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Dry heat and pressure favor bioactive compounds preservation and peptides formation in sorghum [*Sorghum bicolor* (L.) Moench]

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ABSTRACT

Sorghum is a cereal with potential economic and nutritional properties. It has gained headway in the international market because of its nutritional content which is characterized for many bioactive compounds with antioxidant characteristics, and also, because it is gluten free. This work evaluated the proteomic profile of sorghum grains and its nutritional composition and functional profile after exposure to 7 different treatments (control, grind, dry heat, bursting, wet cooking with and without water and wet cooking in pressure). They were analyzed for chemical composition, protein profile, total phenolic compounds, anthocyanin content and antioxidant activity. The dry heat preserves the protein content, phenolic compounds, anthocyanins and presents between 94% and 95% of radical scavenging activity. Heat treatments that use the pressure promote the natural hydrolysis of proteins. Bursting treatment resulted in 45.6% of proteins and peptides in the range of 3.7; 5.93; 8.9 and 14 kDa. Wet cooking in pressure (SPC) showed a similar behavior, with 26.8% being the abundance of 14 and 14.3 kDa proteins and 25.3% of the peptides with less than 10 kDa, making up 52.1% of protein content. This hydrolysis promoted an important percentage of peptides and low molecular mass proteins which can have bioactive profile and improve healthy.

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] has gained headway in the international market, mainly in the United States and Japan, because it is a gluten-free cereal, which has resistant starch, high fiber content, also bioactive compounds with antioxidant characteristics. In Brazil, according to the Systematic Survey of Agricultural Production, between 2018 and 2019 there was an increase of more than 15% in tons produced (IBGE, 2020). In Africa and Asia the biggest source of protein is sorghum (Labuschagne, 2018). In Brazil until nowadays sorghum is mainly used for the nutrition of animals. However, recently sorghum is of increased scientific interest for human nutrition in order to decrease Non-communicable Diseases and Injuries (NSCIs)(Awika, 2017). NDCIs, which comprehends cardiovascular diseases (CVDs), cancers, chronic respiratory diseases, diabetes, and others, are closely related to poor diet and kill about 40 million people annually (Haregu et al., 2018).

In a nutritional context marked by NCDIs, the increase in productivity and consumption of sorghum is very relevant, since it is a low-cost cereal, considered an excellent source of phenolic compounds, such as luteolinidine and apigeninidine, in addition to condensed tannins, dietary fibers and resistant starch. *In vitro* and *in vivo* studies have proved that compounds isolated from sorghum, such as peptides, phenolic compounds and fat-soluble compounds (polycosanols) or the consumption of sorghum extruded flour, promote health benefits, especially in treatment of NCDIs (Arbex et al., 2018; Sousa et al., 2019).

However, the evaluation the influence of processing on chemical

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composition, phenolic compounds, anthocyanins, antioxidant capacity and the production of peptides in sorghum are scarce. The studies focus on the evaluation sorghum flour, wet cooking and fermentation, but do not evaluate other processes such as bursting and wet cooking in pressure (Cardoso et al., 2014; Mohapatra et al., 2019).

Therefore, it is important to evaluate the result of processing and heat treatments on sorghum grains, since it is possible these ones interfere in their functionality.

The aim of the present study was to evaluate the nutritional implications of grinding, dry heat, bursting, cooking with and without excess of water and cooking in pressure, evaluating the influence on the centesimal composition, peptides production, phenolic compounds profile and antioxidant activity.

2. Materials and methods

2.1. Materials

Sorghum grains were purchased from local market. The experiments were performed in the Biopractices Complex Laboratories of Vila Velha University, located in Vila Velha, Espírito Santo – Brazil.

2.2. Experimental design and sorghum grains processing

Grains has been submitted to 7 different processing types, as shown in Table 1.

Each processing was performed in 2 repetitions, totaling 14 experimental units. The experiment was organized following a Completely Randomized Design to assess the influence of the different techniques applied on variables responses. To the protein profile, a Completely Randomized Design in a factorial scheme was used, considering the independent variables Sorghum and Molecular Mass, and the interaction Sorghum*Molecular Mass.

2.3. Proximate composition

The moisture content was determined by drying in an oven at 105 °C until constant weight; ash by incineration at 550 °C; protein content by the Kjeldahl method, followed by conversion of the result into raw protein, using the factor 5.75, referring to cereals; total lipids by the Goldfish extraction method; and the carbohydrate content by difference (AOAC, 1990, 2002). Calories (kcal.100 g^{-1}) were determined by Atwater conversion values, which considers that 1 g of proteins correspond to 4 kcal, as well as carbohydrates, while 1 g of lipids correspond to 9 kcal (Merrill and Watt, 1973).

Table 1
Sorghum grains processing.

Treatment	Adopted Procedures
S	Natural sorghum grain, manually macerated (control treatment)
CF	Grind in a household processor (KitchenAid, Spicy model KJA09BV - KitchenAid Inc., St. Joseph, MI, USA), until a homogeneous flour is obtained (Cardoso et al., 2014).
DF	Grind in a household processor (KitchenAid, Spicy model KJA09BV - KitchenAid Inc., St. Joseph, MI, USA) until obtaining a homogeneous flour, and then submit to dry heat in a combined oven (Wictory, Tedesco), at 121 °C for 25 min (Cardoso et al., 2014).
B	Submit the grains to dry heat, using a household microwave until bursting, approximately 1.5 min (Cardoso et al., 2014).
SWC	Submit the grains to wet cooking (20 g - 500 ml) at 100 °C for 50 min (Borges, 2013).
SWCE	Submit the grains to wet cooking with excess water (20 g - 800 ml) at 100 °C for 50 min (Borges, 2013).
SPC	Submerge the grains to wet cooking in a pressure cooker for 15 min (20 g - 500 ml) (Ezeogu et al., 2005)

2.4. Relative abundance of the sorghum proteins

The relative abundance of the sorghum proteins was determined in 2 steps, initially to determine the proteins with the highest molecular mass, in the range of 210 to 8 kDa and, later, proteins with the lowest molecular mass, in the range of 26.60 to 1.06 kDa.

The proteins of higher molecular weight was evaluated on 10% (w/v) polyacrylamide gel under denaturing conditions, in the presence of sodium dodecyl sulfate (SDS) and β-mercaptoethanol, as described by Laemmli (1970). To estimate the molecular mass of proteins, the marker Sigma ColorBurst Marker (Sigma-Aldrich, MI, USA) was used, composed with the following molecular masses 210, 90, 65, 40, 30, 20, 13 and 8 kDa. The gels were stained with Comassie Brilliant Blue and the relative density of the bands was determined by the Gel Doc ™ EZ image documentation system, using the Image Lab v software. 5.2.1 (Bio-Rad, USA).

The protein of lower molecular weight, between 26.60 and 1.06 kDa, was determined by electrophoresis on tricin-SDS gel, using 8 × 10 cm and 7 × 10 cm glass plates and 0.75 mm spacers, according to the method described by Schägger and Jagow (1987). To estimate the molecular mass, the low molecular weight marker of 26,600 has been used; 17,000; 14,200; 6500; 3496 and 1060 Da (M3546 - Ultra Low Range Molecular Weight Marker, Sigma). The images of the gels were digitized on a LAS 500 photo-documenter (GE Healthcare). The relative density of the bands was determined by the Gel Doc ™ EZ image documentation system, using the Image Lab v. 5.2.1 (Bio-Rad, USA).

2.5. Extract preparation for phenolic compounds and antioxidant activity

A 1 g of the sample was weighed and placed in a plastic tube (Falcon®) covered with aluminum foil. Then 10 ml of NEON® 60% methanol was added, stirring manually until complete solubilization. This mixture was taken to the Elmasonic-P ultrasound bath for 25 min, at 40 °C controlled by a coupled thermometer, 37 kHz and 50% amplitude (Altemimi et al., 2016). After that, the tube was centrifuged at 3500 rpm for 10 min, and the supernatant was filtered, making up to 15 ml with deionized water (Krepsky et al., 2012). All procedures were performed in the dark and the extracts were stored under refrigeration, protected from light, until the analysis performance.

2.6. Determination of phenolic compounds

The determination of phenolic compounds was carried out according to Luo et al. (2018). 20 μl of each extract were pipetted in microplate and 80 μl of 10% folin Ciocalteu (Sigma®). After 4 min, 100 μl of 7.5% Sodium Carbonate (Sigma®) was added. After 2 h, the absorbance was read at 765 nm on a SpectraMax ® 190 spectrophotometer. The blank analysis was performed with deionized water replacing the extract.

An analytical curve of gallic acid (Dinâmica Ltda ®), in concentrations of 10–100% was created, generating the regression equation ($y = 0.221x + 0.0406$; $R^2 = 0.9937$) to express the results in milligrams of equivalents of gallic acid per gram of sample.

2.7. Determination of anthocyanin content

The total anthocyanins concentration was carried out by pH difference, according to Giusti and Wrolstad (2001). Absorbance was measured using a UV/VIS KASUAKI® spectrophotometer at wavelengths of 300 and 700 nm. The reading at 700 nm was performed to discount the sample turbidity. Total anthocyanins were determined in potassium chloride buffer pH = 1, according to Awika et al. (2004a). The results were expressed in mg.g^{-1} of sample, using the formula:

$$\text{Monomeric anthocyanin pigment (mg.g}^{-1}) = (A \times MW \times DF \times 1000) / (\varepsilon \times 1)$$

Where: A = absorbance of the sample; MW is the molecular weight =

449.2; DF = dilution factor; Ɛ = absorptivity = 26,900.

2.8. Determination of antioxidant activity

The analysis of antioxidant activity was performed using the ABTS, DPPH and FRAP radicals.

- ABTS: 30 μl of each extract was pipetted in a microplate and added 270 μl of ABTS radical. After 6 min, the absorbance was read at 734 nm on a SpectraMax® 190 spectrophotometer. The blank analysis was performed with concentrated methanol NEON® (Awika et al., 2003). An analytical curve of gallic acid was prepared from the concentrations of 0.00002–0.00250 μg, generating the regression equation ($y = -364.62x + 0.7521$; $R^2 = 0.9854$) to express the results in milligrams of gallic acid equivalent per gram of sample. The results were also expressed through the radical scavenging activity (I): $I\% = [(Abs_0 - Abs_1)/Abs_1]$ x100. Where: Abs_0 = absorbance of the blank and Abs_1 = absorbance of the sample (32.33).
- DPPH: 20 μl of each extract and 280 μl of the DPPH radical were pipetted in a microplate. The reading was performed at 517 nm after 60 min of incubation, protected from light in a SpectraMax® 190 spectrophotometer. The analysis of the blank was performed with NEON® concentrated methanol (Luo et al., 2018). An analytical curve of gallic acid, in concentrations from 0.00002 to 0.00250 μg, was drawn up using the regression equation ($y = -348.34x + 0.7911$; $R^2 = 0.9811$) to express the results in milligrams of gallic acid equivalents per gram of sample. The results were also expressed through the radical scavenging activity (I): $I\% = [(Abs_0 - Abs_1)/Abs_1]$ x100. Where: Abs_0 = absorbance of the blank and Abs_1 = absorbance of the sample (Scherer and Godoy, 2009).
- FRAP: 30 μl of each extract was pipetted in a microplate and added 270 μl of FRAP solution. The reading was performed at 595 nm after 10 min of incubation, protected from light, in a SpectraMax® 190 spectrophotometer. The analysis of the blank was performed with concentrated methanol NEON®. The results were also expressed through the radical scavenging activity (I): $I\% = [(Abs_0 - Abs_1)/Abs_1]$ x100. Where: Abs_0 = absorbance of the blank and Abs_1 = absorbance of the sample (Benzie and Strain, 1996; Scherer and Godoy, 2009).

2.9. Statistical analysis

The results were submitted to analysis of variance (ANOVA) and those that showed a significant difference at the level of 5% of probability, related to the isolated independent variables or in case of the protein profile, also the interaction "Sorghum*Molecular Mass", were compared by the Duncan test at the same probability.

The results have been analyzed by using the statistical software *Statistical Analysis System* (SAS University Studio Online, Cary, North Carolina), online version.

3. Results and discussion

3.1. Proximate composition

The proximate composition results of each processing to which the sorghum grain was submitted are shown in Table 2.

Among the analyzed constituents, all showed significant differences ($p \leq 0.05$) with the processing or the heat treatments used. The highest moisture averages were found in samples subjected to wet heat (SWCE, SWC and SPC), as the cooking process tends to modify the material of the plant's cell wall, by breaking the fibers, promoted by water absorption and slowing down the pericarp. Such a process involves not only the diffusion of water from the surface to the core, but also the transfer of heat to the center of the grain, promoting the starch gelatinization (Bayram, 2005). On the other hand, lower moisture content is observed in products that have been subjected to dry heat, due to the dehydration process.

Table 2
Average and standard deviation of the proximate composition of sorghum grains submitted to different processing.

Treat	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	CHO (%)	TCV (kcal)
S	10.89 ± 0.16^{c}	0.44 ± 0.03^{f}	1.08 ± 0.11^{cd}	5.95 ± 0.06^{c}	81.64 ± 0.18^{ab}	360.09 ± 0.04^{b}
CF	10.85 ± 0.05^{c}	0.87 ± 0.00^{d}	2.24 ± 0.22^{b}	6.36 ± 0.48^{bc}	79.68 ± 0.30^{b}	364.32 ± 1.29^{b}
DF	5.96 ± 0.70^{cd}	1.04 ± 0.06^{c}	1.45 ± 0.94^{bc}	7.58 ± 0.03^{a}	83.97 ± 0.27^{ab}	379.27 ± 7.30^{ab}
B	2.90 ± 1.42^{d}	1.25 ± 0.05^{b}	3.41 ± 0.27^{a}	6.71 ± 0.14^{b}	85.73 ± 1.86^{a}	400.42 ± 4.51^{a}
SWC	62.85 ± 1.12^{a}	0.88 ± 0.01^{d}	1.30 ± 0.52^{bc}	3.85 ± 0.51^{d}	31.13 ± 1.16^{d}	151.63 ± 1.93^{cd}
SWCE	64.68 ± 1.33^{a}	0.68 ± 0.08^{e}	0.71 ± 0.23^{cd}	3.17 ± 0.15^{e}	30.76 ± 1.17^{d}	142.11 ± 6.16^{d}
SPC	56.86 ± 5.64^{b}	1.45 ± 0.13^{a}	0.13 ± 0.12^{d}	2.91 ± 0.20^{e}	38.65 ± 5.20^{c}	167.43 ± 22.64^{c}

Treat: Treatments; TCV: Total caloric value; S: sorghum grains – control; CF: control flour – no heat treatment; DF: dry flour; B: bursting; SWC: grain submitted to wet cooking – no water in excess; SWCE: grain submitted to wet cooking – with water in excess; SPC: grain submitted to under pressure cooking.

Sorghum is a cereal which is source of minerals such as phosphorus, potassium and zinc (Heiniö et al., 2016). The highest percentage of minerals was observed in the SPC, differing significantly ($p \leq 0.05$) from all the others. It can be justified because of high temperature and pressure.

The minerals are mostly located in the germ of the grain. This location may also explain the predominance of ash in B and DF, in which the exposure of the germ content and the reduction of moisture due to dehydration of the grains occur. The SWC and SWCE treatments showed lower averages due to leaching which is justified by the water solubility during cooking process with subsequent disposal of water (Khan et al., 2013).

Sorghum lipids are also located, the most part, in the grain germ (Heiniö et al., 2016), which explains the higher average ($p \leq 0.05$) observed in B, due to the greater exposure of the grain germ content to the bursting. The results with lower averages coincide with the samples submitted to wet heat (SWC, SWCE and SPC), however, they do not differ from the content found in treatment S (control - $p > 0.05$), suggesting there was preservation of the average content from that nutrient. However, when this grain is crushed (CF), its content are exposed and, consequently, the lipid content of the cereal, making it more susceptible to changes in dry heat (DF).

Regarding the protein content, is believed that the reduction does not refer to the proteins leaching in cooking water, despite of being soluble in alcohol-water solution, because they are prolamines, kafirins have a more hydrophobic profile, are mostly composed of nonpolar amino acids and have more affinity for hydrophobic extractors (Belton et al., 2006). Thus, it is believed this result is related to the moisture and dry matter percentage, so that the lower the moisture content, the greater the amount of dry matter and, consequently, the higher the protein content. It indicates that submitting the grain to dry heat (DF and B) increase the protein concentration, due to the moisture loss, while using humid heat (SWC, SWCE and SPC), promotes its reduction.

The carbohydrate content has been higher in the control samples (S and CF) and those submitted to dry heat (DF and B) ($p \leq 0.05$). Among the grains submitted to wet heat, it is observed that the carbohydrate content was higher in SPC ($p \leq 0.05$), a result of the lower water volume compared to SWCE and shorter cooking time than both.

Regarding the total carbohydrate content, it is known that this content is the most prevalent in cereals, including sorghum and, therefore, is closely related to the total caloric value (TCV).

3.2. Relative abundance of the sorghum proteins

Using the electrophoresis technique, the abundance of molecular masses (MM) separated during the analysis was identified, with a total of 17 bands ranging from 3.7 to 195 kDa, found in the 2 gels of different concentrations, 10 and 16.4%.

There was a significant difference ($p \leq 0.05$) in relation to the relative abundance of the sorghum proteins molecular masses in general and in the "Sorghum*Molecular Mass" interaction. Fig. 1 shows the average values of relative abundance for each molecular mass obtained in the analysis.

The reserve proteins are more than 50% of the mature grain protein content and provide an amino acids and carbon reserve for germination (Cunsolo et al., 2012). The main reserve of cereal proteins are prolamins. In sorghum, prolamins are called kafirins and are rich in proline and glutamine and have low digestibility due to the disulfide bonds presence (Labuschagne, 2018). Kafirins correspond to 70–80% of the endosperm proteins, being classified in α-Kafirin (23–27 kDa) that correspond up to 80% of total prolamins; β-kafirin (16, 18 and 20 kDa) that correspond to approximately 5% and γ-kafirin (28 kDa), that correspond to approximately 15% (Belton et al., 2006; Espinosa-Ramírez and Serna-Saldívar, 2016). Based on these MM, the results suggest that 23.2; 24.7 and 27 kDa correspond, probably to α-kafirins, whereas MM of 15.9, 17.2 and 18 kDa correspond to β-kafirins, and that, unlike the literature, their abundances were 21.3% and 16.8%, respectively, indicating there was hydrolysis of part of the sorghum proteins.

For Nelson and Cox (2014) peptides have MM below 10 kDa, while proteins have higher MM. In the obtained study results, there is a predominance of intermediate MM proteins, from 14 to 24.7 kDa, mainly from 14 to 23.7 kDa ($p \leq 0.05$). Among the peptides, there is a predominance of molecular masses of 3.7 and 5.93 kDa, however, without statistical difference from other MM ($p > 0.05$).

Proteins with molecular mass above 97 kDa are related to the dimers formation or protein agglutination. Tannins can form complexes with proteins and thus decrease the digestibility and palatability of food (Larry G Butler et al., 1984). However, despite the grain containing tannins, such a result was not observed since proteins greater than 97 kDa, represented by the 163 and 195 kDa bands represented only 5.5% of the relative abundance in treatments in general. It can be explained because proteins differ in their affinity for tannins (L G Butler, 1981). Thus, in the study presented here, protein hydrolysis caused by processing and heat treatments probably reduced the affinity between these two compounds.

The interaction "Sorghum*Molecular Mass" with a significant difference ($p \leq 0.05$) indicates that, within each treatment, there was variation in the relative abundance related to each molecular mass found. Fig. 2 shows relative abundance of proteins in the different treatments used, demonstrating the interaction results analysis.

The sorghum grain (S) showed higher relative abundance of 14 kDa proteins, but without significant difference ($p > 0.05$) of MM proteins suggestive of α-kafirin (23.2 and 24.7 kDa - 19.9%), β-kafirin (17.2 and 18 kDa - 17.8%) and the peptide with 3.7 kDa (14.8%). CF and DF, showed a more dispersed behavior, like grain (S), with higher relative abundance of MM that possibly refer to α-kafirin and β-kafirin, in addition to 14 kDa proteins, however, without significant difference ($p > 0.05$) of other MM.

According to Brijs et al. (1999) there are proteolytic enzymes located mainly in the outer nucleus layers. Thus, the mechanical processing used, such as manual maceration in the S and the grinding in the CF preparation may have exposed such enzymes, promoting hydrolysis protein, justifying the higher percentage of low molecular weight proteins observed in the results. Besides that, heat treatment favors protein hydrolysis, promoting partial denaturation (Hamaker and Bugusu, 2003).

Since α-kafirins are the predominant proteins in sorghum, only in treatments B and SPC, they did not present a higher relative abundance, with the 14 kDa protein being the most prevalent ($p \leq 0.05$). In the B treatment, all other proteins/peptides show statistical similarity ($p > 0.05$), however 24.3% of the abundance refers to the peptide content with 3.7; 5.93 and 8.9 kDa which, added to the 14 kDa protein abundance, encompass almost half of the proteins/peptides in the product (45.6%). Similar behavior can also be observed with SPC, with 26.8% being the abundance of 14 and 14.3 kDa proteins and 25.3% of the peptides with less than 10 kDa, making up 52.1% of the proteins/peptides in the product. In B, the explosion occurs due to the superheated steam produced inside it and the starchy endosperm expansion, exposing its internal constituents to extrinsic factors, such as high temperature, like in SPC (Salazar-lópez, González-aguilar, Rouzaud-sández, & Robles-sánchez, 2018), thus being able to favor protein hydrolysis, generating peptides.

The sorghum protein profile in the SWC and SWCE treatments were similar, with higher proteins abundance ($p \leq 0.05$) with 23.2 and 14 kDa, which added up to 45.1 and 40.4% respectively. The peptides were found in shorter proportions in both treatments. The wet heat in the sorghum cooking reduces protein digestibility due to formation of new structures by the protein-protein interaction or by the interaction

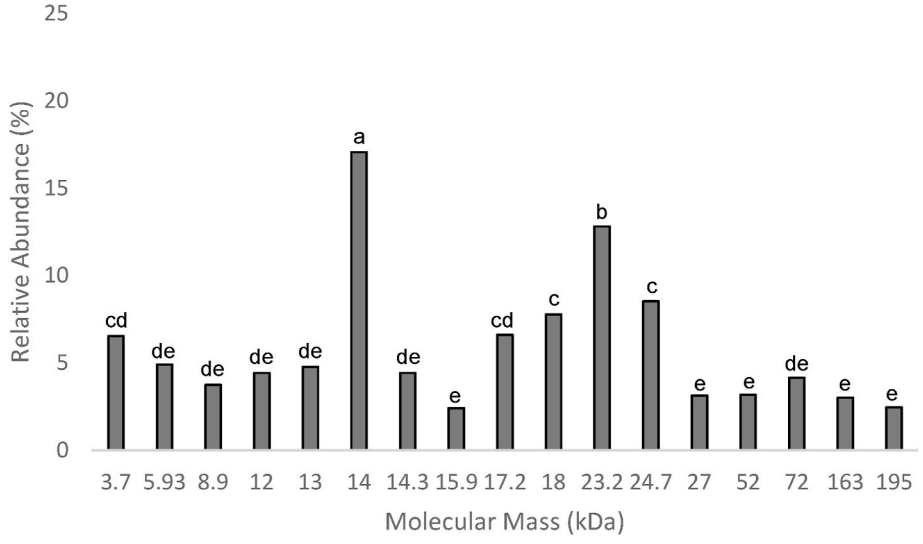

Fig. 1. Relative abundance (%) of the molecular masses (kDa) of sorghum proteins regardless the processes to which they were submitted. Different letters show a significant difference between them by the Duncan test at the 5% probability level.

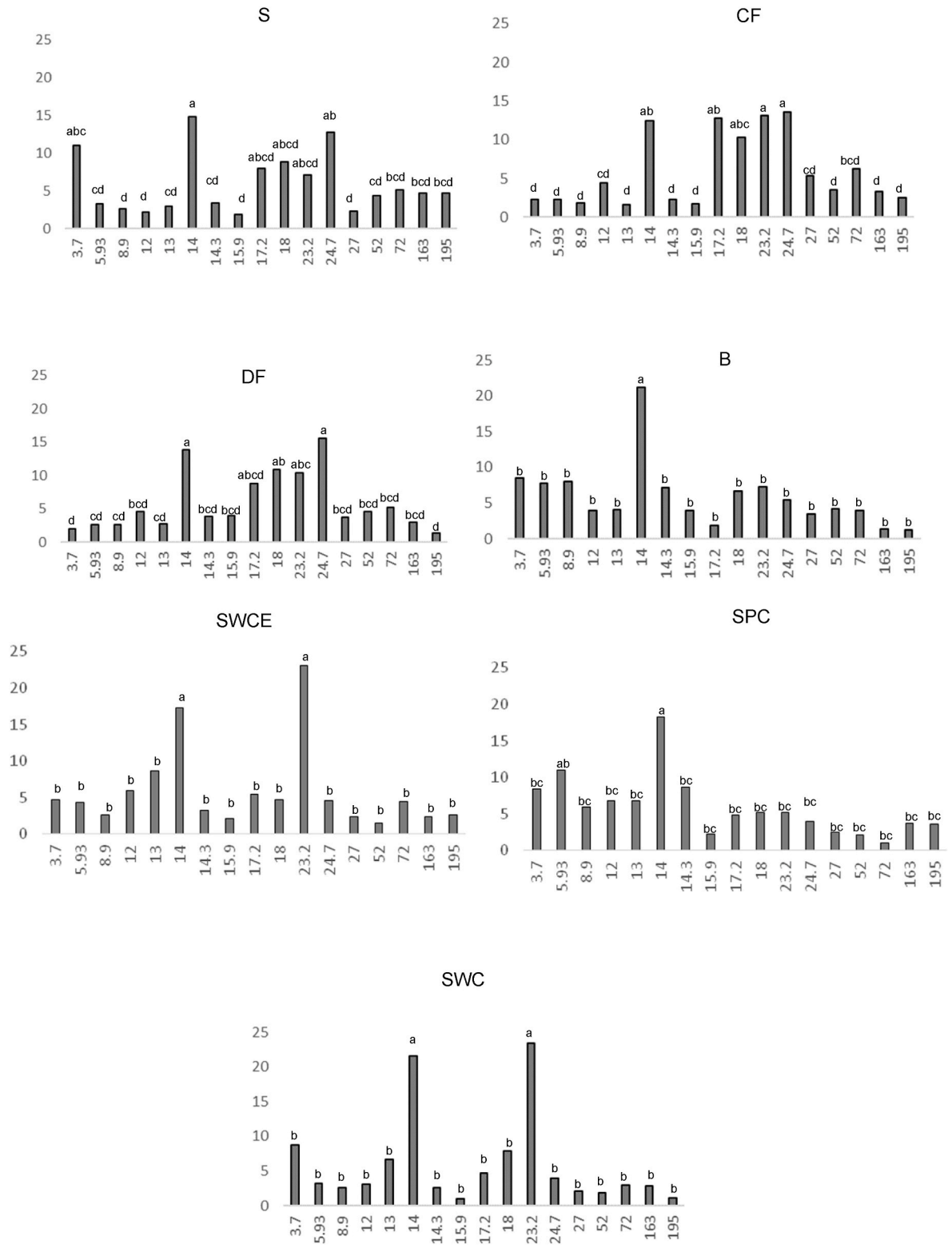

Fig. 2. The relative abundance (%) of protein molecular masses (kDa) in sorghum grains submitted to treatments, from left to right, S: sorghum grains - control; CF: control flour; DF: dry flour; B: bursting; SWC: grain submitted to wet cooking; SWCE: grain submitted to wet cooking with excess water; SPC: grain submitted to under pressure cooking. Different letters show a significant difference between them by the Duncan test at the 5% probability level.

between proteins and other compounds, such as polyphenols, phytates, lipids, starch and cellular components (Hamaker and Bugusu, 2003). On the other hand, Delfino and Canniatti-Brazaca (2010) report that this cooking promotes structural changes in proteins, increasing the susceptibility to enzymatic hydrolysis. Based on our results, there were no sudden changes in proteins or peptides sizes from SWC or SWCE compared to S, allowing us to infer that, given the conditions applied in wet cooking, there was probably no interaction between proteins and other compounds.

In addition, protein hydrolysis promoted by cooking can also be a

positive factor, due to the possibility of generating peptides with bioactive potential, favoring the prevention or even the control of certain diseases. Some studies have shown anticancer, antioxidant and antihypertensive activity of sorghum bioactive peptides, when kafirin was hydrolyzed *in vitro* by enzymes like papain and tripsin (Kamath et al., 2007; Ortíz Cruz et al., 2015; Xu et al., 2019). This is a particular relevance point, because, despite the low protein content of this cereal, the peptides formed have great potential to be beneficial for health, requiring, therefore, further investigation.

3.3. Phenolic compounds and antioxidant profile

Sorghum is considered an excellent source of phenolic compounds, such as 3-deoxyanthocyanidins and tannins, which are closely related to the reduction of chronic inflammation markers, adipogenic markers and reduction of adipose tissue hypertrophy (Arbex et al., 2018). Thus, it is necessary to encourage this cereal consumption, as well as guaranteeing the use of processing or cooking techniques that preserve such compounds. Therefore, this study also proposed to evaluate the impact of these techniques on such compounds.

The obtained results for the phenolic compounds content and antioxidant profile of each processing to which the sorghum grain was submitted are shown in Table 3.

The TPC content has shown a significant difference ($p \leq 0.05$) between the processes that used cooking in dry heat. The highest average was found in flours, especially in DF. According to Heiniö et al. (2016), there are phenolic compounds located unevenly in the outer grain layers and soluble phenolic compounds are compartmentalized inside cell vacuoles, being in free or conjugated form, while the insoluble substances are linked to cell wall structures. Therefore, with processing, they were more exposed to the extracting agent (methanol) and UV waves in the flour than in the grain. In addition, there is a reduction in the moisture content of the sample, which favors the other sorghum constituents' concentration.

Table 3
Average and standard deviation total phenolic compounds (mEq of gallic acid.g sample^{-1}), anthocyanins (mg.g^{-1}), antioxidant profile (mEq of gallic acid.g of sample^{-1}) and radical scavenging activity (%I) of sorghum grains submitted to different processing.

Treat.	TPC	ANT	ABTS		DPPH		FRAP
			mEq	%	mEq	%	%
S	71.47 ± 1.26^{c}	12.70 ± 0.43^{d}	67.10 ± 0.92^{a}	94.91 ± 0.32^{a}	64.92 ± 1.08^{a}	81.56 ± 0.81^{a}	31.87 ± 2.12^{a}
CF	83.11 ± 1.49^{b}	22.67 ± 0.11^{b}	66.64 ± 1.29^{a}	94.45 ± 0.97^{a}	64.65 ± 1.52^{a}	81.56 ± 0.23^{a}	31.59 ± 2.03^{ab}
DF	93.69 ± 2.20^{a}	30.58 ± 0.60^{a}	66.56 ± 0.57^{a}	95.33 ± 0.15^{a}	64.11 ± 0.59^{a}	81.21 ± 0.26^{a}	26.25 ± 0.24^{ab}
B	61.35 ± 6.92^{d}	16.47 ± 1.29^{c}	67.29 ± 1.25^{a}	94.96 ± 0.15^{a}	64.42 ± 0.87^{a}	81.08 ± 1.01^{a}	21.13 ± 2.38^{a}
SWC	6.00 ± 3.03^{e}	4.65 ± 0.09^{e}	43.97 ± 1.83^{c}	36.76 ± 5.89^{d}	43.44 ± 0.79^{bc}	25.52 ± 0.36^{c}	25.88 ± 2.43^{bc}
SWCE	9.40 ± 0.39^{e}	3.87 ± 0.41^{e}	50.77 ± 2.17^{b}	54.83 ± 2.33^{b}	44.14 ± 2.10^{b}	29.46 ± 2.60^{b}	27.82 ± 2.93^{bc}
SPC	4.49 ± 0.35^{e}	3.68 ± 0.53^{e}	46.13 ± 2.12^{c}	43.97 ± 1.50^{c}	41.08 ± 1.44^{c}	22.66 ± 2.55^{c}	21.77 ± 0.19^{c}

TPC: Total phenolic compounds; ANT: Anthocyanins; S: sorghum grains - control; CF: control flour; DF: dry flour; B: bursting; SWC: grain subjected to wet cooking; SWCE: grain submitted to wet cooking with excess water; SPC: grain submitted to cooking under pressure. Different letters in the same column show a significant difference between them by Duncan's test at the 5% probability level.

The grain bursting method produced the highest loss of TPC among the processes submitted to dry heat. Wet cooking significantly reduced ($p \leq 0.05$) the TPC content, however, not differing between the three treatments ($p > 0.05$). The TPC content was at least 6.5 times lower in sorghum submitted to some type of wet heat compared to sorghum treated with dry heat or not submitted to heat treatment, demonstrating a great influence of the presence of water in the reduction of these compounds during cooking.

Cardoso et al. (2014) found results similar to those of this study and pointed out that the higher heat stability of 3-deoxyanthocyanidin, present in sorghum grains, its related to they do not contain a hydroxyl group at the C-3 position. They also report that the reduction in ANT in treatments submitted to wet cooking is due to leaching and, in percentage, to the moisture increase in the grain.

The total phenolic compounds (TPC) of sorghum are divided into three classes: phenolic acids, flavonoids and condensed tannins. Flavonoids are represented by anthocyanins and condensed tannins. Generally, flavonoids are found in greater abundance in vegetables and less in cereals; however, sorghum stands out with important values for this compound (Salazar-lópez et al., 2018).

The evaluation of antioxidant activity has shown similar results both in use of the radical ABTS and DPPH and their respective radical scavenging activity (%I). The grains submitted to wet cooking showed the lowest averages and significantly differed ($p \leq 0.05$) from each other, while the other groups, submitted to dry heat, with higher averages, did not show a difference ($p > 0.05$).

The reduction in TPC, ANT and antioxidant activity in the processes submitted to wet cooking is somehow in reference to the leaching of compounds. As for treatments which used dry heat and those without heat treatment (control), the antioxidant activity did not vary ($p > 0.05$) between them, despite significant variations in the TPC content. This result can be explained due to the higher concentration of bioactive compounds in the food matrix promoted by the humidity reduction, as well as by the exposure of fat-soluble vitamins, present in the germ, such as vitamin E and greater accessibility to carotenoids (Cardoso et al., 2014).

Regarding the results obtained in the analysis of antioxidant activity by the FRAP method, it was possible to observe that this test showed results well below those obtained in the ABTS and DPPH tests. Some limitations from this method are pointed out: the low pH (pH = 3,6) that can prevent the electron transfer from the antioxidant to the compound; the colors interference in plant extracts; and also, the very slow reaction of phenolic compounds in the FRAP assay. In addition, authors also point out a possible interference due to UV–Vis absorption at 593 nm by other compounds and the reaction estimates only the Fe (III) reducing activity (Ou et al., 2002).

In this context, Awika et al. (2003) compared different methods for determining antioxidant activity and concluded that the ABTS method was even more suitable for sorghum than the DPPH method and others under study, as it is a faster method and has consistent results between sorghum evaluated varieties. Given this, the present study also does not recommend the use of the FRAP method to determine the antioxidant activity of sorghum and its products, but also considers the use of ABTS method as the most interesting one.

4. Conclusions

Sorghum has shown very different results when submitted to different processing and heat treatments. As for the proximate composition, a higher concentration of macronutrients was observed in the sorghum submitted to dry heat, as expected, since wet cooking promotes leaching and water absorption, leading to a percentage reduction in components. However, this result was different for ashes, which had a higher average in the SPC, due to the shorter time of exposure to temperature and humidity.

In the electrophoretic profile analysis, a protein predominance in the

14 kDa range was observed, indicating protein hydrolysis, which is related to several factors, among them the proteolytic enzymes exposure, denaturation due to temperature and wet cooking, among other factors. In particular, treatments using pressure have a higher percentage of low MM proteins and peptides.

As for bioactive compounds and antioxidant activity, higher averages were also observed in treatments with dry heat, which are related to the higher total grain content exposure, as well as its moisture content reduction. Therefore, when the objective is to guarantee the TPC and ANT supply, and consequently, higher antioxidant activity, it is more appropriate to make use of dry heat, as they preserve such compounds more efficiently.

It is concluded, then, that the sorghum processing alters its proximate composition, nbioactive compounds and protein profile, generating low MM proteins and peptides. This study reinforces that the sorghum consumption should be more widespread and encouraged, since it is a cereal rich in nutritional and bioactive compounds, with different potential health benefits, as presented.

Declaration of competing interestDoCI

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ana Carolina Bianco-Gomes: Drafting the manuscript, Formal analysis, Conception and design of study, acquisition of data, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. **Luana Dos Santos Nogueira:** acquisition of data, Approval of the version of the manuscript to be published. **Nathiely Ventura Mariano Bono:** acquisition of data, Approval of the version of the manuscript to be published. **Carolina Paula Gouvêa De Souza:** acquisition of data, Approval of the version of the manuscript to be published. **Johara Boldrini-França:** acquisition of data, Approval of the version of the manuscript to be published. **Valdirene Moreira Gomes:** acquisition of data, Approval of the version of the manuscript to be published. **Milena Bellei Cherene:** acquisition of data, Approval of the version of the manuscript to be published. **Natália Elizabeth Galdino Alves Peixoto:** Conception and design of study, Approval of the version of the manuscript to be published. **Christiane Mileib Vasconcelos:** Conception and design of study, Formal analysis, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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