

### EXTRACTION OF FREE AND BOUND POLYPHENOLS FROM BRANS OF *SORGHUM BICOLOR* USING DIFFERENT SOLVENTS

Róbert Nagy<sup>\*1</sup>, Anna Pál<sup>1</sup>, Eszter Murányi<sup>2</sup>, Judit Remenyik<sup>3</sup>, Péter Sipos<sup>1</sup>

Address(es): Róbert Nagy,

<sup>1</sup> Institute of Nutrition, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, 138 Böszörményi street, 4032 Debrecen, Hajdú-Bihar, Hungary. +36306862687.

<sup>2</sup> Research Institute of Karcag, Hungarian University of Agriculture and Life Sciences, 1 Páter Károly street, 2100 Gödöllő, Pest, Hungary.

<sup>3</sup> Center for Complex Systems and Microbiom Innovations, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, 1 Egyetem square., 4032 Debrecen, Hajdú-Bihar, Hungary.

\*Corresponding author: [nagy.robert@agr.unideb.hu](mailto:nagy.robert@agr.unideb.hu)

<https://doi.org/10.55251/jmbfs.10906>

#### ARTICLE INFO

Received 9. 1. 2024  
Revised 21. 1. 2025  
Accepted 11. 2. 2025  
Published 1. 4. 2025

#### Regular article



#### ABSTRACT

Sorghum is a drought tolerant, gluten-free cereal, which is known for the abundance of polyphenol compounds, including flavonoids. It is proved that these compounds have important health benefits as antioxidants and anti-inflammatory agents. They are found in seed walls in a free form or in a bound, esterified form, which affects their digestion and utilization. The main purpose of this research was to evaluate the ratio of free and bound polyphenols in sorghum brans extracted with different solvents, and see how the extraction step can influence the physiology importance of a sorghum extract. Solvents had significant effects on the polyphenol and flavonoid content of free polyphenol extracts, but there was not any difference in terms of their antioxidant activities. In case of bound fractions from different extractions, there were significant differences in all parameters, except for the flavonoid content. Correlation analysis showed strong connection between parameters, and there were differences in correlation between extraction. Among the two varieties, Alföldi1 had significantly higher content of total phenol, flavonoid and higher values of antioxidant capacity as well compared to Zádor in both extractions. In this research only the effect of the extraction solvent was evaluated, but further investigation is necessary to specify more extraction factors. This research aimed to emphasize the importance of extractant selection in determination of bioactive compounds from plants. Our results highlights that selecting the proper extractant is essential for determination of total polyphenolic content, however, the analysis if antioxidant power is not sensitive for extraction agents.

**Keywords:** sorghum, solvent efficiency, bioactive compounds, antioxidant activities

#### INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a staple food source for nearly 500 million people in arid and semi-arid regions of the world, and it is also an important source of polyphenols, and flavonoids and other bioactive compounds (Girard and Awika 2018; Xiong et al. 2019; Li et al. 2021). It is a unique type of grain with one of the most abundant and diverse polyphenol content among cereals, having several positive effects, thus sorghum consumption can influence human health significantly (Ragaei et al. 2006; Awika 2017; Girard and Awika 2018; Pontieri et al. 2021). Depending on genotype and environmental conditions, sorghum seeds can contain a significant amount of condensed tannins, which are powerful antioxidants against reactive oxygen species (ROS) due to their numerous hydroxyl groups, and they also have antibacterial, anticancer properties, and they are also related to the health of the microbiota in the gut (Taleon et al. 2014; Wu et al. 2016; Amoako and Awika 2016; Rao et al. 2018b; Dykes 2019; Sharma et al. 2019). Meanwhile, other sorghum flavonoids are effective agents against inflammation, chronic diseases and aging processes as well (Awika 2011; Xu et al. 2021a). These polyphenols can be found in plant species basically in two forms: free phenols, which are extractable and bound phenols by either ester or covalent bounds to non-starch polysaccharides (Alves et al. 2016; Shen et al. 2018; Rao et al. 2018a; Xiong et al. 2020; Pontieri et al. 2021). While extractable or free phenols and flavonoids are easily available during digestion, until insoluble bound phenols are digested only by the microbiota of the human gut. This also determine their final utilization during digestion.

In the last few years research about sorghum and its biological value increased multiple times as sorghum dishes and products became widespread in several western countries (Rumler et al. 2022; George et al. 2022; Meena et al. 2022). Scientific interest includes the possible biological and industrial utilization of sorghum polyphenols, especially condensed tannins, development of high value gluten free food products, and production of new sorghum breeds/genotype for specific demands and environmental conditions (Girard and Awika 2018; Rashwan et al. 2021; Silva et al. 2021).

Nutrient enrichment and functional food products are considered a fairly popular trends in the food industry nowadays and bioactive compounds such as tannins and flavonoids are the key components to increase the quality of our products (Widowati and Luna 2022; Frankowski et al. 2022; Khalid et al. 2022). Thus, it is increasingly important to maximise the amount of available bioactive compounds for consumption or incorporation into food or medicine.

The chemical structures of polyphenols and other bioactive molecules of a plant sample are crucial factors of the selection of extraction method. One key property is the polarity of a chemical compound, which is attributed to the electron-negativity of the building molecules. It determines the inter- and intramolecular interactions, reactivity and solubility of the compounds, and they are important factors during extraction. Choosing an appropriate extraction solvent for extraction is the first step for making plant extracts with specific biological value. Other parameters, such as temperature, pH, solvent type and composition are key regulators of extraction efficiency and selectivity (Tang et al. 2016; Alves et al. 2016; Wang et al. 2019; Luzardo-Ocampo et al. 2020). According to previous literature, the most frequent solvents used for extraction of polyphenols from plant materials are the following: methanol/ethanol, acetone, water, diethyl ether or ethyl acetate (Rodríguez et al. 2000). Methanol and acetone are common solvents of analytical extraction, and while methanol is more effective to obtain a higher level of polyphenols and extraction of anthocyanins, acetone is highly selective for specific subclasses of polyphenols, for example flavonoids and tannins (Downey and Hanlin 2010; Rao et al. 2018a; Das et al. 2020; Xu et al. 2021a).

In this study our aim was to evaluate the ratio of extractable and bound phenols in different polyphenol-rich sorghum varieties, as well as to evaluate the effect of the applied extraction method during sample preparation in terms of solvent type and ratios. The final aim was to determine if there is any statistical difference between different extraction methods and to obtain some data about the bioactive profile of sorghum varieties.

## MATERIAL AND METHODS

### Experimental design

Sorghum grains were acquired from the Research Institute of Karcag (47°17'27.2"N 20°53'27.8"E), Hungary in 2020. Two red varieties of sorghum bred in Hungary were included in this research, named "Zádor" and "Alföldi" with higher expected polyphenol contents. Grains were grown in a randomized design small plot field experiment. Seeds included in this work was not treated with any supplementation. Grains were cleaned and dehulled with a SATAKE Stone Peeler at maximum power for 50 seconds. Sorghum bran was used for further evaluation and they were stored in plastic bags at -20 °C until further analysis.

### Chemical reagents

Folin-Ciocalteu reagent, sodium-carbonate, hydrochloric acid, ethyl-acetate, sodium-hydroxyde, diethyl-ether, acetone and methanol were sourced from VWR International (Debrecen, Hungary). Gallic acid standard, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, DPPH (1,1-diphenyl-2-picrylhydrazyl), vanillin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and catechin hydrate were bought from Merck (Budapest, Hungary). All chemicals and reagents used in this experiment were at least analytical grade and met quality standards.

### Extraction of free polyphenols from sorghum bran

After homogenization, 5 g bran was weighted on an analytical scale and 50 ml methanol-distilled water mixture (80:20 % by volume) or acetone-distilled water (70:30 % by volume) was added. The mixture was shaken thoroughly and it was put into an ultrasonic water bath (25 °C, W) for 30 minutes. Extracts were centrifuged (EBA-21, Hettich Lab, Tuttingen, Germany) at a maximum of 4020 x g for 10 minutes and the supernatant was decanted. The residues were extracted again twice with the same solvent. After extraction the supernatants were pooled and evaporated until dryness by a rotary evaporator (Hei-VAP, Heidolph Instruments, Schwabach, Germany). Samples were reconstituted in 10 ml methanol-distilled water (80:20 % by volume) in both extraction methods for later analysis, and stored at -20 °C until evaluation. The weight of the dried samples was noted. The remaining residues were put into a drying oven and they were dried until weight equilibrium at a moderate temperature of 40 °C for 24h. All samples were stored at -20 °C until further analysis.

### Acid hydrolysis and extraction of bound polyphenols from sorghum bran

For the extraction of bound phenols the dried residues of previous extractions were used. Extraction of bound phenols were done by the method of Wang et al. (2019) 1 g of sample was weighted on an analytical scale and 10 ml 2M HCl solution was added in a 50 ml centrifuge tube (Wang et al. 2019). Samples were incubated in a shaking water bath at 85 °C for 1 hour. After hydrolysis the samples were filtrated using a filtration paper and pH was set to pH=2 using NaOH solution. Hydrolysed samples were extracted using a separation funnel with 15 ml diethyl-ether: ethyl-acetate (1:1 % by volume) solvent four times and extracts were pooled in centrifuge tubes. After extraction samples were evaporated until dryness by a rotary evaporator, and reconstituted in 10 ml methanol-distilled water (80:20 v/v%), and stored at -20 °C until evaluation.

### Measurement of total polyphenol content and total flavonoid content of methanol and acetone extracts of sorghum

The Folin-Ciocalteu method was used for total phenol content measurement, according to Singleton and Rossi (Singleton and Rossi 1965). 0.5 ml adequately diluted extract was added to a plastic chemical tube, with 2.5 ml Folin-Ciocalteu reagent added, and after 6 minutes, 2 ml sodium-carbonate (7 % m/V) was added and, after a 2h incubation at room temperature in the dark absorbance values were measured at 765 nm. Absorbance values were taken using a two channel spectrophotometer in the UV-VIS range (Perkin Elmer Lambda 2S) with cuvettes. Gallic acid was used as a standard and results were given in milligram per 100 g gallic acid equivalent (GAE).

For total flavonoid content evaluation, the aluminium-chloride method was used according to Zhishen et al. (1999) with some modification (Zhishen et al. 1999). Extracts prepared for TPC measurement were used for this measurement too. 4 ml distilled water and 1 ml of extract or standard was added to each plastic chemical tube, then 0.3 ml sodium-nitrite was added. After 5 minutes incubation 0.3 ml aluminium-chloride was added to the tubes, and after 1 minute 2 ml of sodium-hydroxid was pipetted to the tubes. Solutions were supplemented up to 10 ml with distilled water and absorbance values were taken immediately against distilled water at 510 nm. Catechin was used for standard calibration curve, which was prepared using a 1 mg ml<sup>-1</sup> stock solution. All values were given as dry matter.

### Measurement of condensed tannin content of methanol and acetone extracts of sorghum bran

For measuring condensed tannin (CT) content of sorghum brans vanillin-HCL method was used according to Price et al. (1978) with some modifications (Price et al. 1978; Nemes et al. 2018). The previously described extracts were used after proper dilution. 100 µl sample/methanol/standard, 2000 µl vanillin (4 % m/V) and 1000 µl cc. HCl were pooled together and after 15 minutes incubation absorbance values were measured at 500 nm against methanol blank solution. Catechin was used as standard for calibration curve. 1 mg ml<sup>-1</sup> stock solution was prepared and diluted for twofold calibration curve, values were given as mg g<sup>-1</sup> dry weight values.

### Measurement of antioxidant properties of methanol and acetone extracts of sorghum bran

DPPH and TEAC antioxidant assays were used to measure the antioxidant effects of sorghum extracts according to the methods of Zhu et al. (2009) and Blois et al. (1958) modified by Nemes et al. (2018) (Blois 1958; Nemes et al. 2018). For DPPH assay DPPH reagent (9 % m/V) was prepared on day of measurements and 100 µl sample/methanol/standard, 1400 µl methanol, and 1500 µl DPPH reagent was pooled together and absorbance values were measured at 517 nm after 30 minutes incubation. For TEAC assay ABTS reagent was prepared on the day before analysis using ABTS radical and potassium-persulfate. For analysis 100 µl sample/distilled water/standard, 900 µl ABTS solution, and 1000 µl distilled water were pooled together, and after 20 minutes incubation absorbance values were taken at 734 nm. Trolox calibration curve was prepared using 1mg ml<sup>-1</sup> stock solution, which was diluted to twofold dilution series. All values were given as µmol Trolox equivalent g<sup>-1</sup> dry weight values, using Trolox standard stock solution (1 mg g<sup>-1</sup>) for calibration curve.

### Statistical analysis

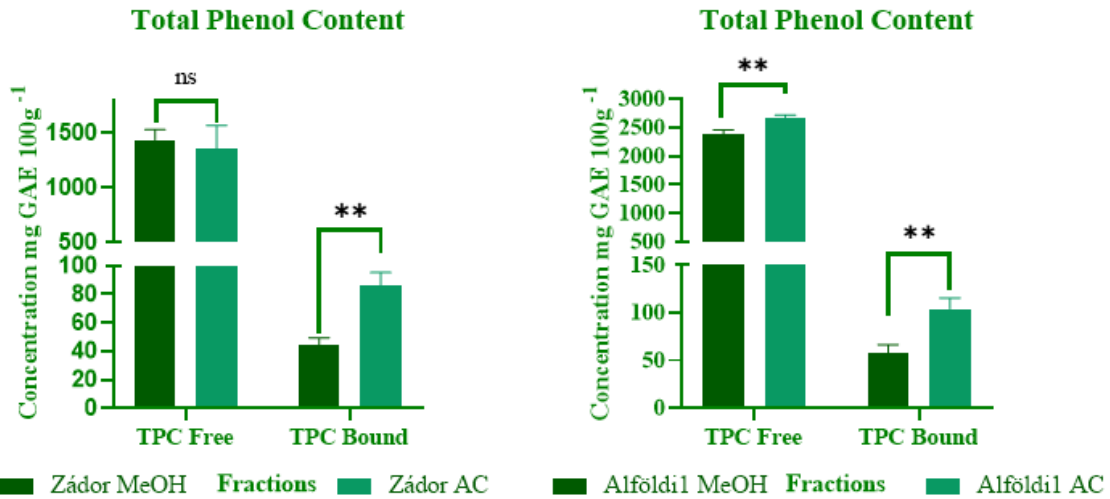
The effect of extraction methods was analysed using GraphPad Prism 8 software. Independent sample T tests were applied to evaluate the effect of extraction solvents on measured variables in case of freely extractable and bound polyphenols. The effect of variety was not included as we already knew there are significant differences between varieties from our previous research (Nagy et al. 2023). Correlation analysis was performed to evaluate the connections between chemical parameters and antioxidant activity values after different extractants using SPSS Statistic Software version 24.

## RESULTS AND DISCUSSION

### Total Phenol and Total Flavonoid content of methanol and acetone extracts

For this research the extraction efficiency of methanol and acetone, two commonly used extraction solvents were compared using two polyphenol rich red sorghum genotypes. The varieties were evaluated for their free and bound phenol and flavonoid contents which can be seen at the following figures below of Figure 1. and Figure 2.

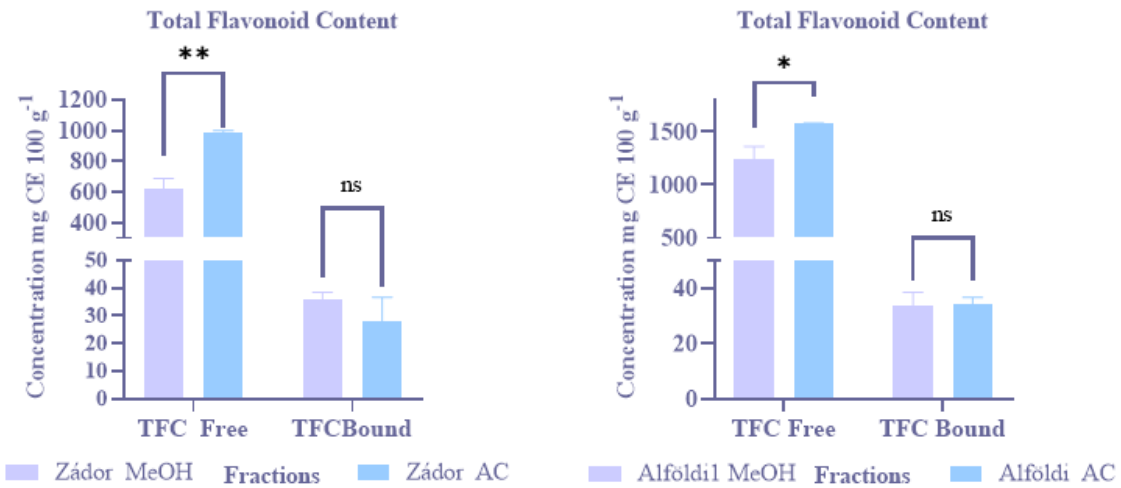
Several previous publications have provided evidence that sorghum can be a rich source of bound polyphenols which only will be fermented by colon bacteria. Depending on genotype, 40% of total phenols and as much as 50% of total flavonoids can be found in an esterified form (Shen et al. 2018; Miafo et al. 2020). As it can be seen at Figure 1., despite these evidences, our samples showed low values of bound phenols and flavonoids in a total 2-5 % of the measured total amounts, which can be attributed to genetic and environmental variables, and to the applied extraction procedure. In most cases, extraction solvents used in this project had significant differences between acetone and methanol extracts for both free and bound polyphenol fractions ( $p < 0.01$ ). There was no significant difference in the case of Zádor free phenol extracts. Alföldi had the highest polyphenol content in both free and bound fractions with 2667±4 mg GAE 100 g<sup>-1</sup> and 104.1 ±11.5 mg GAE 100 g<sup>-1</sup>, and acetone proved to be a more efficient solvent to extract polyphenols for this genotype and conditions ( $p < 0.01$ ). Several previous research measured high levels of polyphenol content of red-brownish sorghum varieties with values of 4000-6000 mg GAE g<sup>-1</sup> TPC concentrations (Salazar-López et al. 2018; Xu et al. 2021b). Shen et al. (2018) analysed several differently coloured sorghum grains for their free and bound phenol content. They found bound phenols in the range of 3.4-194.5 mg 100 g<sup>-1</sup> from white to red or black varieties with free extractable polyphenols ranging from 171 mg GAE 100 g<sup>-1</sup> to 1051 mg GAE 100 g<sup>-1</sup>, respectively (Shen et al. 2018). While Miafo et al. (2019, 2020) found in their continuous research that that dietary fiber isolated from sorghum bran contains different amount of esterified phenols with values of 93-1183 mg GAE100 g<sup>-1</sup>, concentration depending on the extractability of fibers. Meanwhile, whole grains contained around 20 mg FAE 100 g<sup>-1</sup> bound and 290 mg FAE 100 g<sup>-1</sup> freely extractable phenols (Miafo et al. 2019, 2020).



**Figure 1** Total Phenol Content of two red sorghum variety extracted by two different extraction solvents. Notes: MeOH =methanol, AC= acetone n.s.= not significant,  $p>0.05$ , \* and \*\* means statistically significant differences at  $p<0.05$  and  $0.01$ , respectively

Total flavonoid content of evaluated samples can be seen at Figure 2. below. In case of total flavonoid content, statistical differences were found only between the free flavonoid extracts ( $p<0.05$ , and  $p<0.01$ ). Alföldi had the highest flavonoid content with  $1574.9\pm 4.1$  mg CE  $100\text{ g}^{-1}$  concentration, and there were high variations between methanol and acetone extracts. There were no statistically meaningful differences in case of bound flavonoids in case of extraction solvent. Also values were similar to each other for both varieties. Shen et. al. (2018) found that sorghum varieties contained from  $7.11$  to  $194$  mg rutin equivalent  $100\text{ g}^{-1}$

flavonoids depending on genotype (Shen et al. 2018). These values are much lower compared to values measured in this project, but this can be attributed to the sample matrix used for the evaluation. In our research only the bran fraction was evaluated, which is the main place where flavonoids are accumulating, thus high level of flavonoids were expected. All values are given as amount measured in the bran fraction. Furthermore, in our study we used catechin as standard instead of rutin which also influences the final result.

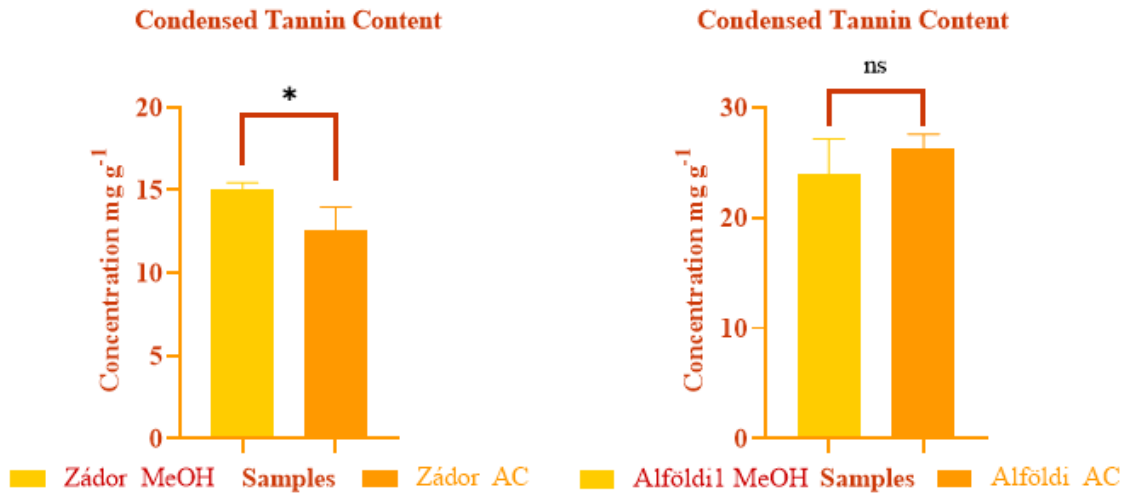


**Figure 2** Total Flavonoid Content of two red sorghum variety extracted by two different extraction solvents. Notes: MeOH =methanol, AC= acetone, n.s.= not significant,  $p>0.05$ , \* and \*\* means statistically significant differences at  $p<0.05$  and  $0.01$ , respectively.

**Condensed tannin content of methanol and acetone extracts**

Condensed tannins (CT) were found only in the freely extractable fraction, and there was not any detectable amount in case of bound fractions as it can be seen at Figure 3. This was against to previous findings described by Terril et. al. (1992) presenting freely extractable and protein or fiber bound CTs in sorghum, which suggests either these sorghum genotypes are not containing protein and fiber bound CTs, or the applied extraction method was not appropriate to extract them (Terrill et al. 1992). In our extraction tests, Zádor showed only significant ( $p<0.05$ )

differences between tannin contents of different extracts, which may be caused by the uncertainty of the applied method as the Alföldi had not have this difference. Measured CT contents were fairly higher compared to our previous research with  $15.9\pm 0.4$ ,  $26.2\pm 1.3$ , and  $5.6\pm 0.6$ ,  $14.8\pm 0.8$  mg  $\text{g}^{-1}$  concentration values, respectively (Nagy et al. 2023). These differences can be caused by the differences between the applied preparation methods, where for the first time a onetime methanol extraction was used, here we changed the polarity of the solvents and used a three-time extraction repetition step as well, increasing the efficiency of our methods (Nagy et al. 2023).

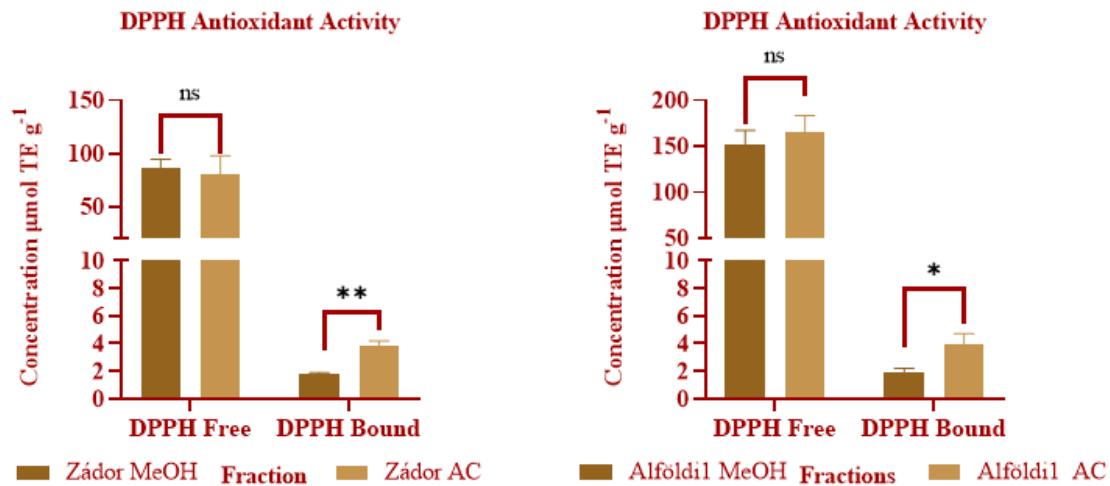


**Figure 3** Condensed of two red sorghum variety extracted by two different extraction solvents. Notes: MeOH =methanol, AC= acetone, \* means statistically significant differences at  $p < 0.05$  and n.s.= not significant,  $p > 0.05$ , respectively.

**Antioxidant properties of methanol and acetone extracts**

To measure the antioxidant properties of the polyphenol rich sorghum extracts two antioxidant assay methods (TEAC, DPPH), appropriate for polyphenolic compounds, were used. Measured values can be seen at Figure 4. and Figure 5.

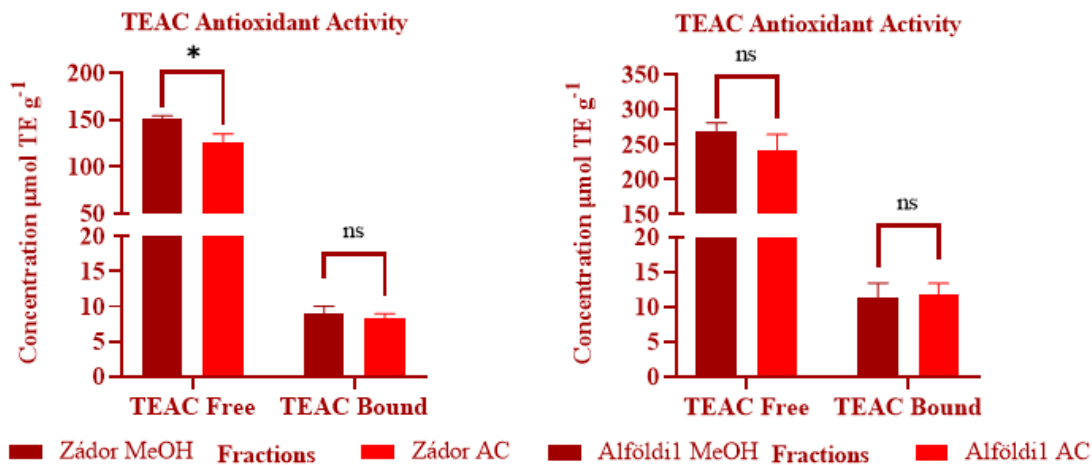
Interestingly, the significant changes between extracts experienced previously were not detectable for the measured antioxidant values. For DPPH only the bound fractions showed any significant discrepancy ( $p < 0.05$ , and  $p < 0.01$ ) between extraction methods, while acetone and methanol extracts of freely accessible bioactive compounds had similar values.



**Figure 4** Antioxidant capacities measured by DPPH assay of two red sorghum variety extracted by two different extraction solvents. Notes: MeOH =methanol, AC= acetone, \*, \*\* means statistically significant differences at  $p < 0.05$ , and  $0.01$ . n.s.= not significant,  $p > 0.05$

In our previous research the measured DPPH values from 100 % methanol extracts were slightly higher compared to TEAC antioxidant capacities (Nagy et al. 2023). Meanwhile in this study TEAC values were significantly higher for both extracts compared to DPPH values with 125-150 μmol TE g<sup>-1</sup>, and 241-269 μmol TE g<sup>-1</sup> concentration values against 80-86 μmol TE g<sup>-1</sup>, and 151-165 μmol TE g<sup>-1</sup> concentration values for DPPH. Again, the different solvents and polarity of solvents can explain these experienced differences in measured values. Antioxidant properties of bound fractions were negligible compared to the free extracts with a maximum of 10 μmol TE g<sup>-1</sup> concentration values. The lack of differences between antioxidant properties of two different extracts can be attributed to the different composition of the aforementioned extracts, which affects the antioxidant effects of the extracts. Luo et. al (2018) compared ultrasound assisted extraction with solvent extraction and they have found

significant differences in composition in terms of extracted amount of specific compounds. This resulted in significant differences between extracts (Luo et al. 2018). Xiong et al. (2021) found similar tendencies to our results with several sorghum varieties where they measured the antioxidant properties of free and bound phenolic extracts of sorghum bran and whole kernel. They experienced higher values for antioxidant effects measured by TEAC compared to DPPH with a two-fold difference between different assays. The ratio of bound phenol antioxidant properties were fairly low compared to free phenol extracts (Xiong et al. 2021). Similar tendencies could be observed for different plant extracts as well in many literature data (Venkatesan et al. 2019; Ghazzawi et al. 2021; El Mannoubi 2023).



**Figure 5** Antioxidant capacities measured by TEAC assay of two red sorghum variety extracted by two different extraction solvents.  
 Notes: MeOH =methanol, AC= acetone, n.s.= not significant,  $p>0.05$ , \*

**Correlation analysis of antioxidant capacities and bioactive profile**

A correlation analysis was done between free and bound polyphenol, flavonoid, and antioxidant contents to evaluate if there is any connection between the experienced differences in antioxidant power and content. Pearson correlation values for free and bound extracts can be observed at Table 1., and Table 2. below.

**Table 1** Pearson correlation analysis of measured parameters for free compounds extracted by two solvents

Methanol					
	TPC	DPPH	TFC	TEAC	CTC
TPC	1	0.940**	0.984**	0.979**	0.909*
DPPH		1	0.964**	0.972**	0.760
TFC			1	0.966**	0.834*
TEAC				1	0.890*
CTC					1
Acetone					
	TPC	DPPH	TFC	TEAC	CTC
TPC	1	0.955**	0.977**	0.955**	0.983**
DPPH		1	0.955**	0.947**	0.914*
TFC			1	0.970**	0.985**
TEAC				1	0.962**
CTC					1

\*= $p<0.05$ , \*\*= $<0.01$

In case of free methanol and acetone extracts strong positive correlations could be observed between measured parameters ( $p<0.05$ , and  $0.01$ ), All parameters correlated stronger for acetone extracts compared to methanol solvent, especially between DPPH, TFC, and CTC content. This suggests acetone was more suitable to extract bioactive compounds with higher antioxidant properties from sorghum. This can be also linked to structural differences and molecule size as there was no significant difference between total condensed tannin content of extracts except for Zádor at  $p<0.05$ . Further HPLC analysis is required to confirm this hypothesis. As for bound polyphenols, correlations between parameters were not significant in most cases, and negative correlation could be observed for acetone extracts, although without statistical significance (Table 2.). There were no clear tendencies between them, which further proved that evaluated varieties did not contain relevant amount of polyphenols using the evaluated extracts.

**Table 2** Pearson correlation analysis of measured parameters for bound compounds extracted by two solvents

Methanol					
	TPC	DPPH	TFC	TEAC	CTC
TPC	1	0.251	0.745	0.970**	
DPPH		1	0.528	0.372	
TFC			1	0.698	
TEAC				1	
CTC					1
Acetone					
	TPC	DPPH	TFC	TEAC	CTC
TPC	1	0.604	0.016	0.451	
DPPH		1	-	0.633	
TFC			1	0.122	
TEAC				1	-0.164
CTC					1

\*\*= $<0.01$

**CONCLUSION**

In this study polyphenol rich sorghum varieties were evaluated for their polyphenol distribution between free and bound forms of phenols with the influence of two different extraction solvents applied during sample preparation. Overall the bound polyphenol content of the analysed varieties was relatively low compared to other literature, but there were significant differences between extraction methods for both free and bound polyphenol content, and free total flavonoid content as well. It was discussed by several earlier publications that acetone is a more appropriate solvent to extract flavonoid type compounds like tannins or flavanones. We confirmed sorghum flavonoids are also more readily available with acetone compared to methanol ( $p<0.05$ ,  $p<0.01$ ). There were evidences from the measured antioxidant properties that the two extracts had different polyphenol composition, which would be worth to further investigate with HPLC technics. Overall, two polyphenol rich sorghum genotypes were identified with the potential to use as a functional food ingredient, and there were significant differences between extraction methods. This is important because the utilization of plant based extracts is an upcoming trend both in science and nutrition. Plants are usually rich source of polyphenols, flavonoids, and other bioactive compounds with strong antioxidant properties. It is necessary to know how these compounds can be extracted from the complex plant matrix effectively. We found significant differences between two commonly used extractants in terms of extractable polyphenols and flavonoids. However, these solvents are not suitable for human consumption, thus in the future other, more compatible solvents should be evaluated for producing food grade extracts. Furthermore, both of the evaluated varieties contained a high amount of freely extractable polyphenols with high antioxidant properties, but the amount of bound compounds were considerably lower. Further investigation is needed to justify these findings, and to investigate how different solvents and sample preparation methods influence the composition of a specific plant extract, thus influencing their biological effects as well. In case of our research it turned out, that red sorghum extracts contain high amount of polyphenol type compounds, and flavonoids were its major constituents. Among them condensed tannins were one of the more important subgroup. As these condensed tannins have anti-nutrition effects as well, their digestion and absorption in human nutrition would be important to evaluate and, if it is justified, their intake should be regulated.

**Acknowledgments:** We thank the Research Institute of Karcag for providing the samples for this research. Also many thanks to Dr. József Prokisch for providing equipment and space to conduct the experiments.

**Funding:** This project was made with the support of the New National Excellence Program of Ministry of Culture and Innovation, code number ÚNKP–22–3-II-DE-225, financed from the national research, development, and innovation fund.

**Authors contribution:** R. Nagy did the conceptualization, carried out the experiment, data analysis, wrote, and revised the manuscript. A. Pál also carried out experiments, and data analysis. E. Murányi provided the samples from the plot field experiment. J. Remenyik and P. Sipos supported the conceptualization, and managed the resources needed for the experiment. All authors took part in the preparation and revision of the manuscript, and in the visualization of data.

**REFERENCES**

Alves G.H., Ferreira C.D., Vivian P.G., Monks J. L. F., Elias M. C., Vanier N. L., Oliveira M. (2016) The revisited levels of free and bound phenolics in rice: Effects of the extraction procedure. *Food Chemistry* 208:116–123. <https://doi.org/10.1016/j.foodchem.2016.03.107>

- Amoako D.B., Awika J.M. (2016) Polymeric tannins significantly alter properties and in vitro digestibility of partially gelatinized intact starch granule. *Food Chemistry* 208:10–17. <https://doi.org/10.1016/j.foodchem.2016.03.096>
- Awika J. (2011) Sorghum Flavonoids: Unusual Compounds with Promising Implications for Health. In: *ACS Symposium Series*. pp 171–200
- Awika J.M. (2017) Chapter 3 - Sorghum: Its Unique Nutritional and Health-Promoting Attributes. In: Taylor J.R.N., Awika J.M. (eds) *Gluten-Free Ancient Grains*. Woodhead Publishing, pp 21–54
- Blois M.S. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* 181:1199–1200. <https://doi.org/10.1038/1811199a0>
- Das A.K., Islam Md.N., Faruk Md.O., Ashaduzzaman Md. (2020) Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany* 135:58–70. <https://doi.org/10.1016/j.sajb.2020.08.008>
- Downey M.O., Hanlin R.L. (2010) Comparison of Ethanol and Acetone Mixtures for Extraction of Condensed Tannin from Grape Skin. *J Enol Vitic* 31: <https://doi.org/10.21548/31-2-1412>
- Dykes L. (2019) Sorghum Phytochemicals and Their Potential Impact on Human Health. *Methods Mol Biol* 1931:121–140. [https://doi.org/10.1007/978-1-4939-9039-9\\_9](https://doi.org/10.1007/978-1-4939-9039-9_9)
- El Mannoubi I. (2023) Impact of different solvents on extraction yield, phenolic composition, in vitro antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit. *JUmm Al-Qura Univ Appl Sci* 9:176–184. <https://doi.org/10.1007/s43994-023-00031-y>
- Frankowski J., Przybylska-Balcerek A., Stuper-Szablewska K. (2022) Concentration of Pro-Health Compound of Sorghum Grain-Based Foods. *Foods* 11:216. <https://doi.org/10.3390/foods11020216>
- George T.T., Obilana A.O., Oyenihni A.B., Obilana A. B., Akamo D.O., Awika J. M. (2022) Trends and progress in sorghum research over two decades, and implications for global food security. *South African Journal of Botany* 151:960–969. <https://doi.org/10.1016/j.sajb.2022.11.025>
- Ghazzawi H.A., Al-Sayyed H.F., Al-Kurd R.A., Mwalla M. M., Arafat T.A., AbdelQader S.M. (2021) Effect of different extraction solvents on the antioxidant content and capacity of nine seasonal fruits. *Clinical Nutrition Open Science* 38:33–42. <https://doi.org/10.1016/j.nutos.2021.06.003>
- Girard A.L., Awika J.M. (2018) Sorghum polyphenols and other bioactive components as functional and health promoting food ingredients. *Journal of Cereal Science* 84:112–124. <https://doi.org/10.1016/j.jcs.2018.10.009>
- Khalid W., Ali A., Arshad M.S., Afzal F., Akram R. (2022) 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* 25:1045–1062. <https://doi.org/10.1080/10942912.2022.2071293>
- Li Z., Zhao X., Zhang X., Liu H. (2021) Bioactive Compounds and Biological Activities of Sorghum Grains. *Foods* 10:2868. <https://doi.org/10.3390/foods10112868>
- Luo X., Cui J., Zhang H., Duan Y., Zhang D., Cai M., Chen G. (2018) Ultrasound assisted extraction of polyphenolic compounds from red sorghum (*Sorghum bicolor* L.) bran and their biological activities and polyphenolic compositions. *Industrial Crops and Products* 112:296–304. <https://doi.org/10.1016/j.indcrop.2017.12.019>
- Luzardo-Ocampo I., Ramírez-Jiménez A.K., Cabrera-Ramírez Á.H., Rodríguez-Castillo N., Campos-Vega R., Loarca-Piña G., Gaytán-Martínez M. (2020) Impact of cooking and nixtamalization on the bioaccessibility and antioxidant capacity of phenolic compounds from two sorghum varieties. *Food Chemistry* 309:125684. <https://doi.org/10.1016/j.foodchem.2019.125684>
- Meena K., Visarada K., Meena D.K. (2022) Sorghum bicolor (L.) Moench a multifarious crop -fodder to therapeutic potential and biotechnological applications: A future food for the millennium. *Future Foods* 6:100188. <https://doi.org/10.1016/j.fufo.2022.100188>
- Miafo A.P.T., Koubala B.B., Kansci G., Muralikrishna G. (2020) Antioxidant properties of free and bound phenolic acids from bran, spent grain, and sorghum seeds. *Cereal Chemistry* 97:1236–1243. <https://doi.org/10.1002/cche.10348>
- Miafo A.P.T., Koubala B.B., Kansci G., Muralikrishna G. (2019) Free sugars and non-starch polysaccharides–phenolic acid complexes from bran, spent grain and sorghum seeds. *Journal of Cereal Science* 87:124–131. <https://doi.org/10.1016/j.jcs.2019.02.002>
- Nagy R., Murányi E., Bíróné Molnár P., Szepesi J., Györi Z., Veres Sz., Remenyik J., Sipos P. (2023) Assessment of Bioactive Profile of Sorghum Brans under the Effect of Growing Conditions and Nitrogen Fertilization. *Agriculture* 13:760. <https://doi.org/10.3390/agriculture13040760>
- Nemes A., Szöllösi E., Stündl L., Bíró A., Homoki J. R., Szarvas M. M., Balogh P., Czíáky Z., Remenyik J. (2018) Determination of Flavonoid and Proanthocyanidin Profile of Hungarian Sour Cherry. *Molecules* 23:3278. <https://doi.org/10.3390/molecules23123278>
- Pontieri P., Pepe G., Campiglia P., Merciai F., Basilicata M. G., Smolensky D., Calcagnile M., Troisi J., Romano R., Del Giudice F., Aletta M., Guida M., Alifano P., Del Giudice L. (2021) Comparison of Content in Phenolic Compounds and Antioxidant Capacity in Grains of White, Red, and Black Sorghum Varieties Grown in the Mediterranean Area. *ACS Food Sci Technol* 1:1109–1119. <https://doi.org/10.1021/acscfoodscitech.1c00115>
- Price M.L., Van Scoyoc S., Butler L.G. (1978) A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J Agric Food Chemistry* 26:1214–1218. <https://doi.org/10.1021/jf60219a031>
- Ragaei S., Abdel-Aal E.S.M., Noaman M. (2006) Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry* 98:32–38. <https://doi.org/10.1016/j.foodchem.2005.04.039>
- Rao S., Santhakumar A.B., Chinkwo K.A., Wu G., Johnson S. K., Blanchard C. L. (2018a) Characterization of phenolic compounds and antioxidant activity in sorghum grains. *Journal of Cereal Science* 84:103–111. <https://doi.org/10.1016/j.jcs.2018.07.013>
- Rao S., Schwarz L.J., Santhakumar A.B., Chinkwo K. A., Blanchard C. L. (2018b) Cereal phenolic contents as affected by variety and environment. *Cereal Chemistry* 95:589–602. <https://doi.org/10.1002/cche.10085>
- Rashwan A.K., Yones H.A., Karim N., Taha E. M., Chen W. (2021) Potential processing technologies for developing sorghum-based food products: An update and comprehensive review. *Trends in Food Science & Technology* 110:168–182. <https://doi.org/10.1016/j.tifs.2021.01.087>
- Rodríguez I., Llompart M.P., Cela R. (2000) Solid-phase extraction of phenols. *Journal of Chromatography A* 885:291–304. [https://doi.org/10.1016/S0021-9673\(00\)00116-3](https://doi.org/10.1016/S0021-9673(00)00116-3)
- Rumler R., Bender D., Schönlechner R. (2022) Sorghum and its potential for the Western diet. *Journal of Cereal Science* 104:103425. <https://doi.org/10.1016/j.jcs.2022.103425>
- Salazar-López N., Aguilar G., Rouzaud-Sáñez O., Sánchez M. (2018) Technologies applied to sorghum (*Sorghum bicolor* L. Moench): changes in phenolic compounds and antioxidant capacity. *Food Science and Technology* 38: <https://doi.org/10.1590/fst.16017>
- Sharma K., Kumar V., Kaur J., Tanwar B., Goyal A., Sharma R., Gat Y., Kumar A. (2019) Health effects, sources, utilization and safety of tannins: a critical review. *Toxin Reviews* 40:1–13. <https://doi.org/10.1080/15569543.2019.1662813>
- Shen S., Huang R., Li C., Wu W., Chen H., Shi J., Chen S., Ye, X. (2018) Phenolic Compositions and Antioxidant Activities Differ Significantly among Sorghum Grains with Different Applications. *Molecules* (Basel, Switzerland) 23: <https://doi.org/10.3390/molecules23051203>
- Silva T.N., Thomas J.B., Dahlberg J., Rhee S. Y., Mortimer J. C. (2021) Progress and challenges in sorghum biotechnology, a multipurpose feedstock for the bioeconomy. *J Exp Bot* 73:646–664. <https://doi.org/10.1093/jxb/erab450>
- Singleton V.L., Rossi J.A. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am J Enol Vitic* 16:144–158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Taleon V., Dykes L., Rooney W.L., Rooney L.W. (2014) Environmental effect on flavonoid concentrations and profiles of red and lemon-yellow sorghum grains. *Journal of Food Composition and Analysis* 34:178–185. <https://doi.org/10.1016/j.jfca.2014.03.003>
- Tang Y., Zhang B., Li X., Chen P. X., Zhang H., Liu R., Tsao R. (2016) Bound Phenolics of Quinoa Seeds Released by Acid, Alkaline, and Enzymatic Treatments and Their Antioxidant and  $\alpha$ -Glucosidase and Pancreatic Lipase Inhibitory Effects. *J Agric Food Chem* 64:1712–1719. <https://doi.org/10.1021/acs.jafc.5b05761>
- Terrill T.H., Rowan A.M., Douglas G.B., Barry T.N. (1992) Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* 58:321–329. <https://doi.org/10.1002/jsfa.2740580306>
- Venkatesan T., Choi Y-W., Kim Y-K. (2019) Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of *Pinus densiflora* Bark Extract. *Biomed Res Int* 2019:3520675. <https://doi.org/10.1155/2019/3520675>
- Wang L., Lin X., Zhang J., Zhang W., Hu X., Li W., Li C., Liu S. (2019) Extraction methods for the releasing of bound phenolics from *Rubus idaeus* L. leaves and seeds. *Industrial Crops and Products* 135:1–9. <https://doi.org/10.1016/j.indcrop.2019.04.003>
- Widowati S., Luna P. (2022) Nutritional and Functional Properties of Sorghum (*Sorghum bicolor* (L.) Moench)-based Products and Potential Valorisation of Sorghum Bran. *IOP Conf Ser: Earth Environ Sci* 1024:012031. <https://doi.org/10.1088/1755-1315/1024/1/012031>
- Wu G., Johnson S.K., Bornman J.F., Benett S. J., Clarke M. W., Singh V., Fang Z. (2016) Growth temperature and genotype both play important roles in sorghum grain phenolic composition. *Sci Rep* 6:21835. <https://doi.org/10.1038/srep21835>
- Xiong Y., Damasceno Teixeira T.V., Zhang P., Warner R. D., Shen S., Fang Z. (2021) Cellular antioxidant activities of phenolic extracts from five sorghum grain genotypes. *Food Bioscience* 41:101068. <https://doi.org/10.1016/j.fbio.2021.101068>
- Xiong Y., Zhang P., Warner R.D., Shen S., Johnson S., Fang Z. (2020) Comprehensive profiling of phenolic compounds by HPLC-DAD-ESI-QTOF-MS/MS to reveal their location and form of presence in different sorghum grain genotypes. *Food Research International* 137:109671. <https://doi.org/10.1016/j.foodres.2020.109671>
- Xiong Y., Zhang P., Warner R.D., Fang Z. (2019) Sorghum Grain: From Genotype, Nutrition, and Phenolic Profile to Its Health Benefits and Food Applications. *Comprehensive Reviews in Food Science and Food Safety* 18:2025–2046. <https://doi.org/10.1111/1541-4337.12506>

- Xu J., Wang W., Zhao Y. (2021a) Phenolic Compounds in Whole Grain Sorghum and Their Health Benefits. *Foods* 10:1921. <https://doi.org/10.3390/foods10081921>
- Xu J., Wang W., Zhao Y. (2021b) Phenolic Compounds in Whole Grain Sorghum and Their Health Benefits. *Foods* 10:1921. <https://doi.org/10.3390/foods10081921>
- Zhishen J., Mengcheng T., Jianming W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64:555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)