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INFLUENCE OF DRY HEAT TREATMENT ON ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOL CONTENT OF DIFFERENT SORGHUM PARTICLE SIZES

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Abstract: *Sorghum is the fifth most used cereal and contains many bioactive compounds which enhance the antioxidant capacity. The aim of this study was to investigate the antioxidant capacity and the total phenolic content by applying different organic solvents and extraction times on sorghum grain at different particle sizes which were subjected dry heat treatment. The antioxidant capacity values varied between 53.5% to 100%, and the total phenols content ranged from 6.58 mg GAE/g to 54.90 mg GAE/g for the sorghum flour particles sizes, indicating a significant increase when the dry heat treatment was applied. From the solvent used point of view, it can be seen a rise in antioxidant capacity and concentration of total phenols extracted in the following order: methanol > water > ethanol. Also, when the particle size of sorghum flour decreased and extraction time increased, the values of antioxidant capacity and total polyphenols components increased. This study revealed the potential of different sorghum particles size from the total polyphenols and antioxidant capacity point of view and to use them as ingredients to raise the shelf life of food products.*

Keywords: *antioxidants; dry heat treatment; gluten-free; particle size; polyphenols; sorghum grains.*

1. Introduction

Sorghum is a gluten-free grain that contains bioactive components (phenolic acids, flavonoids, and anthocyanins), being a good cereal alternative for human consumption, especially for celiac patients [1,2]. Many researchers have focused on the health benefits of sorghum phenolics and their antioxidant capacity, which found that the antioxidant capacity of sorghum is higher than that of any other grain [3].

Sorghum grain contains from the outside to the inner: outer covering - pericarp (5%), layer - testa (1%), storage tissue - endosperm (84%), and embryo - germ (10%) [4]. Testa is placed over the pericarp and under the endosperm, being unique through other cereals [5]. Phenolic substances are mostly placed in the pericarp and testa [6], this fact is influenced by varieties and growing conditions [7]. The principal sorghum phenolic compounds are phenolic acids, flavonoids (anthocyanins, catechins, and leucoanthocyanidins), and condensed tannins [8]. Phenolic compounds from sorghum grains manifest a great antioxidant capacity, which is mainly characterized by scavenging radicals [1,9].

The extraction yield, content, and profile of phenolics compounds in sorghum present significant differences when are used different extraction methods, such as refluxing extraction, water extraction, maceration extraction, Soxhlet extraction, and organic solvent extraction [10,11]. Various solvents including water, ethanol, methanol, ethyl ether, etc. were used for the phenol's extraction [12-14]. Furthermore, physical treatment methods applied to sorghum grains change the phenolic profile and antioxidant capacity. One of the methods that enhance the nutritional quality and phenolic

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compounds of cereal grains is the thermal processes [15], such as baking, roasting, and extrusion which lead to physico-chemical modification when starch, protein, and other, compounds interact, and Maillard reactions are producing [16]. These modifications will improve the antioxidant characteristics, nutritional quality, organoleptic properties, inactivating of high temperature sensitive toxic components and enzyme inhibitors. These increases might be possible due to the damage to cellular components which release phenolic acids and hydrolyze the polyphenols in simple phenols [17,18]. Some authors highlight an increase in vitamin E and a decrease in the carotenoids, flavanones, flavones, and proanthocyanidins when sorghum flours were treated with dry heat in a conventional oven [19]. Contrarily, in the same study, was demonstrated that the antioxidant activity in treated flours with dry heat remained constant or increased [19]. This can be possible due to various bioactive substances which have different sensitivity to heat and the carotenoids, flavanones, flavones, and proanthocyanidins are more sensitive than vitamin E to heat.

The processing technology of sorghum includes the partial removal of the germ, endosperm, and pericarp, through the decortication, malting, fermentation, roasting, flaking, and milling process [20]. For cereal grains, milling represents the operation of the bran and germ separation from the starchy endosperm which results in refined flour, because the pericarp, testa, and aleurone layers are all separated [21]. Some studies reported higher flavonoid content in sorghum bran compared to other fractions [22] and an improvement of total polyphenols content when particle size decreased in sorghum fractions [9], in barley [23], because once the fiber matrix was broken, the phenolic components were released or exposed. These results demonstrated that grinding treatment could effectively improve the antioxidant capacity [24]. This leads to the conclusion that particle size has a great influence on compound present in grains such as minerals, vitamins, fibers, and phytochemicals. Some authors stated that the bran particles represent a very valuable natural source of antioxidants due to phenolics, the bran being a a value-added product in the preparation of functional food ingredients and/or for the enrichment of particular products [16]. The phenolic compounds from sorghum are bound to the cell wall and these makes it difficult to extract it [21], and are important to find the techniques that enhance polyphenol extraction at different temperature conditions and time.

Sorghum, one of the most diverse cereals in terms of the types and amounts of polyphenols [25], presents the following beneficial effects such as reducing the oxidative stress and chronic inflammation, prevention of some types of cancer, enhancement of glucose metabolism and reducing insulin resistance, improving lipid metabolism and changing the gut microbiota for good colon function [8,26]. It has been observed in the literature that recently, studies on sorghum have intensified, regarding bioactive compounds and their effects on health [27-29].

Even though there are many studies about the phenolic compounds and antioxidant activity in the sorghum flour, from our knowledge are no reports about the total phenolic content and antioxidant activity of particle size of dry heat treatment sorghum grains at different temperatures, and the impact of the type of solvent and extraction time on them.

The aim of this research was to assess the influence of dry heat treatment of sorghum grain on phenolic compounds and antioxidant activity in different particle sizes of sorghum flour by using different organic solvents and extraction times.

2. Materials and methods

2.1. Materials

The white sorghum seeds (ES Albanus hybrid) from harvest 2021, were purchased from the Secuieni Agricultural Development Research Station (Neamt, România). The proximate composition includes: 11.50% moisture, 10.35% protein, 3.07% lipids, 1.16% ash, 8.35% dietary fiber and 65.56% carbohydrates.

2.2. Sorghum treatment

Dry heat treatment of sorghum was performed at different temperatures (121 °C, 132 °C, and 140 °C) for 15 min in a Binder ED53L convection oven (Binder, Tuttlingen, Germany). The sorghum grains heat treated and untreated were milled into a laboratory machine (Grain Mill, KitchenAid, Model 106 5KGM, Benton Harbor, MI, USA). Then, the integral flour was sieved on three mesh (Retsch Vibratory Sieve Shaker AS 200 basic, Haan, Germany), and was obtained three flour particle sizes large (L > 300 µm), medium (200 µm < M < 250 µm), small fractions (S < 200 µm). In this study, large particle size was selected from grains treated at 140 °C (LT), medium particle size from grains treated at 132 °C (MT) and small particle size from grains treated at 121 °C (ST). The selection was made based on the results obtained in our previous research [9]. The untreated samples of sorghum grains sieved on the same three meshes, L, M, and S were coded as LN, MN, and SN and considered as control.

2.3. Methods

2.3.1. Antioxidant capacity

To determine the antioxidant capacity of sorghum particle sizes was used the 2,2 diphenyl-1- picrylhydrazyl (DPPH) method at four different times (10, 15, 20, and 30 min). Briefly, 2 mL of DPPH reagent was mixed with 2 mL extract, prepared as reported in a previous study [9]. The absorbance was reading at 517 nm after incubation for 10, 15, 20, and 30 min at room temperature. The DPPH scavenging activity was calculated using Equation (1), where A_{sample} is the absorbance of the sample with sorghum flour extract and A_{blanck}, is the absorbance reading without extract.

$$DPPH\ (\%) = \left(1 - \frac{A_{sample}}{A_{blanck}}\right) \cdot 100 \qquad (1)$$

2.3.2. Total polyphenol content

In order to determinate the total polyphenol content (TPC), 0.2 mL extract was mixed with 2 mL Folin–Ciocâlteu reagent and 1.8 mL sodium carbonate (7.5%) [30]. After resting at room temperature in the darkness for 10, 15, 20, and 30 min, the absorbance was read at 750 nm [30]. The calibration curve made with gallic acid had R^2 = 0.99 and the equation y = 0.00949 x + 0.02950.

2.4. Statistical Analysis

All assays were done in triplicate. Statistically significant differences at a 95% confidence level were evaluated by means of one-way ANOVA with Tukey's test, by using XLSTAT for Excel 2021 version (Addinsoft, New York, NY, USA).

3. Results and discussion

3.1. Effect of dry heat treatment and extraction solvent type on the antioxidant capacity of sorghum particle size

All three types of untreated and treated sorghum flour particle sizes were analyzed from an antioxidant activity point of view obtained after 10, 15, 20, and 30 min extraction times, and the results are presented in Figure 1. The obtained results demonstrated that treatment temperatures remarkable influenced the antioxidant capacity of sorghum flour.

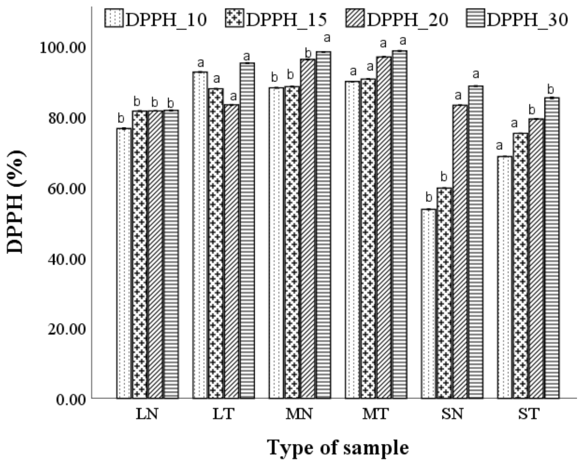

Fig. 1. Effect of the water solvent on samples antioxidant capacity

During phenolic compounds extraction with water, it is observed that particle sizes of sorghum flour obtained from dry heat-treated sorghum grains show higher values of antioxidant capacity. It can also be seen in Figure 1 that the medium particle size has the highest antioxidant capacity, and when the extraction time increase, the antioxidant capacity increases proportionally. This fact can be possible due to higher temperatures during processing which leads to the enhance of total phenolic content and free radical scavenging capacity. Similar results were obtained in other studies, which presented an increase in barley flour antioxidant capacity when was applied a roasted treatment to the grain [31,32].

In the case of ethanol extraction, there were significant differences observed between the particle sizes of thermally and non-thermally treated samples (Figure 2).

Ethanol extraction showed an enhanced antioxidant capacity of the samples as the particle size decreased and the extraction time increased. Sorghum phenolic types influenced the antioxidant activity of the samples. The increase of antioxidant activity with the diminishing of grain granularity was noticed by other authors. This fact can be due to the damage of the fiber matrix [33], and when was applied a heat treatment on sorghum grains it could lead to the release of phenolic compounds from glycosidic components and the hydrolyzation of higher phenolic components into smaller ones [34].

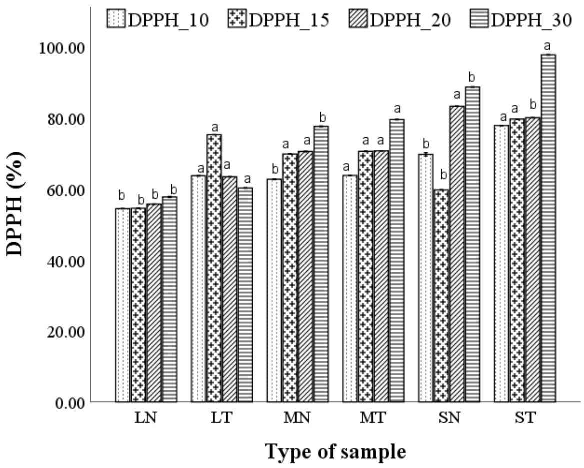

Fig. 2. Effect of the ethanol solvent on samples antioxidant capacity

Extraction with methanol of the phenolic compounds from the sorghum flour different particle sizes showed higher values, a similar trend with the water extraction. Significant differences were observed between samples from the heat treatment applied, the size of the particle, and the extraction time used point of view (Figure 3).

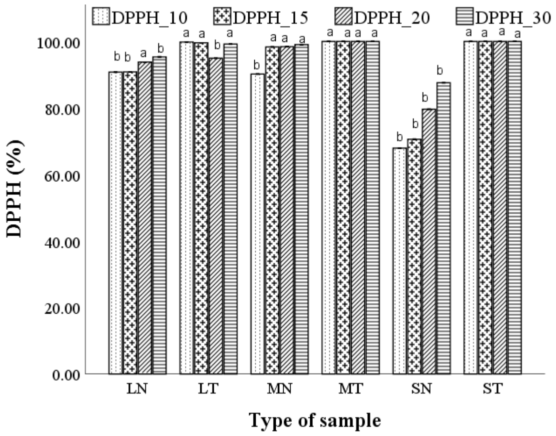

Fig. 3. Effect of the methanol solvent on samples antioxidant capacity

Our results were similar to those obtained by the Morais Carddoso et al. [19].

The increase of antioxidant activity in the dry heat-treated samples can be explained by the formed compounds such as Maillard reaction products [35], and the release of bound phenolic acids from cell walls during baking [36]. This variation in sorghum samples' DPPH values can be based on the chemical extracts structure and on the radical–antioxidant reactions parameters. The value which presented higher antioxidant activity was with methanol extracts and this result can be based on the presence of higher content and synergistic action of antioxidant components with hydrophilic and hydrophobic nature [36]. The use of an alcoholic solvent could be more adequate for extraction of antioxidant compounds from sorghum [6].

3.2. Effect of dry heat treatment and extraction solvent type on the total polyphenol content of sorghum particle size

The extraction of polyphenols from white sorghum flour was strongly influenced by the treatment applied to the grains, the sorghum flour particle size, and extraction time.

The highest particle size values of polyphenols were found in the samples where the extraction time was applied for 20 and 30 min (Figure 4).

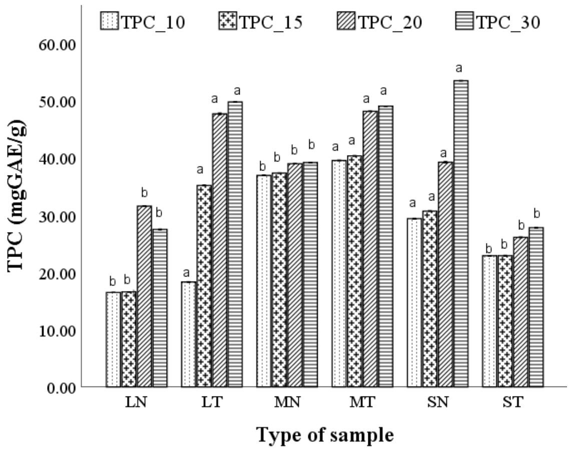

Fig. 4. Effect of the water solvent on samples total polyphenols contents

Statistical analysis of the data shows that the extraction solvent had a positive effect on the phenolic compounds in the following order: methanol > water > ethanol (Figures 4, 5, and 6). The Maillard reaction and chemical oxidation of phenols can be responsable for the increase of total polyphenols content.

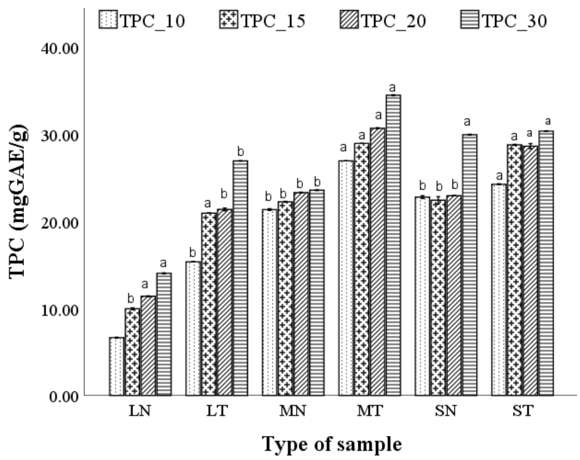

Fig. 5. Effect of the ethanol solvent on samples total polyphenols contents

Some authors demonstrated that heat enhanced the total phenols content [36]. Some phenols can also be deposited in the cellular vacuoles [37], and processing with high temperatures may release unavailable phenolics.

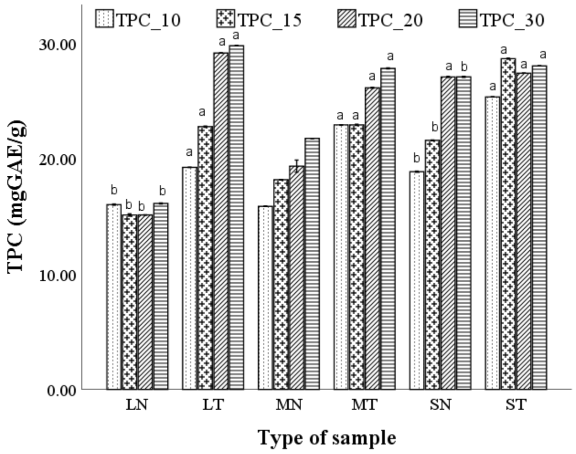

Fig. 6. Effect of the methanol solvent on samples total polyphenols contents

The processing conditions considerable influenced the phenolic compounds. The release of phenolic compounds depend of moisture content, time and temperature during extrusion processing [38].

Based on the indicated results, we found significant differences ($p < 0.05$) on the total phenolic content of non-treated and heat-treated samples. This enhance can be explain by the hydrolization of conjugated phenolic moiety during the thermal process and a polymerization reaction which lead to the obtaining of phenols [16].

This suggests that even though the phenolic components are considered a major group of antioxidants, the non-phenolic components can also significantly contribute to the antioxidant activity of this cereal [6].

4. Conclusion

The processing with dry heat treatment affected the antioxidant capacity and total polyphenols compounds of different particle-size sorghum flour depending on treatment temperature. The type of solvent and time of extraction that can facilitate the release of phenols highlighted an increased antioxidant activity in some sorghum particle sizes. The highest antioxidant capacity was found for medium fractions when methanol was used as extraction solvent at 10 min extraction time.

5. Acknowledgment

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