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Original Paper

Hydrothermal Treatment to Remove Tannins in Wholegrains Sorghum, Milled Grains and Flour

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Abstract

Pigmented sorghum with high content of tannins were studied in this work. Tannins bind to proteins and reduce their availability. A hydrothermal treatment was carried out to reduce tannins. A control sample of non-pigmented pericarp variety was used. After the treatment, grains were milled, and a part was separated for wholegrain flour elaboration. Several determinations were done after treatment: tannins (T), total antioxidant capacity (TAC) and total polyphenols (TPP) content. TPP and TAC in wholegrain pigmented sorghum were 3.9 to 12.3 and 2.3 to 3.5 times higher than those of non-pigmented sorghum, respectively. In all sorghum varieties the extractions of TPP decreased with milling. TAC in flour increased 3.3 times the initial value for non-pigmented sorghum, whereas for the other sorghum samples it increased slightly from 1.1 to 1.3 times the initial value. In flours there was a noticeable reduction in T, with respect to the wholegrain. It was possible to conclude that the hydrothermal treatment allowed lower levels of tannins than those established in the Codex for both wholegrain sorghum and flour. This reduction makes it possible to obtain flour which may be suitable for food processing and the recovery of tannins for other uses.

Keywords

Steeping, Annealing, Milling, Sorghum, Tannin

1. Introduction

Sorghum (Sorghum bicolor (L.) Moench) is the fifth most important cereal crop in the world after wheat, rice, corn and barley (Singh et al., 2011), being an excellent source of energy used for both animal and human feed (Carvalho Teixeira et al., 2016). The world consumption of this cereal is considerable (Althwab et al., 2015) and it is probably due to the ability of the grain to grow over extensive agro-ecological zones (Girard et al., 2018; Taylor et al., 2014). This cereal has nutrients common to all varieties, including several minerals, vitamins and amino acids (Althwab et al., 2015). The presence of polyphenol in the grain is typical in all varieties. The antioxidant level of polyphenols in sorghum is higher than in any other cereal analyzed (Rao et al., 2018). The presence of polyphenols in the sorghum grain provides natural protection against microorganism and insect attacks (Chandrashekar & Satyanarayana, 2006).

The levels of phenols and antioxidant activity are highest when sorghums have secondary purple/red plant color; a black or dark red, thick pericarp and a pigmented testa (Dykes et al., 2005). In pigmented sorghum, condensed tannins, belonging to the group of polyphenols, become important. In this variety, a positive correlation between total phenolic content and proanthocyanidin, flavan-4-ols and 3-deoxyanthocyanidins (condensed tannins) has been reported. In the same study, 55% of polyphenols correspond to proanthocyanidin 18% to flavan-4-ols and 7.5% to 3-deoxyanthocyanidins (Dicko et al., 2005). From this previous study, analyzing tannin content in pigmented sorghum through polyphenol determination leads to a good estimation. Such hypothesis is evaluated in this new study.

Condensed tannins have a negative impact on sorghum flours because they reduce the digestibility of many nutrients, which can affect animal productivity and health (Awika & Rooney, 2004). Its main effect on nutritional value is reducing the digestive availability of protein and starch (Aguiar Moraes et al., 2015). However, there is a tendency to use milled wholegrain (Van der Kamp and Lupton, 2013), because this type of food may be suitable for diets among people with type 2 diabetes, it is proven that polyphenols in sorghum bind to digestive enzymes, specifically alpha-amylase and retard the degradation of starch into glucose, attenuating hyperglycaemia (Links et al., 2015). Therefore, the hydrothermal treatment proposed in this work would has a double benefit, since on one hand it would reduce the tannins in flours, where they are not desired due to their coloration, astringency and reduction of protein and starch availability (Links et al., 2015), and on the other hand their separation by means of a suitable solvent allowing the use of them as an additive in other foods or as nutraceutical, to reduce type 2 diabetes.

The production of wholegrain flour consists of grinding the whole grain to take advantage of the nutrients found in the pericarp and fibers thus improving the gastrointestinal tract health and reducing the incidence of chronic diseases (Van der Kamp & Lupton, 2013). The milling of sorghum could cause contact of the pericap polyphenols and proteins from the inside of the grain and reduce protein availability. On the other hand, the Codex Alimentarius Standard states that wholegrain and flour of sorghum cannot contain more than 0.5% and 0.3% tannins, respectively (Codex Alimentarius

Commission (CAC), 2018).

Previous studies proposed water treatment to reduce the content of polyphenols present in the pericarp of the wholegrain, to take advantage of polyphenols for other uses in food, and to obtain flour with better starch and protein availability (Acquisgrana et al., 2016). After treatment there is still enough polyphenols retaining their antioxidant capacity. For that reason, the purpose of this work is to quantify the amount of the remaining polyphenols in the grain after treatment, after grain milling and in final flour. However, in this work both determination of tannins and polyphenols was done, because tannins are the real problem in food.

The method to quantify the concentration of polyphenols in cereals is not direct, because it is required to extract them from the food matrix and in many cases, extraction is incomplete depending on the solvent used (Tufan et al., 2013). For a better comparison between different types of solvents, tannins in flour is done both, with water and a methanolic extraction.

It is interesting to quantify the residual antioxidant capacity after extraction, because it is an attractive quality in food and it is associated with the presence of polyphenol, then an important loss of this property with the hydrothermal treatment applied to wholegrain is expected. The cupric ion reducing antioxidant capacity (CUPRAC) method has been applied to cereals and has proved to be a reliable determination (Tufan et al., 2013); therefore, this is the method used in this work.

2. Material and Methods

2.1 Steeping

Five samples of sorghum with high content of tannin were obtained from the Experimental Agricultural Station- National Institute of Agricultural Technology (INTA), Argentina. Four of the samples were red or brown sorghum (DK 61T, DOW 108, TOB 60T and Malón– simplified nomenclature: DK, DW, T and M). The other sample was non-pigmented sorghum, named Blank sample (B). Total tannin concentration for each sample was obtained from extractable tannins during hydrothermal treatment and the second extraction used in this study. Total tannin concentrations were summarized in Table 1.

Table 1. Total Tannin Concentration of Each Wholegrain Sorghum Sample

SAMPLE	UMS mg/kg UMS
B	1220 ± 21
DK	7577 ± 54
DW	9252 ± 60
M	4443 ± 26
T	9683 ± 51

Data are mean values ± standard deviation

Sorghum grains (50 g) of each sample were steeped in 100 ml of sodium hypochlorite (NaOCl) solution, containing 0.5% (v/v) available chorine. The procedure was done at 25 ℃ during 18 h (Acquisgrana et al., 2017). The samples were washed to eliminate the NaOCl solution and 100 ml of water was added. The preparation was incorporated to a heat bath at 75 ℃ for 60 min (Acquisgrana et al., 2016), a stage called "annealing" (Singh et al., 2011). Finally, the samples were dried for 12 h at 60 ℃.

2.2 Milling

After annealing and extracting polyphenol, each sample was divided in three samples: unmilled sorghum (UMS), milled sorghum (MS) and flour (F). The MS and F samples were milled with a two-roller mill (CIBART, Argentina) with a separation of 0.5 mm between rollers. MS samples went through the mill once; F samples underwent the same process eight times. F samples were screened through a 500 μm mesh (ASTM 35) to obtain fine flour, according to CAC.

2.3 Polyphenol Extraction

A sample of UMS, MS and F was weighed, and polyphenols were extracted with double the amount of water. Each preparation was incorporated to a heat bath at 75 ℃ for 120 min. Samples were taken every 30 minutes to analyze total polyphenols (TPP) and total antioxidant capacity (TAC).

2.4 Measures

TPP were estimated using the Folin-Ciocalteu method (Singleton et al., 1999) and tannis (T) were estimated using the HCl- vainillin midific method (Price et al., 1978). Both methods were expressed in mg catechin/Kg of solid matter, UMS or F. TAC was estimated with the CUPRAC method (Özyürek et al., 2011) and expressed as mmol trolox equivalents (mmol TE/Kg of solid matter, UMS or F) (Tufan et al., 2013). Measurements were carried out in triplicate.

2.5 Statistical Analysis

Mean values were calculated, and the software Infostat (2002) was used to analyze variance. Tukey test was carry out at the 0.05 significance level.

3. Results and Discussion

3.1 Polyphenol Determination

In all UMS samples, it was observed that even after treatment to reduce the content of polyphenols, concentration was still significant (Figure 1). Polyphenols were extracted using different solvents: water and methanol. Table 2 shows the final concentration of polyphenols and tannins for each sample at the end of the extraction process. Total values correspond to extraction at 120 minutes. The same table shows the values for the extraction of tannins in aqueous solution and methanolic solution.

For UMS (Figure 1), it is observed that pigmented sorghum T contains more polyphenols than the rest of the samples (0.40%), while non-pigmented sorghum shows the lowest value (<0.03%). For UMS, extractable polyphenols corresponds to those found in the pericarp. In all the UMS analyzed a second-order polyphenol extraction kinetics could be obtained, which may be modeled with a quadratic polynomial similar to those previously obtained by Acquisgrana et al. (2016). This fact suggests that the treatment could continue for longer than the expected 60 minutes, from previous results (Acquisgrana et al., 2016). Sorghum with lower tannin content was obtained with longer extraction time, but as it will be seen in the MS and F samples, it would not be necessary since the final concentrations were adequate according to the Codex. On the other hand, an excessive reduction may cause a greater loss of antioxidant capacity, which is not be desirable for food. Furthermore, in previous studies it has been observed that the implementation of the proposed treatment greatly improves availability of soluble proteins (Acquisgrana et al., 2017).

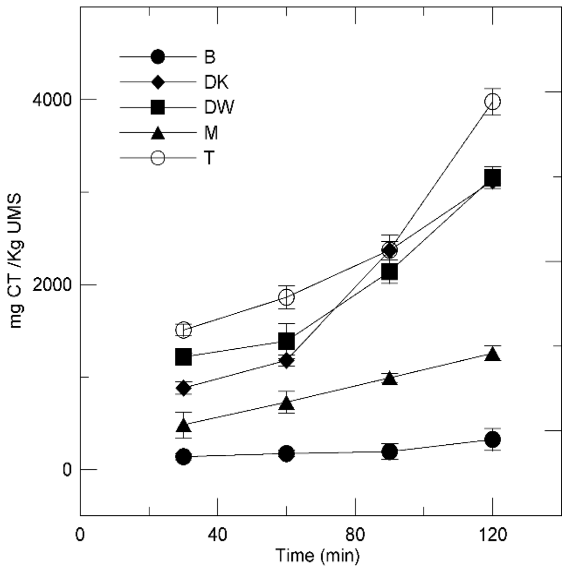

Figure 1. Polyphenol Extraction vs. Extraction Time for Unmilled Sorghum (UMS)

Table 2. TPP, T-WE and T-ME and AC after 120 min of Extraction for UMS, MS and F Samples

		UMS	MS	F
		mg/kg UMS	mg/kg UMS	mg/kg F
TPP	B	324 ±31	377 ±9	145 ±47
	DK	3119 ±11	1515 ±13	1194 ±21
	DW	3156 ±62	1710 ±50	983 ±14
	M	1255 ±41	1008 ±3	1179 ±41
	T	3977 ±36	1537 ±12	1068 ±39
T-WE (Water extraction)	B	440 ±22	608 ±23	180 ±13
	DK	2622 ±16	3150 ±38	2787 ±30
	DW	2828±9	2900 ±39	2056 ±43
	M	1093 ±25	2218 ±26	2181 ±35
	T	3417 ±55	3471 ±52	2861 ±28

T-ME (Methanolic extraction)	B		640 ±38	284 ±18
	DK		2678 ±56	2613 ±32
	DW		3524 ±83	2144 ±71
	M		1790 ±45	2258 ±55
	T	*	3375 ±72	1930 ±39
AC** mmol TE/kg	B	1.2 ±0.2	2.9 ±0.4	4.0 ±0.4
	DK	4.2 ±0.4	5.2 ±0.2	4.7 ±0.4
	DW	3.8 ±0.1	5.5 ±0.1	4.2 ±0.1
	M	2.7 ±0.1	4.4 ±0.3	3.4 ±0.3
	T	3.5 ±0.2	6.1 ±0.3	5.1 ±0.2

* Determination of T-ME in UMS was not done. Data are mean values ±standard deviation

** AC units are mmol TE/kg instead of mg/kg

Figure 2 shows the extraction of polyphenols for MS. It is observed that practically in all samples, the values are stabilized at 90 minutes. The concentrations of polyphenols in MS samples of all pigmented sorghums showed lower values than the UMS samples: DK was reduced in 51.4%, DW in 45.8%, M in 19.6% and T in 61.3% in relation to UMS. White non-pigmented sorghum presented an increase with respect to UMS by 16.4% (Table 2).

The decrease of polyphenols in all sorghum varieties could be directly linked with their interaction with proteins. When the grain is milled, polyphenols could interact with proteins and could not continue to be extracted, which does not necessarily imply the reduction of the proteins, because they continue in the ground matrix.

In the flour samples (F), since they are completely ground and have been separated from the pericarp by sieving, extraction speed is higher than for UMS and MS samples, reaching stability within the initial 30 minutes and remaining invariable the rest of the time, therefore only the final value of extraction is indicated in Table 2. Since most polyphenols have been extracted during the annealing process and the retention of most of the pericarp and germ during sieving, the flours of all the variety present a lower concentration of polyphenols than the UMS and MS. Extraction stability after 30 minutes at 75 ℃ could indicate the appropriate time to extract all the polyphenols present in the F samples.

Previous studies have reported the high TAC present in sorghum grains, regardless of their variety (Dlamini et al., 2007). This previous study considered pigmented sorghums with and without tannins. However, it is known that the presence of tannins confers the greatest antioxidant capacity in sorghum varieties, due to the presence of proantocyanidin and other condensed tannins (Rao et al., 2018). For example, in the case of proanthocyanidin, mean values of 9400 mg/kg for the red variety, against 1300 mg/kg for the white variety have been reported. Furthemore, the proanthocyanidin levels were positively correlated with the total phenolic content. Other examples and quantities can be comparated in Bröhan et al. (2011).

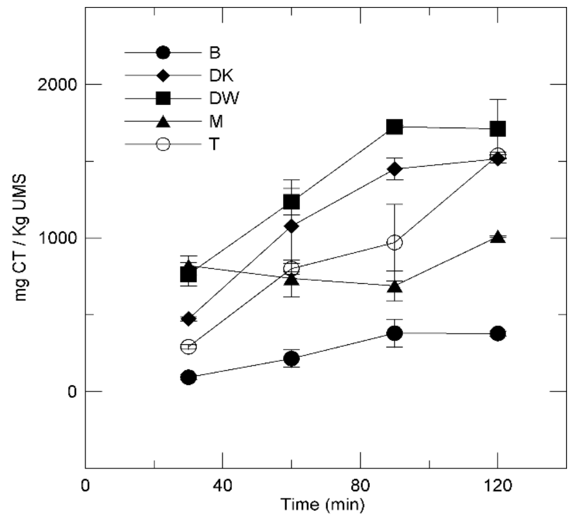

Figure 2. Polyphenol Extraction vs. Extraction Time for Milled Sorghum (MS)

Other previous studies have reported a strong correlation between the content of polyphenols and tannins (Dicko et al., 2005). This observation could be verified in the present report work (Figure 3), but only in whole sorghum the content of tannins is lower than that of polyphenols, finding the linear adjustment that is reported in Table 3, with the setting parameters of Equation 1:

$$TPP = a \cdot T \tag{1}$$

Table 3. Setting Parameters for Eq. (1) Correlation of Tannins and Polyphenols in UMS, MS and F, with Water (WE) and Methanolic (ME) Extraction

T	a	R^2
UMS-WE	00.91a	0.985
MS-F-WE	2.11b	0.914
MS-F-ME	2.02b	0.949

Mean in same row in different lowercase are significantly different (p<0.05).

From the linear adjustments through the origin for UMS samples, it is obtained that 91% of the polyphenols correspond to tannins. However, a strong correlation between tannins and polyphenols is possible for MS and F samples, but tannins exceed more than twice the content of polyphenols, obtaining no significant differences between both extractions, with water or methanol. There were also no significant differences between F and MS samples (Table 3). Probably, no significant differences are observed due to the high temperature used for the different types of solvent. The main difference between tannins and polyphenols may be the limitation of the Folin-Ciocalteu method (Singleton et al., 1999) which does not allow adequate quantification of condensed tannins. Recent studies provide more evidence on this behavior where a similar relationship between tannins and popyphenols was found (Adetunji et al., 2015). In all cases the tannins values found are much lower than that established in the CAC 0.5% for wholegrains and 0.3% for flours, therefore with the treatment proposed by Acquisgrana et al. (2016) it is possible to obtain a flour suitable for human consumption from the varieties of colored sorghum studied and that can be used for the production of gluten-free foods for people with celiac disease.

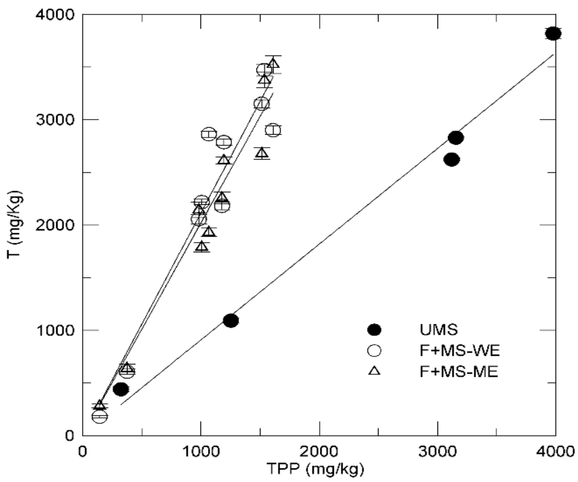

Figure 3. Correlation between Tannins (T) and Total Polyphenols (TPP) in UMS, MS and F Samples. Full Lines Represent Eq. (1) with Constant Values in Table 2. Vertical Bars Represent the Standard Deviation in Each Value

3.2 Antioxidants

TAC determination in Table 2 shows that pigmented sorghum contain a greater quantity of antioxidants than non-pigmented sorghum in the UMS. In all cases, the TAC is the highest in the UMS samples, and the pigmented varieties show higher TAC values than the non-pigmented sorghum variety. This is a nutritional advantage of the wholegrain of treated pigmented sorghum since it is possible to reduce the content of tannins to suitable levels according to the CAC and that preserve an antioxidant capacity greater than the white variety. However, during flour production, the pericarp part containing most of the fiber and probably the tannins, is separated with the sieving process, leaving the flour of pigmented sorghum with an TAC similar to the non-pigmented variety (Table 2). In the case of flour, it is observed that in all cases the values obtained are lower than those found by Tufan et al. (2013) for the aqueous extract of 18.28 mmol TE/kg Barley, Rye 8.64, Wheat 4.31 and 7.51 Oat, with the same CUPRAC method. It is interesting to show that the previous cereal did not undergo sieving, and probably in this samples the TAC will be reduced. However, the values of TAC obtained in the sorghum samples are of the same order of magnitude obtained with the other cereals studies and similar to milling wheat.

The reduction in the TAC is due to the initial annealing procedure to extract polyphenols. The methodology used in this work and in the previous studies (Acquisgrana et al., 2016) can be used to regulate the content of tannins to meet the requirements of the CAC and present a residual antioxidant capacity that makes it attractive to obtain products with the selected flour.

4. Conclusion

From the result obtained, it was possible to conclude that the treatments of steeping and annealing allowed lower levels of tannins than those established in the Codex for both UMS and F samples. It may also be possible to handle this reduction to improve the antioxidant capacity of flour. This reduction makes it possible to obtain flour, from pigmented varieties, which may be suitable for food processing and the recovery of tannins for other uses.

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