

## Condensed tannin content and antioxidant activity of Hungarian sorghum varieties grown at Research Institute in Karcag

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### SUMMARY

*Cereal-based products are one of our main energy sources, and are consumed on a daily basis. One of the weaknesses of wheat based products is their low antioxidant content. Sorghum is a minor cereal, mostly consumed in Africa and Asia. Amongst other phenolic components it contains tannins, which are potent antioxidants and have other positive effect on human health, for example anti-tumor, anti-inflammatory and anti-viral/bacterial effect. We evaluated the tannin content (vanillin-HCL) and antioxidant capacity (DPPH) of sorghum varieties (Alföldi1, Zádor, Foehn, Albita, Albanus) grown in Hungary, with two type of agronomy technology. Red varieties especially Alföldi1 and Zádor had higher tannin contents than white varieties. The highest condensed tannin content was 1470±73 mg 100 g<sup>-1</sup> (Control, Alföldi1), 1810±154 mg 100 g<sup>-1</sup> (Fertilized, Alföldi1), and the highest total antioxidant capacity was 2099±19 mg 100g<sup>-1</sup> (Control, Alföldi1) and 2117±26 mg 100 g<sup>-1</sup> (Fertilized, Alföldi) We found that sorghum type, variety and color influence their tannin and antioxidant contents in general.*

**Keywords:** *Sorghum bicolor, condensed tannins, antioxidants, cereals*

### INTRODUCTION

Cereals are major sources of nutrients in our diet. Besides their high carbohydrate content, which are our main energy source, they contain antioxidants and other biologically active compounds in significant amounts, for example tocopherols, phenolic acids and carotenoids (Akkoc et al., 2019). There are minor cereals, like millets, sorghum or teff which are grown in a smaller amount compared to wheat or maize, and contains these compounds in greater amount, for example sorghum species. Sorghum (*Sorghum bicolor*, L.) is a tropical cereal, which comes from the hot and arid areas of Africa (Potgieter et al., 2016). Sorghum varieties are grown mainly in Africa and Central-Asia as food ingredients. In developed countries, like in the USA it is grown as an industrial plant, or animal feed (Girard et al., 2018). They are accustomed to extreme environmental circumstances and are resistant to drought and heat exposure (Józsa, 1976; Awika et al., 2017).

Antioxidants have important role living organism in the regulation of the level of free radicals. These reactive oxygen species (ROS) and reactive nitrogen species (RNS) can arise during biochemical reactions in the cells, and although they are needed in small amount, they can denature proteins, decrease enzyme activity and cause various chronic diseases in humans as well (cardiovascular diseases, kidney damage) if produced in huge amount (Rasheed et al., 2019; Kunwar et al.; 2011). These antioxidative compounds like phenols, tocopherols, ascorbic acid or flavonoids play great role in the regulation of the amounts of these radicals, thus prevent illnesses related to free radicals.

Sorghum varieties are rich in such phenolic compounds, flavonoids, and minerals (Althwab et al., 2015; Girard et al., 2018; Wu et al., 2017). One of their main phenolic components are tannins., which have potent antioxidant activity, thanks to their many hydroxyl (-OH) groups (Macáková et al., 2014). There are 2 major groups of tannins: hydrolysable tannins and condensed tannins. Hydrolysable tannins are polyols with gallic acid or ellagic acid-esters, while condensed tannins are polyhydroxi-flavan-3-ol oligomers or polymers, also called proanthocyanidins because their depolymerisation yields anthocyanidins (Koleckar et al., 2008; Macáková et al., 2014; Sekowski et al., 2020). Their major characteristics is chelating ability with metals proteins and lipids, thus reduce their bioavailability. Due to this property they are mostly excluded in animal feeding, but they can be used in human food industry as energy reducers. Tannins have many positive effect on human health, they prevent chronic diseases like cardiovascular diseases, or their anti-inflammatory effect, they also have a prooxidative enzyme inhibition effect, for example they inhibit nitric-oxid (NO) synthetase and xanthine oxidase activity. These enzymes are responsible for producing free radicals. (Kolarec et al., 2008; Macáková et al., 2014) The aim of this study was to analyse the antioxidant capacity and condensed tannin contents of 5 red and white sorghum varieties (Albita, Albanus, Alföldi1, Zádor, ES Foehn), and the evaluation of differences amongst the varieties and agronomic treatments.



## MATERIALS AND METHODS

The examined varieties were grown at the Institute for Agricultural Research and Educational Farm of University of Debrecen in Karcag, at the plot B1 (47.28957; 20.88794), in the year 2019. Crops were grown on 4 parcels per variety as replicates. Soil analysis were carried out in an accredited laboratory of Research Institute. The pH of soil was 5.1, its total salt content was 0.02 m/m%, carbonated lime content was less than 0.05 m/m%. Ammonium lactate soluble phosphorus peroxide (P<sub>2</sub>O<sub>5</sub>) and potassium oxide (K<sub>2</sub>O) were 136 and 486 mg kg<sup>-1</sup>, respectively. Nitrate and nitrite derived nitrogen was 6.4 mg kg<sup>-1</sup>. The soil humus content was 3.4 m/m%. Amongst meteorological properties mean precipitation and mean temperature (Table 1) were taken into consideration. There was an increased rainfall in May, compared to other months of the year. During the experiment 5 varieties of sorghum were investigated, white varieties (Albita, Albanus), and red varieties (Alföldi1, ES Foehn and Zádor). They were sown and cultivated in 2019 at 19.1 m<sup>2</sup> parcel size. Beside control (Control), samples of one mineral fertilizer treatment with 60 kg ha<sup>-1</sup> (Fertilized) was evaluated in this study. The fertilizer used was NH<sub>4</sub>NO<sub>3</sub> + CaMg(CO<sub>3</sub>)<sub>2</sub> with 27% nitrogen content. The samples were stored in textile sacks in storage room after harvest.

Table 1. Climatic data of 2019

	Precipitation (mm)	Average temperature (°C)
January	31.2	-1
February	6.2	3.7
March	8.8	9
April	47.3	12.9
May	116.7	14.6
June	65.5	23.1
July	59.5	21.8
August	14.6	23.8
September	40.6	17.2
October	8.4	12.9
November	72.2	9.1
December	34.1	3.1
<b>Total</b>	<b>505.1</b>	
<b>Mean</b>		<b>12.5</b>

### Sampling and measurements

After homogenization, 150–160 g of grains were taken from each sack, and were peeled with a Satake peeler. The peeled kernels were milled with a roller mill. The flour and bran were weighted on an Ohaus analytical scale. Weighted samples were put in an airtight plastic bag and were stored at -20 °C before analysis. The antioxidant capacity, condensed tannin contents and dry matter contents were examined from sorghum brans and flours as a preliminary measurement.

### Determination of dry matter content

Dry matter content was analyzed using a drying oven. Two grams of brans and flours were measured in heat resistant cups and put in oven at 105 °C for 3 hours. Samples were dried until reaching constant mass.

### Determination of antioxidant capacity

For antioxidant measurement DPPH assay was used according to Blois (1958). Spectrophotometry measurement was made by a Spectrostar spectrophotometer. The DPPH is an artificial, purple free radical which is protonated by antioxidants, like tannins and phenols. Two mg of DPPH reagent was solved in 50 ml methanol and stored in a dark bottle until measurement. Standards were made from 1.4 mg ml<sup>-1</sup> Trolox stock solution. Each standard point was made with 2x methanol dilution. Samples were extracted with concentrated methanol (100%), using ultrasonic water bath for 20 min, homogenized and centrifuged at 3000 rpm for 10 min. The supernatant was saved and used later in analysis. Spectrophotometry analysis was concluded on a 96-plate, the necessary reagents were added to the methanol extract and after incubation at 25 °C and for 30 min, measurement was carried out at 517 nm. Results were given on dry matter (DM) base

### Determination of condensed tannin content

Vanillin-HCL assay as colorimetric method was used for the determination of condensed tannin content of flours and brans. It based on the reaction of tannins with vanillin in the presence of acid. This reaction yields a red complex. Vanillin binds to the phenol rings. For standard (+) catechin was used. Vanillin (0.83 g) was solved in 20 ml methanol, standards were prepared from 1.4 mg ml<sup>-1</sup> stock solution, with twofold dilution. Samples were extracted with methanol (100%) using an ultrasonic water bath for 20 min. Samples were centrifuged at 3000 rpm for 10 min, and supernatants were saved. Reagents were added to the extract on a 96-plate and was incubated at 25 °C for 15 min. Samples were analyzed at 500 nm, using a Spectrostar spectrophotometer. Results were given on DM base.

### Statistical analysis

Statistical evaluations were performed by Microsoft Excel. The effect of treatments were evaluated by t probe after the homogeneity analysis of averages by Fisher's test.

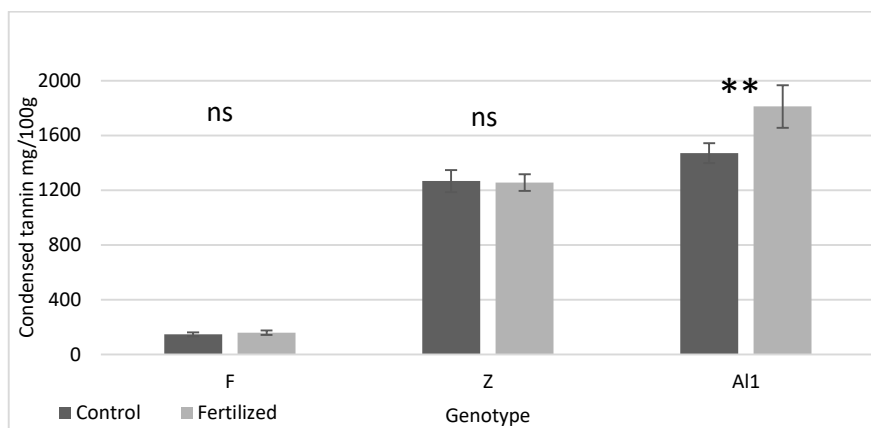
## RESULTS AND DISCUSSION

Figure 1 and Figure 2 show the condensed tannin (CT) contents of white and red sorghum varieties respectively. Statistically significant differences between the control and fertilized samples can be seen only in the case of Alföldi1 red sorghum variety (p<0.01). Among red sorghums Alföldi1 had the highest CT content with 1470±73 mg 100 g<sup>-1</sup> at control, and 1810±154 mg 100 g<sup>-1</sup> for fertilized. Lowest values were found for ES Foehn, CT; they were at 148±14 and

158±15 mg 100 g<sup>-1</sup>. This is lower than values can be seen at white varieties. White varieties showed much lower values of CT content than red hybrids, except of ES Foehn, which showed a very low CT content despite being a red variety. CT contents varied depending on

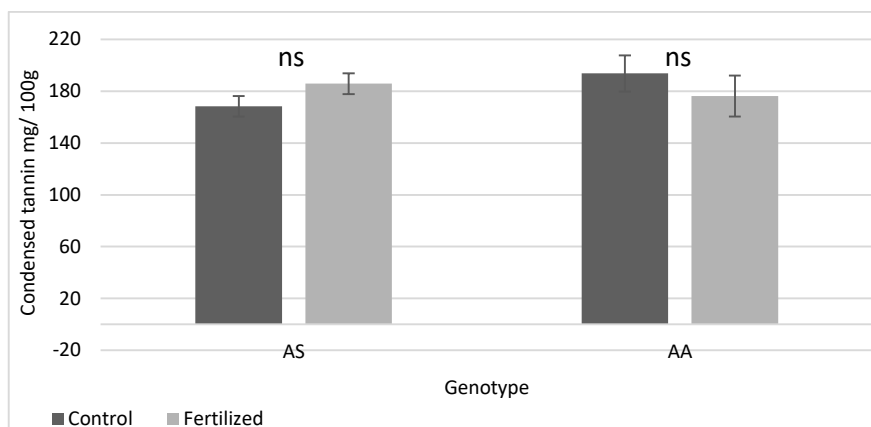
varieties and treatments in the case of white sorghum varieties, as can be seen in *Figure 2*. Albita (control) and Albanus (fertilized) had the highest values, 193±13 and 185.83±8.01 mg 100 g<sup>-1</sup> respectively.

*Figure 1. Condensed tannins values of red sorghum brans*



Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant, \*\*: p≤0.01 (n=3 ±s.e). F= Foehn, Z= Zádor, Al1= Alföldi

*Figure 2. Condensed tannins values of white sorghum brans*



Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant. (n=3 ±s.e) AS=Albanus, AA=Albita

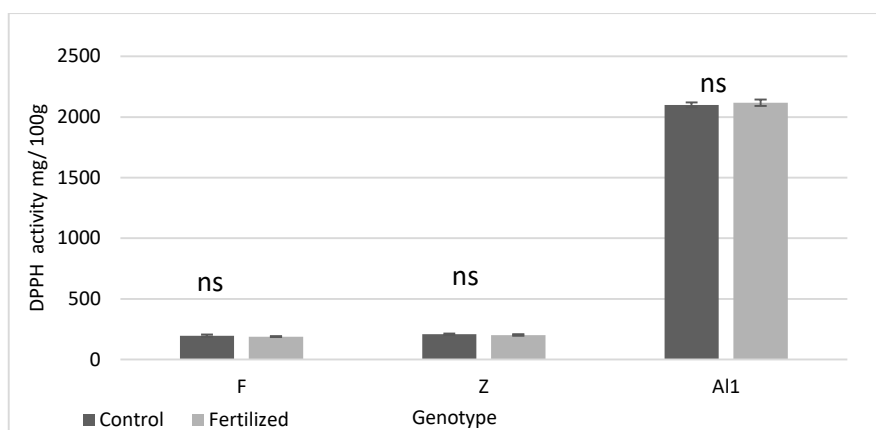
Total antioxidant capacity values did not show significant differences (p<0.05) between treatments, but differed between sorghum varieties, and agronomy treatments (Control, Fertilized) as it is shown in *Figure 3* and *Figure 4*. Red varieties had higher values than white varieties, which can be related with their higher CT contents.

Highest antioxidant capacity was measured in Alföldi sample at 2099±19 and 2117±26 mg 100 g<sup>-1</sup>. Among white varieties Albanus had a higher antioxidant capacity at 183±11 and 166±12 mg 100 g<sup>-1</sup>, and Albita showed low capacity at 17±0.62 and 16±1.12 mg 100 g<sup>-1</sup> respectively.

Brans aside, flours were also examined for CT content and DPPH activity. Except the flour of Albanus, there weren't statistically significant differences between treatments in the examined parameters. Albanus showed great difference at DPPH values for Control and Fertilized. Antioxidant capacity of Albanus flours were 31.94±4.16 and 4.37±0.71 mg 100 g<sup>-1</sup> respectively. The CT content and DPPH activity of flours were very low and measured values were out of linear range. Results can be seen in *Figure 5–8* and are indicative ones only. The CT content may come from residues of bran, while antioxidant activity values may come from accompanying components of carbohydrates or from the germ.

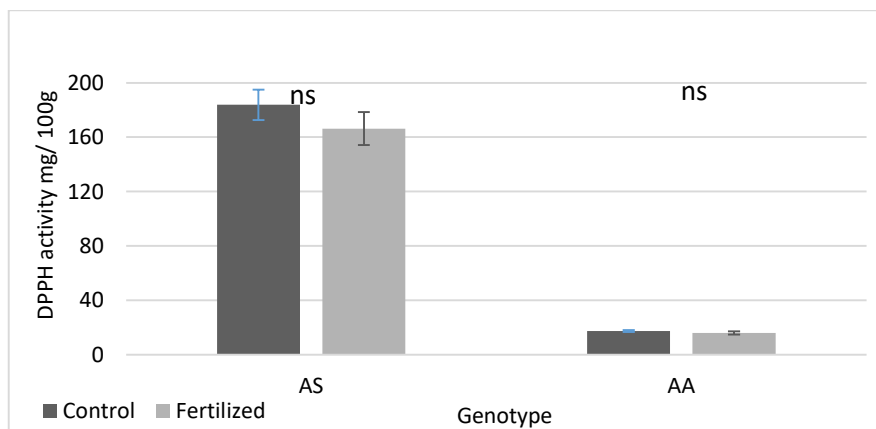


Figure 3. DPPH values of red sorghum brans



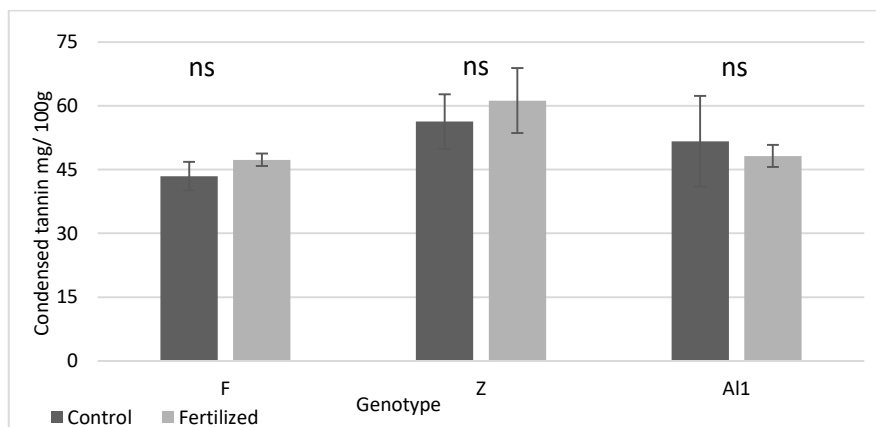
Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant. (n=3 ±s.e). F=Feohn, Z=Zádor, Al1= Alföldi

Figure 4. DPPH values of white sorghum brans



Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant. (n=3 ±s.e) AS=Albanus, AA=Albita

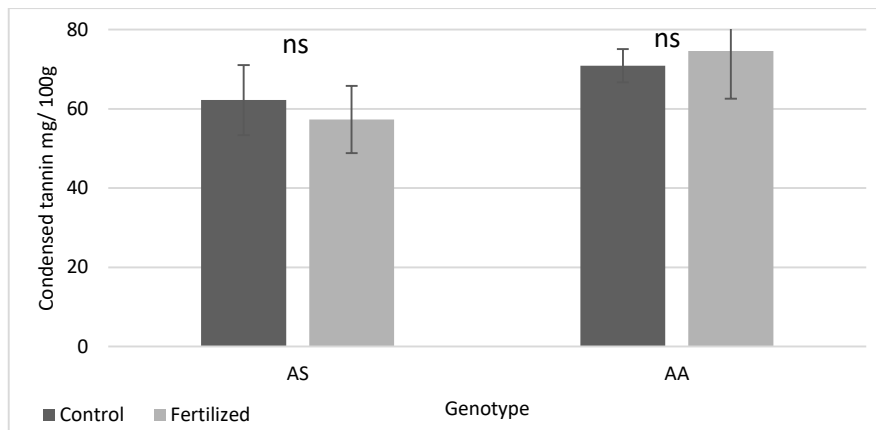
Figure 5. Condensed tannins values of red sorghum flours



Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant. (n=3 ±s.e). F=Feohn, Z=Zádor, Al1= Alföldi Results are out of linear range of used standards.

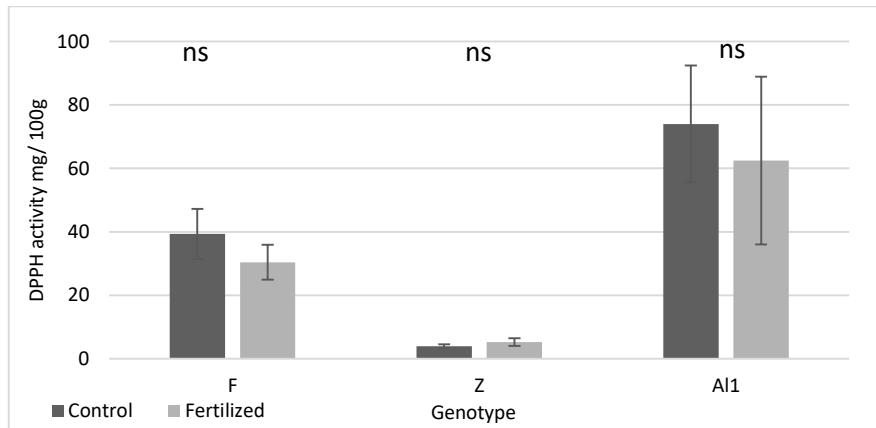


Figure 6. Condensed tannins values of white sorghum flours



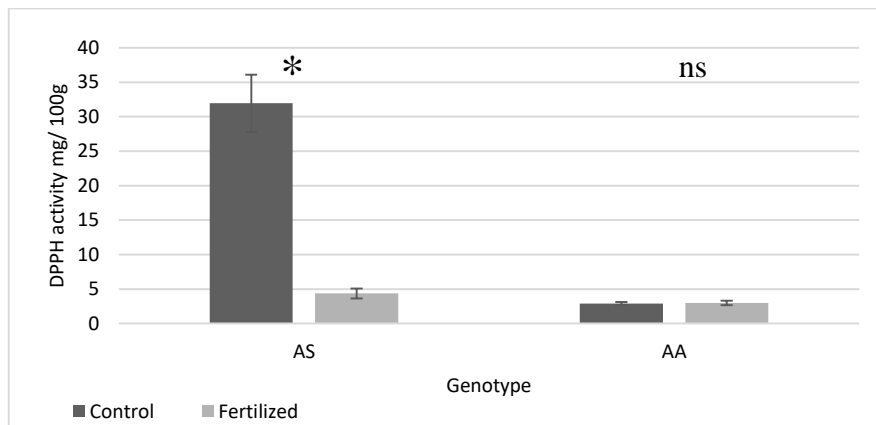
Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant. (n=3 ±s.e) AS=Albanus, AA=Albita. Results are out of linear range of used standards.

Figure 7. DPPH values of red sorghum flours



Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant. (n=3 ±s.e) F=Foehn, Z=Zádor, Al1= Alföldi1 Results are out of linear range of used standards.

Figure 8. DPPH values of white sorghum flours



Note: Values shown as mg 100 g<sup>-1</sup> in dry matter. Significance values between treatments are shown as ns: not significant, (\*) p ≤ 0.05. (n=3 ±s.e) AS=Albanus, AA=Albita\*. Results are out of linear range of used standards.

## CONCLUSIONS

After evaluating our results, it can be seen that there were clear differences between sorghum types and varieties in considering CT content and total antioxidant capacity. In general, we can say that grain color determines their phenolic compound contents and antioxidant capacity in general. Most red sorghum varieties had higher CT content and antioxidant activity

than white ones. Also in each groups of sorghum varieties determined their tannin content. However, *ES Foehn*, despite being a red variety, had low CT content, and *Zádor* with a higher tannin value showed low antioxidant capacity. Other phenolic compounds, like flavonoids, phenolic acids could also contribute to the DPPH values. Much more and detailed measurements needed to get a clearer picture about these parameters.

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