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Total phenolic compounds and antioxidant activity of sorghum flour

Compostos fenólicos totais e atividade antioxidante de farinhas de sorgo

Abstract

Introduction: Sorghum, a cereal native to Africa, and little explored as food in Brazil, has bioactive compounds, which can scavenge free radicals and be a potential in promoting health. ***Objective:*** To determine, by spectrophotometric methods, the levels of bioactive compounds and antioxidant activity of sorghum, native sorghum flour and after phosphating, and commercially obtained flours. ***Materials and Methods:*** Five samples were analyzed: whole sorghum, native sorghum flour, phosphate sorghum flour, and red and white commercial sorghum flours. Total phenols, flavonoids, anthocyanins, total tannins, and antioxidant activity were determined. ***Results:*** In general, there was no significant difference in the levels of bioactive compounds between whole sorghum and native sorghum flour. Phosphating reduced the levels of total phenols and anthocyanins in sorghum flour. The commercial red sorghum flour showed higher levels of total phenols and flavonoids than the other flours, and the commercial white sorghum flour showed the lowest levels of flavonoids, anthocyanins, and total phenols. ***Conclusion:*** In comparison to other foods studied in the literature, sorghum exhibited higher values of total phenols and anthocyanins, which suggests that sorghum flour has considerable potential to be used as an ingredient in preparations.

Keywords: Anthocyanins. DPPH. Flavonoids. Phosphating. Tannins.

Resumo

Introdução: O sorgo, cereal nativo da África, e pouco explorado como alimento no Brasil, apresenta compostos bioativos, os quais têm a capacidade de sequestrar radicais livres e potencial na promoção da saúde. ***Objetivo:*** Determinar, por métodos espectrofotométricos, os teores de compostos bioativos e a atividade antioxidante do sorgo, da farinha de sorgo nativa e após fosfatação, e de farinhas obtidas comercialmente. ***Materiais e Métodos:*** Foram analisadas cinco amostras, sendo elas sorgo integral, farinha de sorgo nativo, farinha de sorgo fosfatado e farinhas comerciais de sorgo vermelho e de sorgo branco. Foram determinados os teores de fenóis totais, flavonoides, antocianinas, taninos totais e a atividade antioxidante. ***Resultados:*** De maneira geral, não houve diferença significativa nos teores de compostos bioativos, entre o sorgo integral e a farinha de sorgo nativo. A fosfatação reduziu os teores de fenóis totais e antocianinas da farinha de sorgo. A farinha comercial de sorgo vermelho apresentou teores de fenóis totais e de flavonoides maiores que as outras farinhas, e a farinha comercial de sorgo branco exibiu os menores teores de flavonoides, antocianinas e fenóis totais. ***Conclusão:*** Em comparação com outros alimentos estudados na literatura, o sorgo exibiu valores superiores de fenóis totais e antocianinas, o que sugere que a farinha de sorgo possui considerável potencial para ser usada como ingrediente em preparações.

Palavras-chave: Antocianinas. DPPH. Flavonoides. Fosfatação. Taninos.

INTRODUCTION

Sorghum (*Sorghum bicolor L. Moench*) is a cereal native to Africa and presents itself as a species well adapted to extreme environment of abiotic stress, especially air temperature and soil moisture, which provides favorable conditions for its adaptation compared to other grains.[1] This cereal is classified into four groups: grain; forage for silage and/or sugar; forage for grazing/cutting/haying/covering; and broom. Of the four groups, grain sorghum is the most economically significant and is among the five most cultivated cereals in the world, behind rice, wheat, corn, and barley.[2] In Brazil, sorghum is little explored as human food, despite its great potential, since its flour can be used as a substitute for wheat flour, in gluten-free bakery and confectionery products, being an alternative for consumers with gluten-related disorders.[3]

Sorghum has advantages for use in human food, as it contains phenolic compounds with potent antioxidant activity, which can contribute to health promotion.[4] Phenolic compounds are included in the category of free radical scavengers and are effective in preventing auto-oxidation.[5] The concentration of tannins, a type of phenol, in sorghum cultivars varies according to genetic characteristics, where sorghum genotypes that have grains with a pigmented testa have higher tannin contents, as well as higher concentrations of total phenolics and higher antioxidant activity.[6]

The way cereals are processed (whole meal flour, sifted, decorticated) can result in differences in nutritional composition when different layers of the grains are removed.[7] Sorghum is a cereal composed mainly of starch (32.1 to 72.5%), and can be modified to change its properties with a view to its application in bakery products, including gluten-free products. Modifications can be physical (gelatinization) or chemical (oxidation, phosphation, acetylation).[8] Phosphating is a low-cost, easy-to-perform chemical modification performed using reagents such as sodium tripolyphosphate, which introduces phosphate groups through phosphorylation, controlling process conditions such as pH, time, and temperature.[9] As a result of this modification, there is an increase in the swelling power and solubilization of the granules, in addition to a reduction in the gelation temperature, as well as an increase in the clarity of the paste and the viscosity of the gel.[10] However, these modifications can result in changes in the nutritional composition of cereals that are still poorly explored.

In order to verify whether there is a change in the composition with the processing and phosphating of sorghum starch, and due to the lack of studies on the bioactive compounds in sorghum flour in Brazil. The present study aimed to determine, by spectrophotometric methods, the contents of bioactive compounds and the antioxidant activity of sorghum, native sorghum flour and after phosphating and commercially obtained red and white sorghum flours.

MATERIALS AND METHODS

Grain red sorghum grains (Sorghum bicolor) were used from Embrapa Clima Temperado from Pelotas, Rio Grande do Sul, Brazil. Two brands of sorghum flour acquired at random (1 lot of each), in the market in the city of Santa Maria, Rio Grande do Sul, Brazil. Brand 1 was red sorghum (Red S.), and Brand 2, white sorghum (White S.).

Sample preparation

Whole sorghum grains were ground in a Willey TE-650 type macro-knife mill (Tecnal). A portion of the flour was stored in whole form (whole sorghum flour) and the other portion was sieved until obtaining a flour with a granulometry of 0.250 mm (native sorghum flour), thus eliminating the outermost parts of the grain.

Phosphating sorghum flour

Phosphating was performed according to the methodology described by Paschall[11] with modifications. Phosphating was carried out using 167 mL of a 5% tripolyphosphate solution (Ph 6.0) in 100 g (on a dry basis) of native sorghum flour. The mixture was stirred in a mechanical shaker (IKA RW 20 digital) for 20 minutes, filtered in a vacuum pump, and the sediment was dried in a forced-air oven (De Leo) for 48 hours at 45°C ± 2°C. The dried sample was pulverized in a knife mill and placed in an oven with forced air circulation at 65°C ± 2°C for 90 minutes. Then, it was transferred to a stationary oven (FANEM model 515) at 155 ± 2°C for 40 minutes. After cooling, 300 mL of 50% ethanol was added, and the samples were centrifuged at 2200 rpm for 5 minutes. The supernatant was discarded, the resulting product was transferred to aluminum trays and oven-dried at 45°C ± 2°C for 24 hours.

After the phosphating process, the dialysis process was carried out, necessary to remove the phosphorus salts not bound to the starch, as described by Limberguer.[12] A 10% (w/v) suspension of 4hosphate starch was placed in cellophane bags, submerged in distilled water for five days, and water changed daily. Subsequently, the gel was placed on aluminum trays, dried in a forced-air oven at 45°C ± 2°C, and pulverized to a granulometry of approximately 0.250 mm. After the dialysis process, the samples of phosphate sorghum flour were stored in hermetically closed containers, under refrigeration.

Production of extracts

For the quantification of total phenols, total flavonoids, total tannins, and analysis of antioxidant activity, extracts were produced, according to Queiroz et al.[13] The samples were extracted with acidified methanol (1% HCl in methanol),centrifuged in a centrifuge (CELM Brand / Combat Model) for 5 minutes at 2400 rpm to separate the liquid phase, and stored under refrigeration (4 ± 2 ºC) until the time of analysis.

For the extraction and quantification of anthocyanins, the samples were mixed and homogenized in acetone. Then, chloroform was added, which partitions the aqueous phase (contains anthocyanins, phenols, sugars, and other water-soluble organic compounds) and the lipid phase (contains immiscible organic compounds, lipids, carotenoids, and other nonpolar compounds). The aqueous phase was transferred to a flask for evaporation of acetone on a rotary evaporator (Fisaton®, model 0241) at 40°C under vacuum. This method has the advantage of producing a lipid-free extract.[14]

Quantification of Total Phenols

This assay was performed according to the method proposed by Roesler et al.[15] In the extracts (2.5 mL) was added 2.5 mL of Folin Ciocalteau reagent diluted 10 times, and 2.0 mL of 7.5% sodium carbonate solution. The mixture was heated at 50 °C for 5 minutes and cooled. The reading was performed in a spectrophotometer at 760 nm. In this study, the quantification of total phenols in the extracts was performed using a standard curve prepared with gallic acid and expressed in gallic acid equivalents (GAE/g).

Quantification of Total Flavonoids

The total flavonoid content was determined according to the method proposed by Zhishen, Mengcheng & Jianming,[16] by reacting the extracts with $NaNO_2$, $AlCl_3$, and NaOH, followed by reading the absorbance in a spectrophotometer at 510 nm. The quantification of total flavonoids in the extracts was performed using a standard curve prepared with catechin and expressed as catechin equivalent (CE). The result was expressed as mg of catechin equivalent per 100 g of sample.

Quantification of Total Tannins

For the determination of total tannins, the method of Hagerman and Butler[17] was used. This method is based on the property of tannins to precipitate, in an aqueous solution, in the presence of protein. For this technique, a 1 mg/mL solution of bovine serum albumin in 0.2 M sodium acetate buffer solution (Ph 4.9) containing 0.17 M sodium chloride was used to precipitate the tannins in the solution. The LSS/Triethanolamine detergent solution was used to separate the tannins from the protein in the precipitate, and the $FeCl_3$ solution was used as the chromogenic solution.

From an aqueous solution of tannic acid of concentration 1 mg/mL, a standard curve was prepared. In test tubes, 1 mL of the standard sample and 2 mL of the albumin solution were added. After 15 minutes, centrifuged at 3000 RPM for further 15 minutes. After centrifugation, the supernatant was discarded, dissolving the precipitate with 4 mL of LSS/Triethanolamine solution. A volume of 1 mL of the $FeCl_3$ solution was added and after 15 minutes the absorbances were read at 510 nm. The quantification of total tannins in the extracts was performed using a standard curve prepared with tannic acid and expressed in mg/100g.

Quantification of Anthocyanins

The total anthocyanins content was determined by the Ph difference method, based on the methodology of Fuleki and Francis.[18] For this, buffer solutions of Ph 1.0 and 4.5 were used. The Ph 1.0 solution was prepared by mixing KCl (0.2N) and HCl (0.2N) solutions in a 25/67 ratio. The Ph 4.5 buffer was prepared from sodium acetate solution (1N), HCl, and water in the proportion 100/60/90. Aliquots of the concentrated extract were quantitatively transferred to volumetric flasks of 25 mL and 10 mL, and their volumes were completed with buffer solutions of Ph 1.0 and Ph 4.5, respectively. The reading was performed in a spectrophotometer at 485 nm and 700 nm, respectively.

Antioxidant Activity Analysis – Determination by DPPH Free Radical Capture

In a test tube, an aliquot of 500 µL of each extract was added to 2500 µL of DPPH solution (0.004% v/v). The mixture was left in the dark until it reached the reaction plateau at room temperature. The control was prepared in the same way, without the extract, using methanol to correct the baseline. The DPPH solution was prepared daily, stored in an amber bottle, and at 4ºC between measurements. Absorbance was read at 517 nm. With the values, the percentage of inhibition was calculated, according to equation 1:[19]

$$\% \text{ Inhibition} = [(ADPPH - AExtr)/ADPPH]\ x\ 100 \qquad (1)$$

where ADPPH is the absorbance of the control and Aextr is the absorbance of the sample at a given concentration.

A curve was constructed using Trolox as a standard, based on the method by Silveira et al.[20] The result of the antioxidant activity of the extracts was expressed in TEAC values (Trolox equivalent antioxidant capacity), defined in μmol of Trolox equivalent per mg of extract. Using the straight line of the calibration curve, the concentration of antioxidants was calculated, according to equation 2:

$$CA = (Abs_{am} - b)/a \quad (2)$$

where Abs_{am} is the absorbance of the sample, a is the slope obtained for the calibration curve, and b is the linear coefficient obtained for the calibration curve.

Statistical analysis

All treatments and analyzes were performed in triplicate. The results were compared by the analysis of variance test and the means were compared by the Tukey test with 5% of significance, using the statistical program IBM SPSS Statistics 23.

RESULTS AND DISCUSSION

The data regarding the bioactive compounds present in whole, native, and phosphate sorghum flours, as well as in the commercial brand flours analyzed in this study, are presented in Table 1.

.Table1. Bioactive compounds from whole sorghum, native sorghum flour, phosphate sorghum flour, commercial red sorghum flour, and commercial white sorghum flour. Santa Maria, RS, 2017.

Sample	Total phenols	Flavonoids	Total tannins	Anthocyanins	DPPH
Whole Sorghum	79.0 ^{b} ± 2.16	412.1 ^{bc} ± 2.65	10.4 ^{a} ± 0.85	0.71 ^{b} ± 0.10	3.5 ^{ab} ± 0.12
Native sorghum flour	80.3 ^{b} ± 3.26	405.8 ^{c} ± 1.08	12.5 ^{a} ± 2.58	0.93 ^{a} ± 0.02	3.3 ^{b} ± 0.19
Phosphated sorghum flour	68.6 ^{c} ± 3.01	435.6 ^{ab} ± 7.91	10.3 ^{a} ± 0.59	0.34 ^{c} ± 0.06	3.6 ^{ab} ± 0.02
Brand 1 – Red S	155.2 ^{a} ± 4.23	446.2 ^{a} ± 2.64	12.4 ^{a} ± 0.44	0.20 ^{cd} ± 0.07	3.6 ^{ab} ± 0.10
Brand 2 – White S.	61.2 ^{c} ± 1.55	244.1 ^{d} ± 9.19	9.2 ^{a} ± 0.60	0.08 ^{d} ± 0.03	3.8 ^{a} ± 0.18

Data are expressed as mean values ± standard deviation. Total phenols are expressed as equivalent mg of gallic acid (GAE) per 100g of sample. Flavonoids are expressed as μg of catechin equivalent (CE) per 100 g of sample. Tannins are expressed in mg/100g. Anthocyanins are expressed in mg/100g. DPPH are expressed in Equivalent Trolox Concentration (TE) (μM = μmol/mg). Samples with different letters within each column are significantly different ($p<0.05$).

Quantification of Total Phenols

It was observed that brand 1 red sorghum flour had the highest total phenol content, while the sorghum phosphating process probably reduced the total phenol content of sorghum flour.

This fact may be associated with the drying carried out in the phosphating process, which promotes the degradation of thermosensitive food components. White sorghum flour had the lowest phenolic content among the samples (61.2 mg GAE/100g), an expected result, since white sorghum has low levels of total phenolic compounds.[21]

Rao et al.[22] analyzed black, brown, red, and white sorghum, finding total phenols values of 11.5 mg GAE/g, 3.58 mg GAE/g, 0.66 – 0.88 mg GAE/g, and 0.24 mg GAE/g, respectively. Punia et al.[23] also analyzed samples of black, brown, red, and white sorghum, but found 844.21 mg GAE/ 100g, 955.88 mg GAE/ 100g, 1040.73 mg GAE/ 100g, and 173.68 mg GAE/ 100g, respectively, corroborating with the results found in the present study, where red sorghum presented a higher amount of total phenols when compared to other cultivars. However, all these researchers observed values higher than those verified in this research, probably due to cultivation, climatic and genetic factors that can influence this characteristic, since the sorghum phenolic profile differs greatly between varieties, genotypes, growth, and environmental conditions,[24] such as also the extraction methodology and the analysis methods used.

However, studies found the levels of total phenols were lower in various foods such as buriti (90.24 mg GAE/100g), cagaita (143.81 mg GAE/100g) and cajá (58.27 mg GAE/100g),[25] purple star apple (14.91 mg GAE/100g), green star apple (18.10 mg GAE/100g), red cashew (115, 53 mg GAE/100g), yellow-green cashew (130 73 mg GAE/100g) and pitaya (58.89 mg GAE/100g).[26] Thus, it is observed that sorghum, even influenced by factors such as cultivation, climate, and genetics, has considerable levels of phenolic compounds, such as flavonoids, tannins, and anthocyanins, considered desirable components due to their antioxidant activity, capable of inhibiting the oxidation of low-density lipoproteins (LDL). In addition to the consumption of foods rich in these compounds, which is associated with a reduction in the tendency to thrombotic diseases, and has already been described for its antimicrobial actions, anti-inflammatory and effects on weight control.[27]

Quantification of Total Flavonoids

Flavonoids are found mainly in sorghum bran, being associated with the color and thickness of the pericarp and the presence of a pigmented testa.[28] According to Table 1, it is observed that the milling and phosphating process of native sorghum flour did not cause significant losses of flavonoids, compared to whole sorghum. The highest content was found in brand 1 flour since it comes from red sorghum, which is a positive factor since commercial flours are available to the population. As expected, white sorghum flour had a lower number of flavonoids. Shen et al.[29] analyzed sorghum and found 181.07 mg rutin equivalent (RE)/100 g of flavonoids, while Punia et al.[23] analyzed black, brown, red, and white sorghum and found 30.54, 36.73, 42.84, and 21.34 mg RE/100g, respectively, indicating that red sorghum has the highest amounts of flavonoids, corroborating the results of the present study.

Possibly, these differences are due to the cultivation characteristics, since the phenols varied significantly due to numerous environmental factors. Flavonoids act to save the consumption of vitamin C, preventing the formation of free radicals.[30]

Quantification of Total Tannins

The tannin contents did not show significant differences between the five samples of the study, that is, the milling and phosphating did not influence the contents of these compounds, as well as the coloring. On the other hand, Punia et al.[23] analyzed black, brown, red, and white sorghum, and found 4.32, 1.13, 1.44, and 10.39 mg/100g, respectively, concluding that white sorghum has higher levels of tannins than other varieties.

Sorghum cultivars with tannin are slowly digested by the body and can be used in foods for patients with diabetes, in which the delay in gastric emptying allows slow absorption of glucose.[31] The tannins found in sorghum are of the condensed type, also known as proanthocyanidins, high molecular weight compounds.[31] Due to the ability to bind free radicals, sorghum genotypes that contain tannins have greater antioxidant activity than sorghums that do not contain tannins.[21]

Quantification of Anthocyanins

As for the anthocyanin content, it was observed that the native sorghum flour has a higher content than the whole sorghum flour, probably due to the increase in the contact surface, which may have facilitated the extraction. Verificou-se também que a fosfatação diminuiu o teor de antocianinas, supostamente porque a secagem promoveu a degradação desses compostos, que são termossensíveis. It was also found that phosphating decreased the anthocyanin content; supposedly however drying promoted the degradation of these thermosensitive compounds. Commercial flours exhibited lower levels of anthocyanins, of which white sorghum flour (brand 2), as expected, had the lowest content, as it is white sorghum. On the other hand, anthocyanins are red, blue, or purple pigments, present in greater amounts in red sorghum.

It is known that fruits are the main sources of anthocyanins, such as purple star apple (3.0 mg/100 g), green star apple (1.8 mg/100 g), red cashew apple (1.8 mg/100 g), red cashew (1.8 mg/100 g).[26] Despite this, sorghum exhibited anthocyanin levels similar to some fruits, such as yellow plum (0.4 mg/100 g), red plum (0.9 mg/100 g), and pitaya (0.4 mg/100 g).[26] In this way, sorghum can contribute to the consumption of anthocyanins in human food. Studies have linked the consumption of anthocyanins to a reduced risk of obesity, heart disease, degenerative conditions, and several types of cancer.[32]

Antioxidant Activity Analysis - Determination by DPPH Free Radical Capture

DPPH is one of the most used methods to assess antioxidant activity, as it is considered a fast, practical method with good stability.[33] DPPH is a stable chromogen radical with deep purple color. It is commercially available and does not need to be generated before testing. The DPPH scavenging assay is based on electron donation from antioxidants to neutralize the DPPH radical.[34] However, this method has limitations, such as the need for dissolution in organic solvents;[35] tendency to react with other radicals present in the sample;[36] the absorbance of DPPH tends to reduce when exposed to light;[37] in addition to having no physiological similarity due to the absence of DPPH radicals in the human body.[38] According to Sadeer et al.,[39] this method and other in vitro antioxidant assays, such as ABTS, FRAP, TEAC, and ORAC present numerous controversies that affect their reliability. However, there is no universal, optimized, and standardized method for determining antioxidant capacity. Thus, a combination of at least three assays is recommended whenever possible.

In the antioxidant activity of sorghum against the free radical DPPH, there was no difference between the analyzed samples, except for native sorghum flour and brand 2 flour (white sorghum), which showed a

significant difference. Rao et al.[22] found 18.04, 21.02, 0.41 to 1.17, and 0.33 mg TE/g in black, brown, red, and white sorghum samples, respectively, showing that brown sorghum showed the higher antioxidant activity against DPPH. However, Punia et al.[23] observed higher antioxidant activity in red sorghum (20.55 mg TE/100g), when compared to black sorghum (14.55 mg TE/100g), brown (15.96 mg TE/100g), and white (7.97 mg TE/100g). Therefore, these studies verified superior antioxidant activity to the studied samples, indicating that several factors can influence this characteristic, although the pigmentation of the pericarp did not present a correlation with the antioxidant activity.[22]

The present study faced difficulties in comparing the results obtained with others reported in the literature, due to the differences in the methods used for the analysis and the different measurement units used. However, even with difficulties in comparison, it was found that sorghum and sorghum flour had higher or similar levels of total phenols and anthocyanins to other foods studied, which may contribute to the consumption of bioactive compounds in human food.

CONCLUSION

Sorghum flour has considerable potential to be used as an ingredient in foods, as this flour contains a considerable number of bioactive compounds. Phosphating reduced the levels of total phenols and anthocyanins in sorghum flour. Both commercial flours showed a considerable number of bioactive compounds. However, the brand composed of red sorghum exhibited higher levels of total phenols, flavonoids, and anthocyanins, compared to the brand composed of white sorghum

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Contributors

Montagner GE worked at all stages from the conception and design of the research, performing all the practical parts of the work, interpreting the results to write and reviewing the article. Fogaça AO and Storck CR worked on the conception and design of the research, supervision, and guidance of the work, analysis, and interpretation of results, review, and final approval of the article.

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