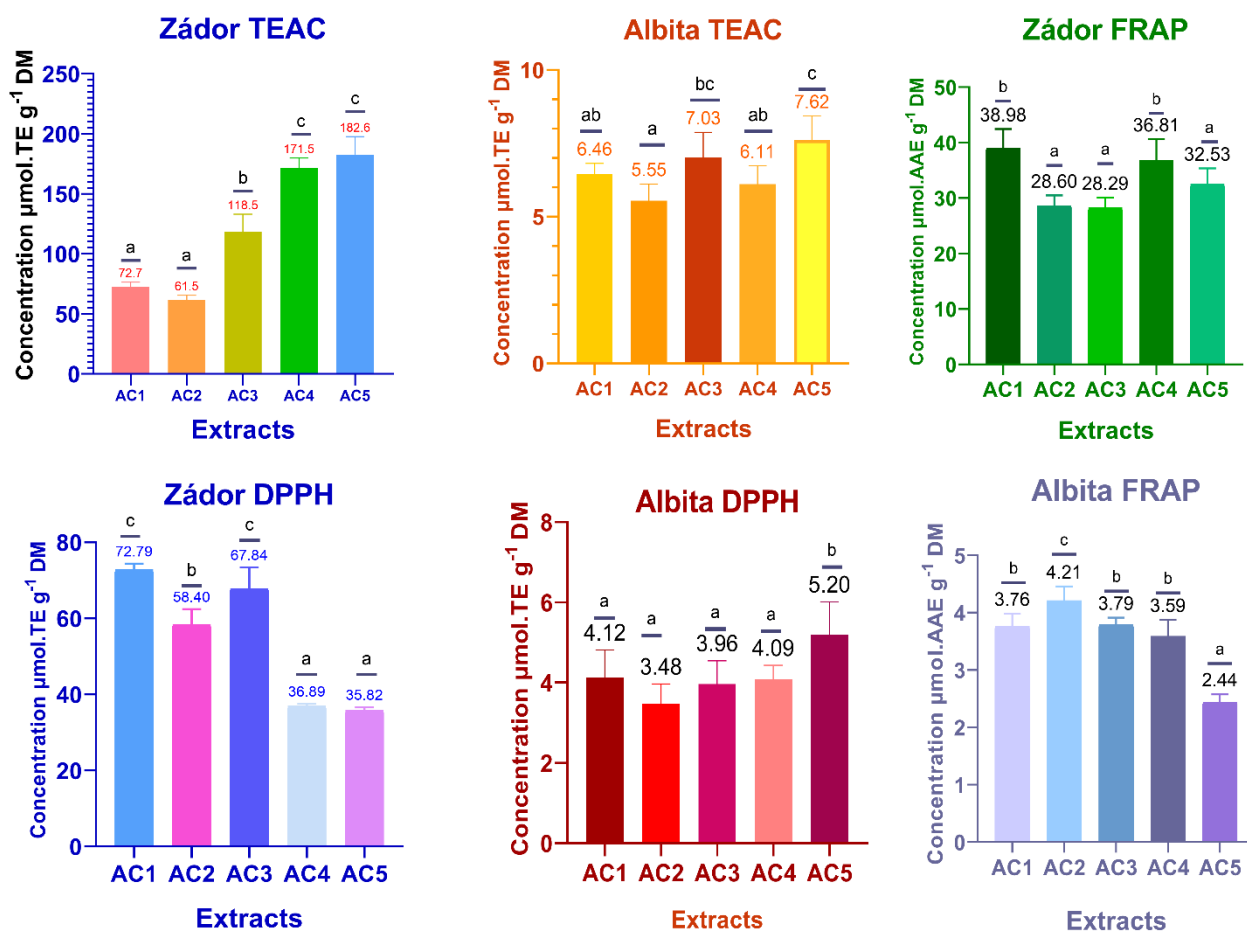


Figure 4.: Antioxidant content of acetone-water extracts measured by TEAC, DPPH and FRAP. Note: Values are given on a dry matter basis. Values with the same letter are not statistically different from each other.

The antioxidant values measured indicate that the red variety is particularly rich in large tannin oligomers and polymers with strong antioxidant activity, as well as other polyphenolic antioxidants, which are particularly important for both human and animal consumption for their anti-inflammatory, antitumor and antibacterial properties.

3.4 DETERMINATION OF THE FLAVONOID COMPOSITION OF RED AND WHITE SORGHUM

The composition of each acetone-water extract was analysed by HPLC MS/MS technique. Among the varieties tested, red Zador contained a large number of flavan, flavone, flavanone and proanthocyanidin compounds, of which the various Procyanidin A, B and C dimer and trimer isomers were predominant, but also several eriodictiols and naringenin glucosides were present in the bran fraction. In contrast, the white Albita variety was dominated by the flavone

subgroup, with apigenin, luteolin and trihydroxyflavones detected. In the white variety, no procyanidin-type components were detected. However, in both varieties, 3-deoxdiantocanidins (apigeninidin and luteolinidin), unique flavonoids of sorghum, were present, so far detected only in algae and brassicas and in purple maize hybrids, and their main characteristic is their extreme stability compared to normal anthocyanidins. Among the identified components, procyanidin B1, naringenin, and apigenin and luteolin were quantified by HPLC, showing that the tannin dimer procyanidin B1 was the most significant flavonoid in Zador with a concentration of 81 mg.100 g⁻¹, while in Albita variety naringenin was detected with a concentration of 2 mg.100 g⁻¹.

3.5 RED AND WHITE SORGHUM VITAMIN COMPOSITION RESULTS

Cereals are an essential source of vitamins E and B. In our studies, the distribution and vitamin B content of tocopherol isomers of 1 red and 1 white variety were analysed by HPLC. The results showed that all four tocopherol isomers were present in the 2 varieties, but sorghum was basically considered as a gamma- and beta-tocopherol accumulating grain. The total amount of gamma- and beta-tocopherols measured was 6.68 and 7.75 µg.g⁻¹ dry matter concentrations for Zador in bran and flour fractions, respectively. Due to their structural features, the chromatographic characteristics of γ- and β-tocopherols are very similar and therefore difficult to separate. For this reason, these two forms of tocopherols were determined together in our studies. In addition, alpha-tocopherol was also present in significant amounts in both fractions tested, with values of 2.75 and 1.7 µg.g⁻¹, respectively. Delta-tocopherol was detected only from the flour fraction at concentrations of 0.42 and 0.53 µg.g⁻¹ for the Albita and Zador varieties, respectively. Among the two varieties studied, the red Zador variety contained higher amounts of gamma- and beta-tocopherols, while the differences between the varieties were smaller for alpha-tocopherol and delta-tocopherol. Thus, based on the literature values and our measurement results, it can be concluded that sorghum is basically a gamma and beta tocopherol accumulator, thus it may play a major role in maintaining colon health, but its alpha-tocopherol content is also significant.

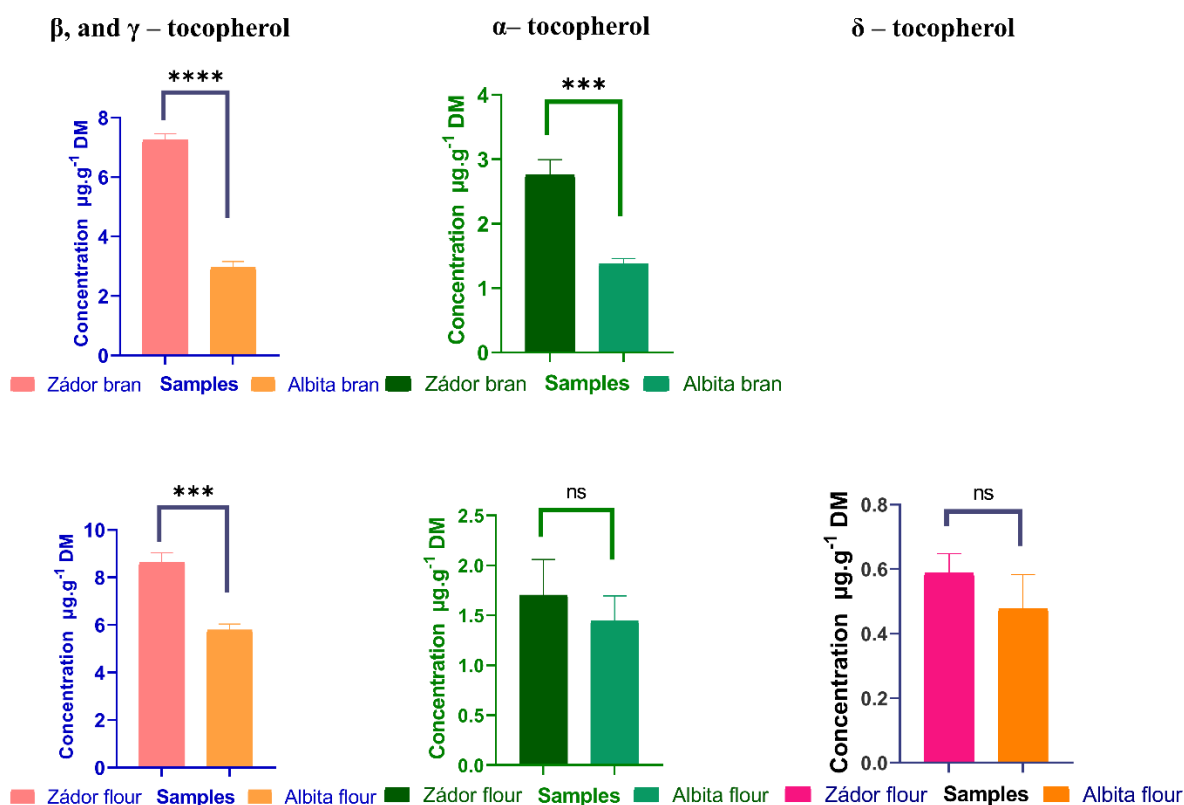


Figure 5.: Tocopherol content of red and white sorghum varieties. *Notes:* *: $p < 0.05$, **: $p < 0.001$, ***: $p < 0.0001$. Values are expressed on dry weight basis.

Among the B vitamins, niacin, thiamine, pyridoxine and riboflavin were determined from bran and flour fractions in Zádor and Albita varieties. Of the two varieties tested, Albita contained the highest levels of pyridoxine. In addition, Albita bran was also particularly rich in vitamin B₃, with concentrations of $3.4 \pm 0.1 \mu\text{g.g}^{-1}$ and $9.3 \pm 1.7 \mu\text{g.g}^{-1}$ for the red and white varieties, respectively. The B vitamins are basically accumulated in the husk, resulting in lower amounts in the flour fractions. Exceptions to this were thiamine, for which levels similar to bran were also measured in the flour fraction for Albita, and riboflavin only in the bran and pyridoxine in the flour fraction. Compared to previous tocopherol concentrations, it can be concluded that white grain sorghum varieties are considered to be a more significant source of vitamin B compared to red varieties, while red varieties are considered to be more of a source of vitamin E.

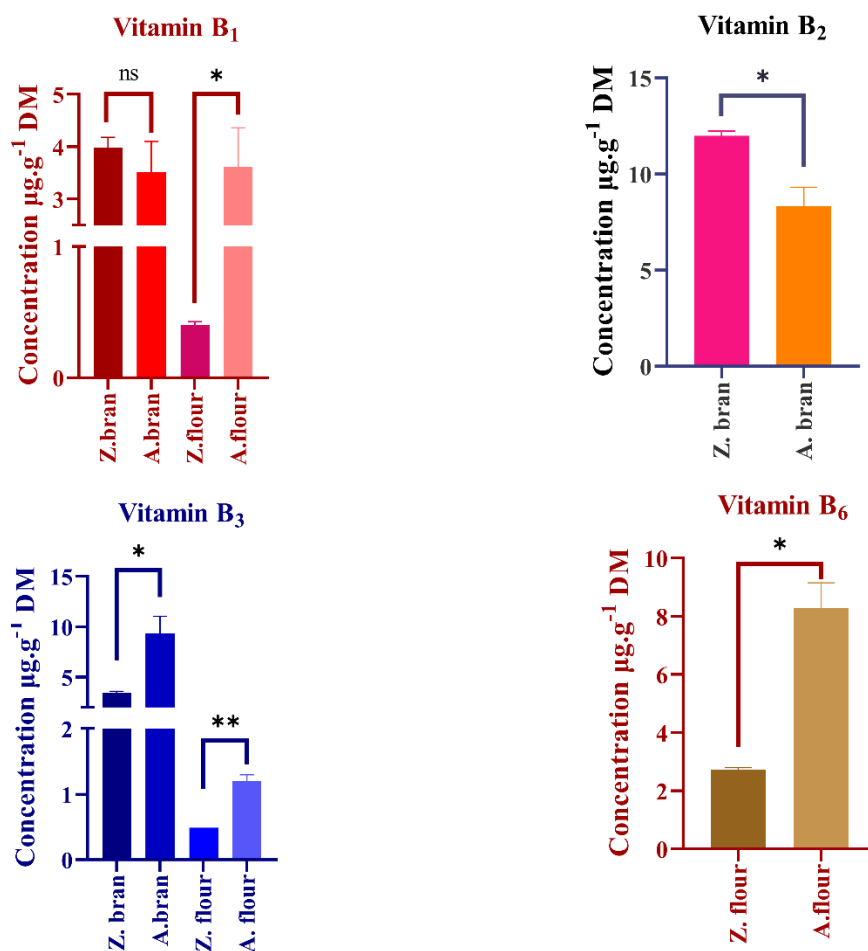


Figure 6.: Vitamin B content of red and white sorghum. *Note: Z.=Zádor, A. = Albita,*
 *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

3.6 FUNCTIONAL PROPERTIES OF SORGHUM FLOUR AND CHANGES DUE TO PHYSICAL TREATMENTS

The functional properties of the different flours used in the production of bakery products are the main determinants of the potential uses of each type of flour. In the case of sorghum flour, the water and oil absorption capacity and the swelling capacity of the proteins were determined, which basically determine the consistency and structure of the dough. We also studied the variation of these parameters under physical thermal treatments. Due to the higher fibre content, the water absorption capacity of sorghum flour was higher compared to wheat with a water absorption capacity of 129% compared to 76% for wheat fine flour. This water absorption capacity can be further increased by thermal treatments or by additional fibre addition. During the thermal treatment, the partial or total moisture content of the flour is removed and the starch structure is also changed, partially chiralized. This also increases the water absorption capacity of the flours. Psyllium husk seed coat further increased the water binding capacity, with the

highest water absorption of 290% in heat-treated flour enriched with 10% psyllium husk flour. Thus, a large structural improvement can be achieved by fibre enrichment. There were significantly smaller variations in oil absorption capacity. The highest value was measured for dry heat-treated sorghum flour at 86%, and there was no significant difference between treated and untreated flours. Since cereals are generally not high in fat and the product did not contain added fat, the OAC values obtained were as expected. The swelling capacity is an important property of structural proteins and they affect both the structure and quality of the final product. The main structural protein in sorghum is kafirine, which has a complex structure and therefore a rather low digestibility, but its functional properties can be affected. In our studies, the measured swelling capacity of sorghum flour was significantly lower than that of gluten-free millet flour, and not statistically significantly but visibly lower than that of rye and wheat flour, with values of 5.90, 6.37, 6.60 and 6.43 g.g⁻¹, respectively. Dry air heat treatment and freeze drying, probably due to protein degradation and bond breaking, showed a visible increase in the swelling capacity of sorghum flour proteins.

3.7 DEVELOPMENT AND QUALITY TESTING OF A SORGHUM-BASED BAKERY PRODUCT

The doctoral research also involved the development of a sorghum-based gluten-free bread with the addition of psyllium husk flour. Psyllium husk were added at 5; 7.5; and 10% and thermal pre-treatment was also used in the development. The fibre-enriched breads had a visibly larger volume, but also a much denser casing with small pores. Compared to the control breads, however, the colour of the fibre-enriched products was significantly improved, with a lighter, less burnt appearance. Furthermore, the control breads had a more porous and crumbly interior compared to the enriched products, so the addition of psyllium husk flour improved the structural properties of the breads as expected. In addition to the stock analysis, the finished product was tested for quality parameters such as volume, specific volume and aspect ratio. Even when fortified with the lowest level of psyllium husk (5%), a significant increase in volume was observed, 420±14 cm³ and 518±4 cm³ in volume for breads fortified with control and 5% psyllium husk meal, respectively. Further increase of the psyllium husk flour ratio did not cause a significant increase in volume.

In terms of shape quotient, the current regulations for wheat bread specify a maximum shape quotient of 2.2 (Codex Alimentarius Hungaricus, 1-3/16-1). In comparison, the shape quotient of sorghum-based breads with high added fibre content ranged between 1.5 and 2, and an increase in the proportion of psyllium husk flour resulted in a decrease in the shape quotient for

the 7.5% and 10% psyllium husk flour ratios. However, it is important to note that this specific value is used to grade wheat breads and can therefore only be used as a reference.

The stock of the finished products was tested on the day of baking and after 2 days of storage under normal conditions using a stock analyser. The tests were carried out to determine the hardness and elasticity of the bread. The control products had an unstable, crumbly, dry texture, which was indicated by a low strength (80.7 g). With the addition of psyllium husk flour, this firmness was significantly increased in the control breads due to its excellent water binding capacity (209.9 g). The combination of heat treatment and psyllium husk flour resulted in a dough structure that was elastic, yet soft and non-crumbly in fresh products. The treated control product had the lowest elasticity at 43% on the day of baking, but the addition of high-fibre psyllium husk flour resulted in a significant improvement to an elasticity of 63-68%. However, after 48 h of storage, a significant water loss occurred due to starch retrogradation, which caused an increase in product hardness and a decrease in elasticity. The psyllium husk flour had a positive effect on the structure of the products, as the greatest increase in hardness was observed in the treated and control breads, with a six fold and fourfold increase respectively compared to the first day values. For the breads with added fibre, this increase was only twice that of the control, and the heat treatment applied reduced this increase slightly further. Overall, of the product prototypes, the breads with 7.5% added psyllium husk husks had the most favourable properties and consistency, with the negative effects of excessive fibre content, such as hard texture and unpleasant taste, not yet being pronounced.

For new foods, meeting consumer needs is one of the most important issues and sensory testing is used to measure this. In our organoleptic survey, five parameters were assessed: appearance, texture, smell, taste and overall impression. Of the breads tested, the tasters found the breads enriched with 7.5 and 10% psyllium husk flour the most appealing, with an average score of 5-7 points, while the control products received the lowest scores. The key points in the rating were the appearance, texture and texture, which basically determined the scoring of each bread. For appearance, the lowest scores were 3.64 and 4 in the appearance and texture categories for the treated control product. Tasters felt that the appearance of the 7.5 and 10% enriched products was appropriate and desirable to them. The tasters experienced a slightly unpleasant bitter aftertaste during the tests, most of them rated this negatively, while some tasters described this as a positive attribute based on personal preference.

4 NEW SCIENTIFIC RESULTS

1. The quantity, composition, and nutritional significance of six sorghum cultivars in domestic production were estimated during our research in response to environmental factors and nutrient supplementation. Among the factors, the effect of weather and genotype were statistically significant, while nutrient supplementation resulted in only small or neglectable differences. Among the cultivars, the strongest effects of different years were observed for the cultivars Alföldi1 and Farmsugro 180. For Alföldi1, total polyphenol concentrations ranged from 945-1802 mg.100 g⁻¹ GAE over the 3 years. For Farmsugro 180 this value ranged from 73.9 to 174.10 mg.100 g⁻¹ GAE.
2. Condensed tannin contents of some of the red varieties showed similar tendencies to the polyphenol content. During the 3 years studied, an increase in tannin content was observed in the third year, with values of 47.4 and 8.81 mg.g⁻¹ in case of Alföldi1 and Zádor, which correlated with the extreme weather conditions experienced in that year. It can therefore be concluded that the polyphenol content of sorghum is essentially determined by the growing and weather conditions of plant production. This may play an important role in the later use of product, as they are not only important anti-nutritive compounds, but also potent antioxidants.
3. Literature has suggested that sorghum may contain up to 50% polyphenolic compounds in ester-bound, including tannins and other flavonoids, in forms that can only be obtained by hydrolysis. This proportion was significantly lower for the studied varieties, with the varieties with proven high polyphenol content reaching a maximum concentration of 100 mg.100 g⁻¹ GAE after the applied extraction. Furthermore, the literature also described a condensed tannin content in bound form, whereas the studied genotypes did not contain bound tannin compounds.
4. We investigated the solvent and polarity dependence of extraction efficiency on the polyphenolic components of sorghum by testing two solvents: acetone, and methanol. The results demonstrated that the polyphenolic components of sorghum can be extracted more efficiently with acetone compared to methanol, especially for flavonoid-type compounds, where the amount of flavonoids extracted with methanol and acetone is 620 and 980 mg.CE 100 g⁻¹ for Zádor, while in the case of Alföldi1 the values reached 1200 and 1500 mg.CE 100 g⁻¹.
5. Our measurements revealed the flavonoid profile of 1-1 red (Zádor) and white (Albita) sorghum cultivars and the detectable isomers of each major component. Based on our

analyses, the main flavonoids of Zádor include the different procyanidin A, B, and C isomers, as well as apigeninidin, luteolinidin and their methyl derivatives, which are only among the deoxyanthocyanidins found in sorghum.

6. In my research, I developed sorghum-based breads enriched with psyllium husk flour, the technology of which was supplemented with thermal pre-treatment (dry heat) to improve the structural properties and texture of the raw material and the finished product. The combination of heat treatment and high-fibre psyllium husk flour significantly improved the physical and mechanical properties of the flours. The sorghum flour is more efficient at absorbing water (130% WAC) compared to refined wheat flour (76% WAC), which was more than doubled by the addition of psyllium husk flour (290% WAC, 10% psyllium husk). The heat treatment improved the water absorption slightly (150% WAC), which was partly due to the amount of water evaporated during the treatment.
7. The combination of psyllium husk flour addition and dry heat treatment positively influenced the physical properties, texture and consistency of the sorghum-based breads. The elasticity of high fibre breads showed a 20% increase in both treated and untreated products compared to the control product. The hardness of the heat-treated control products increased by four times compared to untreated control bread, while no change was observed for the fibre-enriched breads. This has resulted in more acceptable sensory properties for consumers in heat-treated control bread. The fibre content added to the products helped to preserve the favourable organoleptic properties of the breads during the storage experiment. After a short storage period, the hardness of the breads increased fourfold in the case of control breads and doubled in the case of fibre-enriched breads.

5 PRACTICAL RESULTS

1. We have confirmed during our investigation, that Zádor and Alföldi1 red sorghum varieties are excellent source of polyphenols depending on the used extraction techniques. Furthermore, environmental conditions and water supply have great influence on the accumulation on these compounds. Knowing this, farmers could be able to improve on the quality of the harvest. In case of Alföldi1 total polyphenol content increased two-fold in the second and third year compared to the more balanced first one.
2. Acetone was significantly more effective in the extraction of flavonoids compared to methanol, which could be used later in other plant sample matrixes to extract polyphenols and flavonoids more efficiently.

3. The developed sorghum based bread supplemented with fibre can be a stable base for a new group of products aimed at people with celiac disease, which is not only gluten free, but also a rich source of bioactive compounds, fibre and minerals. The developed test products achieved promising results in fulfilling customer demands, with breads supplemented with 7.5 and 10% fibre achieving the highest scores.

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7 PUBLICATIONS



Registry number: DEENK/443/2024.PL
Subject: PhD Publication List

Candidate: Róbert Nagy
Doctoral School: Doctoral School of Nutrition and Food Sciences
MTMT ID: 10078220

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (1)

1. **Nagy, R.**, Szöllősi, E., Bíró Molnár, P., Murányi, E., Czibalmos, R., Sipos, P.: Condensed tannin content and antioxidant activity of Hungarian sorghum varieties grown at Research Institute in Karcag.
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Foreign language scientific articles in international journals (4)

2. **Nagy, R.**, Kun-Nemes, A., Szöllősi, E., Bíró Molnár, P., Cziáky, Z., Murányi, E., Sipos, P., Gálné Remenyik, J.: Physiological potential of different Sorghum bicolor varieties depending on their bioactive characteristics and antioxidant potential as well as different extraction methods.
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IF: 3.4 (2023)
3. **Nagy, R.**, Murányi, E., Bíró Molnár, P., Szepesi, J., Győri, Z., Veres, S., Gálné Remenyik, J., Sipos, P.: Assessment of Bioactive Profile of Sorghum Brans under the Effect of Growing Conditions and Nitrogen Fertilization.
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Foreign language abstracts (3)

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List of other publications

Foreign language scientific articles in Hungarian journals (1)

9. Törös, G., Prokisch, J., Peles, F., **Nagy, R.**, Nagy, J., Béni, Á.: The effect of sous-vide cooking on the antioxidant properties of oyster mushroom (*Pleurotus ostreatus* L.).
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10. Khalfalla, M., Zsombik, L., **Nagy, R.**, Győri, Z.: Promoting the elemental profile of sorghum grain: Driving factors affecting nutritional properties under nitrogen fertilizer conditions.
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IF: 3.4 (2023)





Foreign language abstracts (1)

11. Törös, G., Béni, Á., Nagy, J., **Nagy, R.**, Peles, F., Prokisch, J.: Impact of sous-vide cooking on the antioxidant properties and β -glucan content of oyster (*Pleurotus ostreatus* L.) mushroom. In: ISPEC 14th International conference on agriculture, animal science & rural development: Proceedings book (Abstracts & Full Texts) / eds Banu Yücel, Seyithan Seydoşođlu, IKSAD Publishing House, Golbasi, 54-55, 2024. ISBN: 9786257720991

Total IF of journals (all publications): 14,052

Total IF of journals (publications related to the dissertation): 10,652

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

27 August, 2024

