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Evaluation of the efficacy of toasted white and tannin sorghum flours to improve oxidative stress and lipid profile *in vivo*

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Abstract: The objective of the present work was to evaluate and compare the effect of toasted white and tannin sorghum flours on lipid metabolism and antioxidant potential *in vivo*. Male spontaneously hypertensive rats (SHR) were induced to oxidative stress with paracetamol and fed a normal diet (AIN-93M) and diets containing toasted tannin sorghum flour and toasted white sorghum flour (without tannins), replacing 100% cellulose, during 29 days. Hepatotoxicity was assessed by biochemical tests and by quantifying oxidative stress markers. Groups that received toasted sorghum flour with and without tannins showed reduction of alanine aminotransferase (ALT) concentration and improvement of lipid profile, with increase of high-density lipoprotein (HDL) compared to paracetamol control, and did not differ statistically from the AIN-93M control. Moreover, toasted white sorghum flour presented similar efficacy in reducing oxidative stress in liver tissue compared to toasted tannin sorghum flour, although the former had lower total phenolic content and antioxidant capacity, suggesting a greater effect of small phenolic compounds, such as phenolic acids, in the prevention of oxidative stress. Therefore, toasted white and tannin sorghum flours had similar efficacy to improve the lipid profile and oxidative stress in rats treated with paracetamol, constituting potential sources of antioxidants, which can be used as promising ready-to-eat foods and as ingredients for the development of sorghum-based products.

Keywords: bioactive compounds, condensed tannins, oxidative stress, phenolic acids, Sorghum bicolor L.

Practical Application: The health benefits of sorghum coupled with the growing interest of the food industry in producing healthier food products have motivated the development of toasted sorghum flours as potential sources of antioxidants and dietary fiber. We have demonstrated that consumption of toasted white and tannin sorghum flours by rats treated with paracetamol had similar efficacy to improve oxidative stress and lipid profile. Thus, these toasted sorghum flours have great potential to be used by the food industry as ready-to-eat foods or as ingredients in the development of various food products.

1. INTRODUCTION

There is a growing interest in the consumption of sorghum as a staple food because of its facile cultivation, tolerance to unfavorable weather compared to other cereals, gluten-free and health benefits which are associated with the presence of dietary fiber and antioxidants such as phenolic acids, 3-deoxyanthocyanidins, and condensed tannins (Awika & Rooney, 2004; Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2017). Tannins are known to have high antioxidant capacity and they act in reducing starch digestibility (Barros, Awika, & Rooney, 2012). Thus, sorghum is a potential food for the prevention of chronic noncommunicable diseases (NCD), such as cardiovascular diseases, and can be ingested by individuals with celiac disease (Asif, Rooney, Acosta-Sanchez, Mack, & Riaz, 2010).

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Several studies have been developed to elucidate the functional and nutritional properties of sorghum. *In vitro* and *in vivo* studies have demonstrated the effects of sorghum phenolic compounds in the modulation of markers related to NCD such as low- and high-density lipoproteins (LDL and HDL) and C-reactive protein (CRP) (Arbex et al., 2018; Awika, Yang, Browning, & Faraj, 2009; Moraes et al., 2012; Ritchie et al., 2017). In humans, the consumption of extruded tannin sorghum with unfermented probiotic milk has been linked to reduced inflammation and oxidative stress in individuals with chronic kidney disease (Lopes et al., 2018). Moreover, the consumption of extruded sorghum combined with a calorie restricted diet has been associated with reduced body fat in obese men (Anunciação et al., 2019). In animals, the consumption of whole sorghum flour of different genotypes was related to reduced inflammatory response and oxidative stress, maintenance of jejunal morphology, improved glucose tolerance, insulin resistance, and preservation of pancreatic islet function in obese rats (Moraes et al., 2018).

Thus, studies demonstrating the health benefits of sorghum have motivated the development of a variety of sorghum-based products such as cookies, cereal bars, popcorn, pasta, among others (Infante et al., 2017; Paiva, Queiroz, & Garcia, 2019; Palavecino et al., 2019). However, the content of bioactive compounds in

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sorghum is affected by the processing method employed. According to Cardoso et al. (2014), the dry heat method stands out for preserving a higher proportion of antioxidants, such as phenolic compounds and vitamin E, compared to the moist heat method. Thus, the use of dry heat (e.g., toasted flour production) is attractive for the production of foods with high bioactive compounds.

There are currently no studies relating the intake of toasted sorghum flour to the modulation of metabolic and inflammatory changes. Therefore, due to the potential health benefits of sorghum and the growing interest of the food industry in developing healthier foods to meet consumer demands, toasted sorghum flour stands out as a promising food ingredient, source of antioxidants and dietary fiber, to be used in the development of sorghum-based products. The objective of this work was to evaluate and compare the effect of ready-to-eat toasted white and tannin sorghum flours on lipid metabolism and antioxidant potential in spontaneously hypertensive rats (SHR) treated with paracetamol.

2. MATERIALS AND METHODS

2.1 Sorghum genotype samples

The grains selected and analyzed included sorghum genotype BRS 501 with white pericarp, without tannin, and BRS 305 grains, brown pericarp with tannins, from the germplasm bank of Embrapa Milho e Sorgo, research station, located at Sete Lagoas, Minas Gerais, Brazil.

2.2 Toasted sorghum flour preparation

Sorghum grains were ground in a knife mill (C.W. Brabender, Dusburg, Germany) with a 1.7 mm sieve, in order to obtain the flours. To determine flour particle size distribution of the sorghum genotypes, 100 g of each flour sample was sieved, in duplicate, for 10 min in vibratory sieves with mesh openings (mm) of 0.84 - 0.42 - 0.25 - 0.21 - 0.177 - 0.149 and 0,00 (AACC, 2000). The amounts retained on each sieve were weighed and expressed in percentages, and the first sieve (0.84 mm) was discarded. The flours were exposed to 35 °C in an oven with air circulation for 15 h. Flours with adjusted granulometry were submitted to heat treatment (dry heat: direct heat, average temperature of 200 °C) for 6 min. The toasted flours were stored in polyethylene bags at −22 °C until used in the physicochemical analysis and *in vivo* studies.

2.3 *In vitro* characterization of toasted sorghum flour

Total phenolic content of the toasted flours was determined using the Folin–Ciocalteu method, as described by Singleton, and Rossi (1965). The phenolic concentration was expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE/g). The antioxidant capacity (μmoles Trolox equivalent/g sample) of the toasted flours was determined according to the ABTS method described by Re et al. (1999) and the DPPH according to Kim, Lee, Lee, and Lee (2002). The condensed tannins content (mg catechin equivalent/g sample) of the flours was measured using the vanillin/HCl reaction method as described by Price, Scoyoc, and Butler (1978).

The proximate composition analysis (moisture, proteins, lipids, ash, and carbohydrates) was determined by the AOAC (1998). Total dietary fiber (TDF) and insoluble dietary fiber (IDF) contents of the flours were determined according to the enzymatic gravimetric method (AOAC, 1992). The soluble dietary fiber (SDF) content was obtained by the difference between TDF and IDF.

Table 1–Composition of the experimental diets (g/100g).

Ingredients	AIN93-M[a]	ST[b]	SW[c]
Cellulose	5	–	–
Albumin	14	9.88	7.29
Corn starch	46.57	30.72	14.84
Dextrinized starch	15.5	15.5	15.5
Sucrose	10	10	10
Soybean oil (mL)	4	3	2.11
Mineral mix	3.5	3.5	3.5
Vitamin mix	1	1	1
L-cystine	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25
Toasted sorghum flour with tannin	–	25.96	–
Toasted sorghum flour without tannin	–	–	45.33
Calories (kcal)	447.35	446.27	445.30
Carbohydrate (%)	75.80	77.15	77.02
Protein (%)	14.73	13.56	13.92
Lipids (%)	9.47	9.27	9.06
Caloric density (kcal/g)	4.47	4.46	4.45

[a]Standard diet for rodents.
[b]Standard diet for rodents +100% substitution of cellulose for toasted sorghum flour with tannins.
[c]Standard diet for rodents +100% substitution of cellulose for toasted sorghum flour without tannins.

2.4 Biological assay

2.4.1 Experimental design. Twenty-seven adult male rats (*Rattus norvergicus*, albinus, lineage SHR), supplied by the Animal Laboratory of Biological Science and Health Center (Universidade Federal de Viçosa, Brazil) were used in this study in order to maximize the effects of paracetamol in inducing oxidative stress (Binda et al., 2001). They were randomly divided into four groups, so that the difference between mean weights did not exceed 2 g in each group. The animals were housed under standard conditions in individual cages with 12-h light/dark cycles, and a temperature of 22 °C. Distilled water was provided *ad libitum*. The animals were acclimated for 1 week before induction of oxidative stress. All experimental procedures were performed in accordance with the Ethics Committee for Animal Research of the Universidade Federal de Viçosa (approval number 16/2018).

The experimental diets (Table 1) were based on the formulations of AIN-93M diet with modification to provide toasted sorghum flours as a source of 100% of the recommended fiber to rodents (Reeves, Nielsen, & Fahey, 1993). The other nutrients of the diet (albumin, corn starch, and soybean oil) were adjusted to provide isoproteic, normolipidic, and isocaloric diets with or without the addition of toasted sorghum flour. Briefly, the minor ingredients, such as vitamins, minerals, L-cystine, and choline bitartrate, were mixed with sucrose until obtaining a homogeneous mixture. Then, the others ingredients were added, from small to large amounts, mixing all ingredients except the oil. The mixture was placed into the mixing bowl. The bowl with cover was attached to the mixer and the ingredients were mixed for 5 min at the slowest speed. Then, the oil was slowly added and the diet mixed for about 15 min at slow speed, stopped every 5 min to avoid heat generation and possible loss of vitamins and oxidation of fatty acid. The diets were packed in dark polyethylene bags and stored at −20 °C to minimize oxidation.

After the first induction of oxidative stress, the animals received the following experimental diets: normal control group was fed a standard diet (AIN-93M); paracetamol control group received standard diet plus paracetamol administration (AIN-93M + P); test groups were fed toasted sorghum flour with tannins plus paracetamol administration (ST + P); and toasted sorghum flour without tannin plus paracetamol administration (SW + P) (Figure 1).

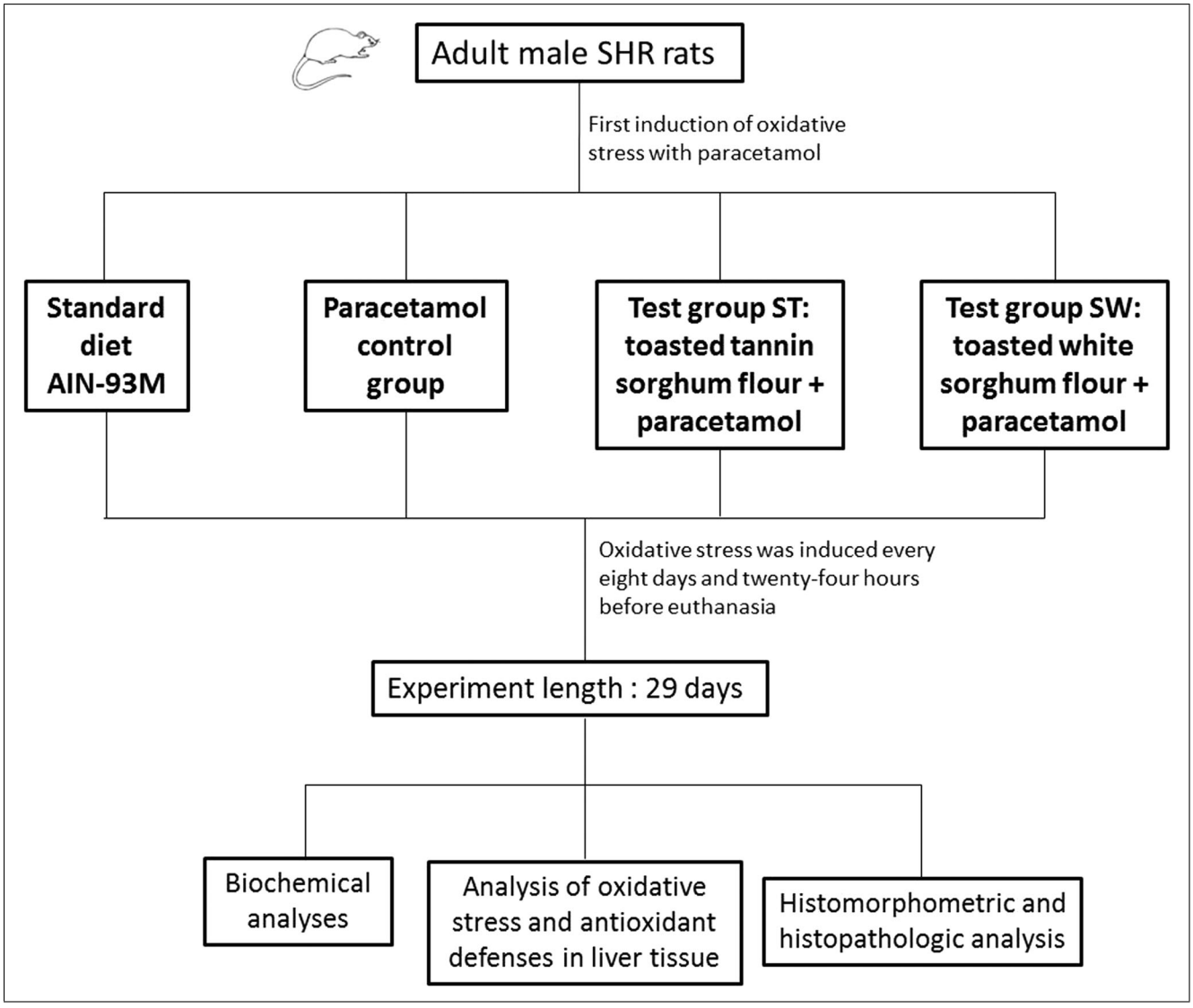

Figure 1—Experimental design.

The toxicity assay was based on Noh et al. (2015) with modifications. Oxidative stress was induced every 8 days and 24 hr before euthanasia by oral gavage at a concentration of 500 milligram per kilogram in a volume of approximately 1 mL per animal dissolved in warm water (37 °C). The doses of 500 milligram per kilogram were chosen according to the literature and paracetamol solubility limit (Arnaiz, Llesuy, Cutrin, & Boveris, 1995; Kelava, Ćavar, & Čulo, 2010; Pang et al., 2016).

Animals were weighed weekly during the experimental period and the feed efficiency ratio (FER) was determined, which represents the relationship between weight gain (WG) and food intake (FI) by the animals: FER = WG (g)/FI (g) (Silva et al., 2020).

After 29 days, the animals were fasted overnight, anesthetized with isoflurane (Isoforine, Cristália®), and euthanized via cardiac puncture. The collected blood was centrifuged in test tubes at 4 °C and 2865 × *g* for 10 min (Fanem–204, São Paulo, Brazil) to obtain serum. The liver was isolated, immediately frozen in liquid nitrogen, and stored at −80 °C. Some samples of the liver tissue were washed in saline solution, fixed in 10% formaldehyde, and kept at room temperature for further histological analysis.

2.4.2 Biochemical analyses. The lipid profile (total cholesterol [TC], high-density lipoprotein-cholesterol [HDLc], low-density lipoprotein-cholesterol [LDLc] and triacylglycerol), renal function (creatinine, uric acid, urea), hepatic function (aspartate aminotransferase, alanine aminotransferase), C- reactive protein (CRP), and fasting glucose were assessed in the Laboratory of clinical Analysis of the Health Division of UFV, Viçosa, MG, Brazil.

2.4.3 Analysis of oxidative stress and antioxidant defenses in liver tissue. Liver samples (200 mg) were macerated in microtubes before the addition of 800 μL 50 mM phosphate buffer (pH 7.4). The samples were macerated and centrifuged at 10,000 × *g* and 4 °C for 15 min, and then supernatants were collected and stored in an ultrafreezer until analysis.

Malondialdehyde (MDA) in liver homogenates was determined via the thiobarbituric acid reactive substances (TBARS) assay (Kohn & Liversedge, 1944; Pyles, Stejskal, & Einzig, 1993). The final values were calculated using an extinction coefficient 1.56 × 10^5 mol/L/cm (Buege & Aust, 1978). The results were expressed as μM MDA per milligram of protein (μM MDA/mg protein).

Protein concentrations in the liver homogenates were quantified according to the Bradford method (Bradford, 1976).

The activity of superoxide dismutase (SOD) was expressed in relative units (U), with a unit of SOD defined as the amount of enzyme that inhibits the oxidation rate of pyrogallol by 50%. For the sample assay, 30 μL of liver homogenate was used. The samples were incubated in an oven at 37 °C for 8 min. The absorbance was measured at 570 nm, and the results were expressed as U of SOD/mg protein (Marklund, 1985).

Catalase activity (CAT) was determined based on its ability to convert hydrogen peroxide (H_2O_2) into water and molecular oxygen, as described by Aebi (1984).

In a polypropylene tube, 20 μL of the supernatant (diluted 1:10, in water), 1 mL of 100 mM phosphate buffer (pH 7.2) + H_2O_2 (40 μL H_2O_2 30% in 25 mL of buffer). The absorbances at 240 nm were determined at 0, 30, and 60 s in a spectrophotometer (T70 + UV/VIS Spectrometer, Taylors, USA). The absorbance used for the calculation was the delta obtained from the measured absorbances at times 0 and 60 s (final absorbance−initial absorbance). One unit (U) of catalase is equivalent to the hydrolysis of 1 mol of H2O2 ($\varepsilon = 39.4$ L/mol/cm) per minute. CAT was calculated according to the Lambert Beer law and expressed as nmol.min/mg of protein.

Nitric oxide concentration was determined in liver homogenates according to Griess (1879). A standard curve was prepared by the addition of sodium nitrite standard and buffer. The absorbance was measured at 570 nm on a Thermo Scientific-Multiskan™ GO reader (Thermo Fisher Scientific, Waltham, MA, USA) and the results were expressed as μM.

2.4.4 Hepatic tissue: Histomorphometric and histopathologic analysis. The hepatic tissue samples were fixed in resin. Sections were cut at 3 μm thick, mounted on glass slides, and stained with toluidine blue and sodium borate. Glass slides analysis was performed under a color microscope (Olympus, BX53, Tokyo, Japan). The histological sections images were captured in a 40 × objective. Fat vesicles, cytoplasm, nucleus, macrophage, and blood vessels were analyzed using Image J® version 1.5 software (Wayne Rasband). The quantitative analysis of hepatocytes diameter was conducted by the system ImagePro-Plus® version 4.5 (Media Cybernetcs, Rockville, USA), using the mean of 50 cells for each group.

2.5 Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS) software version 9.1 and expressed as the mean ± standard deviation. Statistically significant differences between groups were calculated using variance analysis (ANOVA) followed by the Tukey test for characterization analysis and SNK test to compare means of test groups with control group. *P*-values lower than 0.05 ($P < 0.05$) were considered statistically significant. Correlation between consumption of total phenolics, consumption of tannins, and SOD activity was measured by Pearson's correlation coefficient (r) ($\alpha =$ 5%).

3. RESULTS AND DISCUSSION

3.1 Physicochemical characterization

3.1.1 Particle size distribution of sorghum flours. The ground sorghum flour was sifted through a sieve for particle size analysis. The properties of the retained flour are presented in Table 2. Currently, there is no standard method for the classification of sorghum flour, therefore, we followed the 66-20 method proposed by AACC International (2000). The particle size of the flour was determined in a preliminary study (unpublished data) aimed at producing a model flour with acceptable grain size. The highest percentage of flour was retained in a 0.42 mm mesh sieve. Similar results were obtained by Martino et al. (2012), who used the same methodology and genotype evaluated in this study. In addition, the authors characterized the flour as coarse and hard grain, similar to whole corn flour. Thus, the sorghum flour used in our study has similar particle size distribution as other cereals, and can be used for different food preparations.

Table 2–Particle size distribution (%) of sorghum flours.

	ST	SW
Sieves openings (mm)	Particles retention (%)	
0.84[a]	8.22 ± 0.43	5.16 ± 0.00
0.42	63.43 ± 1.77	65.72 ± 1.87
0.25	3.94 ± 1.14	5.20 ± 0.33
0.21	6.98 ± 0.06	10.41 ± 2.27
0.177	14.47 ± 2.35	9.85 ± 1.63
0.149	1.24 ± 0.70	2.09 ± 0.66
0.00	1.00 ± 0.74	0.79 ± 0.80

ST, sorghum flour with tannin; SW, sorghum flour without tannin.
Data are expressed as means values ± standard deviation.
[a]Amount of flour in the first sieve (0.84 mm) was discarded. Flours with adjusted granulometry (from 0.42 to 0.00 mm) were used in this study.

Table 3–Proximate composition of toasted sorghum flours (g/100g).

	Flours	
Nutrients	ST	SW
Proteins	13.17 ± 1.19a	12.22 ± 0.42a
Carbohydrates	59.15 ± 1.08b	68.62 ± 1.01a
Lipids	3.83 ± 0.13a	4.16 ± 0.26a
Total dietary fiber	20.81 ± 2.33a	12.02 ± 0.55b
Soluble dietary fiber	1.56 ± 0.65a	1.00 ± 0.09a
Insoluble dietary fiber	19.25 ± 1.67a	11.02 ± 0.65b
Ash	1.53 ± 0.01b	1.72 ± 0.04a
Moisture	1.56 ± 0.01a	1.33 ± 0.10b
Kcal/100 g	323.16 ± 9.5b	359.46 ± 1.64a

ST, toasted sorghum flour with tannin; SW, toasted sorghum flour without tannin.
Data are expressed as means values ± standard deviation; same letters on the line do not differ by Tukey test at 5% probability.

3.1.2 Proximate composition analysis of toasted sorghum flours. Sorghum-based toasted flours showed differences in the contents of carbohydrate, dietary fiber, ash, moisture, and calories ($P < 0.05$) (Table 3). Toasted tannin sorghum flour (ST) presented higher dietary fiber content, while toasted white sorghum flour (SW) presented a higher amount of carbohydrates, ash, and calories (Table 3). Dietary fiber content was much higher in ST because the sorghum genotype BRS 305 has naturally a high resistant starch content (Teixeira et al., 2016), which is a type of dietary fiber. For both toasted flours, the fraction of IDF was higher than soluble fiber, which is expected given that most cereals have more IDF (Wu, Qiu, Wang, & Li, 2020). However, the quantity of IDF observed was higher compared to another study conducted on sorghum flour of the same genotype (Martino et al., 2012).

Carbohydrates are the main macronutrient found in cereals, which was also observed in our results (Table 3). Similar results were obtained by Martino et al. (2012) regarding both carbohydrate and ash content, as the authors also reported higher amounts

Table 4–Antioxidant capacity, total phenolics, and condensed tannins of toasted sorghum flours.

Analysis	Flours	
	ST	SW
Antioxidant capacity- ABTS (µMol de TE/g)	81.10 ± 2.74a	1.51 ± 0.05b
Antioxidant capacity- DPPH (µMol de TE/g)	80.72 ± 4.02a	2.02 ± 0.63b
Total phenolics (mg GAE/g)	12.44 ± 0.31a	0.69 ± 0.03b
Condensed tannins (mg CE/g)	75.32 ± 5.13	nd

ST, toasted sorghum flour with tannins; SW, toasted sorghum flour without tannins; nd, not detected; TE, trolox equivalent; GAE, gallic acid equivalent; CE, catechin equivalent.
Data are expressed as means values ± standard deviation; same letters on the line do not differ by Tukey test at 5% probability.

of them in white sorghum flour when compared to sorghum flour with tannins.

On average, toasted tannin sorghum flour presented higher protein and dietary fiber whereas the toasted white sorghum flour had high carbohydrate, lipid, and ash contents. These results may be related to differences in genotypes. Moreover, the moisture content of the toasted flours (less than 2%) was lower than sorghum flours reported in the literature (10% to 13%) (Antunes et al., 2007; Martino et al., 2012), due to the dry heat method they underwent. In general, the toasted flours presented similar composition to sorghum flours (Antunes et al., 2007; Martino et al., 2012; Ragaee et al., 2006), except for dietary fiber and moisture contents.

3.1.3 Antioxidant capacity, total phenolics, and condensed tannins. Sorghum-based toasted flours showed significant differences in total phenolic content and antioxidant capacity ($P < 0.05$) (Table 4). Toasted sorghum flour with tannins have higher phenolic content, as well as higher antioxidant capacity ($P < 0.05$), which may be due to differences in genotypes. White sorghum has phenolic acids in its composition, such as coumaric and ferulic acids; whereas, brown sorghum has phenolic acids in addition to flavonoids and condensed tannins, resulting in higher total phenolic content and antioxidant capacity (Hahn et al., 1983; Rao et al., 2018).

Toasted sorghum flour with tannins had a high content of condensed tannins, the same was absent in white sorghum flour (Table 4). Besides their high antioxidant capacity, condensed tannins can interact with starch, decreasing its digestibility and thereby increasing resistant starch content, which acts as dietary fiber (Barros et al., 2012). In this sense, sorghum with tannins can play an important role in the prevention of NCD and in the protection against oxidative stress. The high antioxidant capacity of the sorghum genotype BRS 305 may be associated to its greater potential in controlling biochemical changes and in preventing oxidative stress in the rats evaluated in this study, which are more sensitive to metabolic alterations. The result of the present study is in agreement with that observed by Oliveira et al. (2017) and Moraes et al. (2015) who analyzed sorghum genotypes with brown pericarp and pigmented testa (SC 21, SC 319).

3.2 *In vivo* study

3.2.1 Food intake. Diets had similar energy density and, thus, they provided similar WG among the experimental groups ($P \geq 0.05$) (Table 5). There were no differences in food intake (FI) and food efficiency coefficient (CEA) between the control and test groups ($P \geq 0.05$) (Table 5). Similar results were observed in the study of Moraes et al. (2012) which evaluated the effect of adding three different sorghum genotypes (BRS 305, BRS309, and BRS 310) to high fat diets. Sorghum grains, especially those with pigmented testa and high tannin content, may decrease FI due to astringency and low starch digestibility (Barros et al., 2012). Despite this fact, no difference in FI and WG was observed in the present study. This result may be due to the dry heat treatment applied to the flours, as reported by Moraes et al. (2012), where dry heat did not alter the food consumption of animals fed with sorghum flours of different genotypes (BRS305, BRS309, and BRS310).

3.2.2 Biochemical tests. TC increased in the SW + P group compared to paracetamol control. This change is probably associated with increased high-density lipoprotein in the test groups that matched the normal control group (Table 6). The addition of paracetamol in the control group increased low-density lipoprotein compared to normal control ($P < 0.05$). The tests groups were not able to reduce LDL-c levels compared to paracetamol control, however, they kept the LDL-c levels equivalent to the normal control ($P \geq 0.05$) (Table 6). These changes were beneficial to the test groups, since atherogenic index (AI) decreased and became similar to the normal control group, whereas in the paracetamol control group this index increased ($P < 0.05$). A trend similar to that of AI was observed in the ratio of TC/HDL-c, indicating the protective effect of toasted sorghum flour on lipid metabolism. On the contrary, Moraes et al. (2018) observed that the addition of 19% whole sorghum (SC 21 genotype) to the diet of obese rats reduced HDL-c levels without altering TC and LDL-c. This discrepancy may be related to differences in sorghum varieties, experimental model, and the percentage of sorghum added to diet. We utilized approximately 26% of sorghum with tannins and 45% of white sorghum, suggesting that higher sorghum consumption is associated with increased HDL-c.

On the other hand, Arbex et al. (2018) evaluated the effect of extruded sorghum flour on adiposity modulation and inflammation in obese rats and found high HDL-c levels in the group that received extruded sorghum flour in replacement of 100% of the recommended dietary fiber. Compared to the present study, despite the difference in heat treatments, both toasted flours were efficient in promoting high HDL-c, indicating the protective effect of sorghum consumption on lipid profile improvement.

Increased AST and ALT concentration was observed in the paracetamol control group ($P < 0.05$) (Table 6). However, AST and ALT concentrations were similar in the test and normal control groups. In the present study, the inhibition of ALT in the test groups may be related to the suppression of factors related to lipid metabolism, leading to an improvement in the lipid profile of the animals. This indicates that toasted sorghum flour promotes liver protection, preventing the occurrence of lipid accumulation in the liver, in agreement with the study of Sousa et al. (2018), where hepatic steatosis reduction was observed. In addition, the reduction of these markers is a protective factor against inflammation, although c-reactive protein (CRP) was not altered, which is justifiable since it presents early elevation and rapid return to basal levels after improvement of the inflammatory process due to the shorter half-life. Similar results were observed by Arbex et al. (2018), who evaluated the effect of combining extruded sorghum flour and high fat diet on the modulation of adiposity and inflammation in obese rats. Another possible contributing factor to the reduction of these markers is the phenolic compounds present in sorghum, especially phenolic acids, considering that both tannin

Table 5–Biometric measurements, changes in food intake, and consumption of bioactive compounds of the experimental animals after the treatments with toasted sorghum flours, for 4 weeks (g).

Measures	AIN-93M	AIN-93M+P	ST+P	SW+P
Initial weight	189.20 ± 20.83a	203.91 ± 16.54a	197.53 ± 9.59a	188.62 ± 17.84a
Final weight	251.59 ± 29.09a	245.39 ± 21.21a	255.64 ± 15.49a	263.37 ± 25.22a
Food intake	556.49 ± 64.01a	519.32 ± 38.45a	566.94 ± 29.36a	570.06 ± 15.77a
FER	0.11 ± 0.05a	0.08 ± 0.04a	0.10 ± 0.03a	0.13 ± 0.04a
Tannin intake	–	–	11.27 ± 0.57	–
TP intake	–	–	1.86 ± 0.09a	0.18 ± 0.00b

AIN-93M = standard diet;
AIN-93M+P, Standard diet + paracetamol.
ST+P, toasted sorghum flour with tannins + paracetamol.
SW+P, toasted sorghum flour without tannins + paracetamol.
FER, feed efficiency ratio; TP, total phenolics.
Data are expressed as means values ± standard deviation; same letters on the line do not differ by SNK test at 5% probability.

Table 6–Blood biochemistry values and oxidative stress markers of experimental animals after the treatment with toasted sorghum flours, for 4 weeks (mg/dL).

Variables	AIN-93M	AIN-93M + P	ST + P	SW + P
TC	56.29 ± 7.36ab	51.75 ± 5.83b	60.60 ± 5.27ab	62.86 ± 7.86a
HDL-c	28.33 ± 2.42a	19.25 ± 3.62b	30.60 ± 2.61a	30.33 ± 2.25a
LDL-c	16.43 ± 3.87b	22.25 ± 3.06a	19.80 ± 1.79ab	21.29 ± 4.49ab
AI*	0.26 ± 0.10b	0.43 ± 0.11a	0.21 ± 0.08b	0.27 ± 0.05b
TC/HDLc	1.92 ± 0.10b	2.74 ± 0.34a	1.98 ± 0.07b	2.12 ± 0.15b
Triglycerides	51.00 ± 13.05a	51.25 ± 6.43a	50.60 ± 8.88a	57.00 ± 3.74a
Uric acid	1.54 ± 0.57a	1.03 ± 0.40a	1.20 ± 0.75a	0.99 ± 0.30a
Glucose	143.33 ± 8.04a	181.75 ± 41.67a	135.20 ± 33.84a	147.71 ± 22.63a
Creatinine	0.64 ± 0.07a	0.64 ± 0.03a	0.67 ± 0.02a	0.66 ± 0.05a
Urea	31.57 ± 3.41a	32.38 ± 4.03a	30.60 ± 6.11a	30.14 ± 5.58a
CRP (mg/L)	<6a	<6a	<6a	<6a
AST (U/L)	304.14 ± 158.45ab	466.25 ± 111.86a	300.00 ± 157.58ab	232.50 ± 58.19b
ALT (U/L)	49.29 ± 17.51b	200.88 ± 125.62a	52.20 ± 13.39b	68.43 ± 35.65b
Nitric oxide	13.25 ± 2.64a	10.52 ± 3.45a	12.07 ± 4.95a	11.81 ± 2.85a
MDA	1.63 ± 0.75a	2.07 ± 0.84a	1.57 ± 0.35a	1.63 ± 0.65a

AIN-93M, standard diet.
AIN-93M+P, Standard diet + paracetamol.
ST+P, toasted sorghum flour with tannin + paracetamol.
SW+P, toasted sorghum flour without tannin + paracetamol.
AI, atherogenic index *Niroumand, Khajedaluee, & Khadem-rezaiyan, (2015)
TC, total cholesterol; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MDA, malondialdehyde.
Data are expressed as means values ± standard deviation; same letters on the line do not differ by SNK test at 5% probability. Nitric oxide is expressed as μM. MDA is expressed as μM/mg of protein.

sorghum and white sorghum were effective in not altering the levels of liver enzymes, keeping CRP concentration within a normal range.

Thus, our study confirmed that although paracetamol increases ALT and AST, which may impair liver function, and consumption of toasted sorghum flour exerted a protective effect against liver damage as observed by previous studies. Girish et al. (2009), evaluated the hepatoprotective effect of antioxidants on paracetamol-induced toxicity in mice, and demonstrated that animals receiving pretreatment with antioxidants had reduced AST and ALT levels compared to group treated with paracetamol. Similar results were reported by Pang et al. (2016) in the evaluation of protective mechanism of caffeic acid in paracetamol-induced liver injury in mice. The authors demonstrated a reduction in serum AST and ALT levels in the groups on 30 mg/kg of caffeic acid when compared to the group on paracetamol alone.

The presence of paracetamol in the control diet did not alter the levels of triglycerides, uric acid, glucose, creatinine, urea and CRP, nor did the addition of toasted sorghum flours in the groups receiving paracetamol ($P \geq 0.05$) (Table 6), indicating that paracetamol combined with the sorghum flour diet or not, did not alter markers of renal function and glycemia. In addition, the levels of nitric oxide and MDA did not differ between the experimental groups ($P \geq 0.05$) (Table 6). The results demonstrate that the oxidative stress induction in SHRs with paracetamol was a good experimental design to evaluate lipid profile and hepatic function.

The groups treated with toasted sorghum flour with and without tannins had similar catalase enzyme activity as the control group with paracetamol. The normal control group had the highest average catalase enzyme activity, differing statistically from the other groups ($P < 0.05$) (Figure 2A). The expression of the antioxidant enzyme SOD was higher ($P < 0.05$) in the normal control, paracetamol control, and SW + P groups (Figure 2B). On the other hand, SOD showed lower activity in the group that received toasted tannin sorghum flour ($P < 0.05$). This result may be due to the high antioxidant capacity observed in the tannin sorghum (approximately 81 μMol TE/g), suggesting that the bioactive compounds present in sorghum, such as phenolic acids, flavonoids, and condensed tannins act directly on the elimination of reactive oxygen species as well as in conjunction with antioxidant enzymes (Bellassoued et al., 2015). White sorghum cultivars have phenolic acids as their main phenolic compounds, while sorghum cultivars

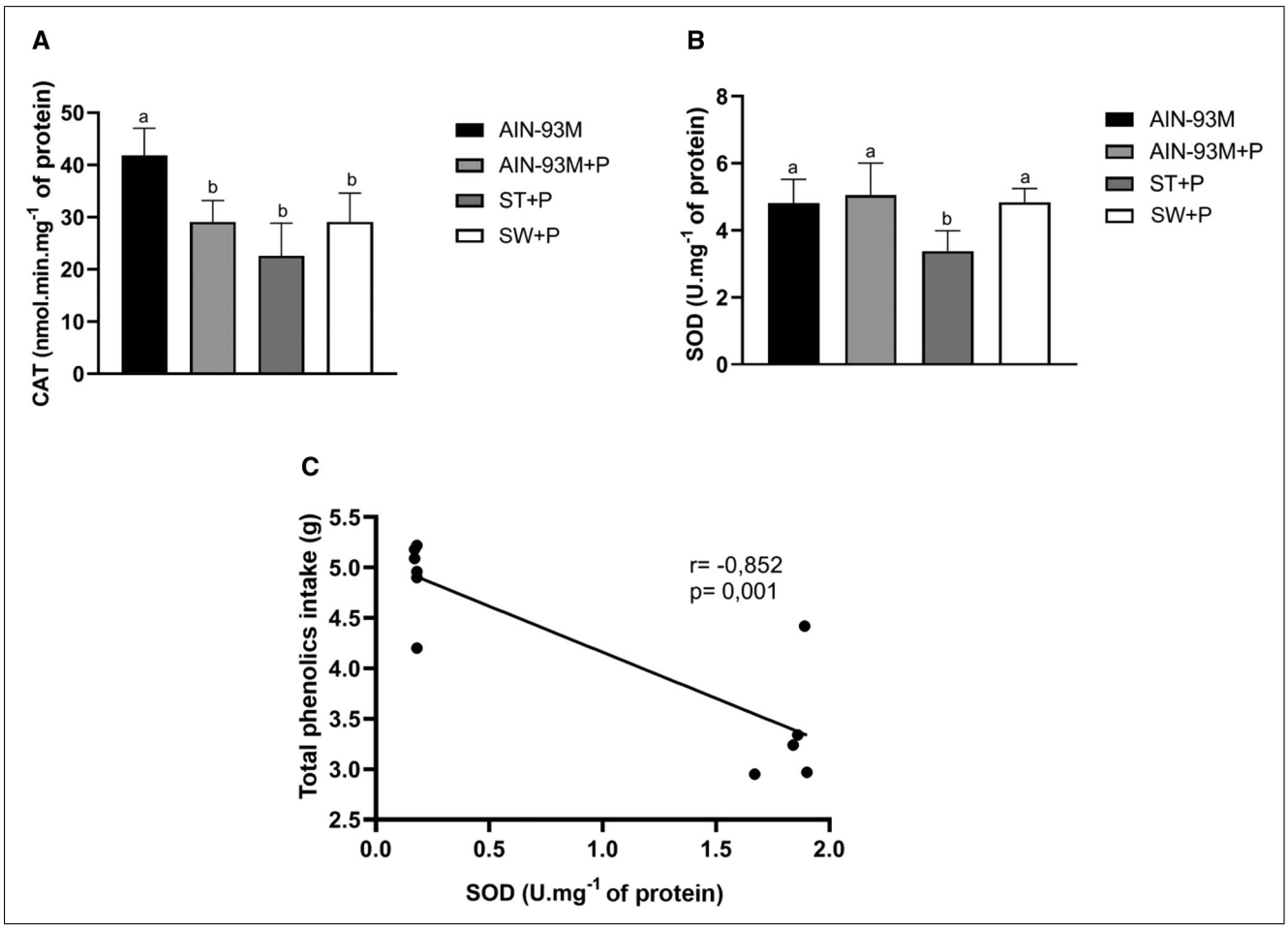

Figure 2–Liver antioxidant enzymes activity (A and B) of experimental animals after the treatment with toasted sorghum flours, for 4 weeks; and the correlation between total phenolic consumption and superoxide dismutase (SOD) activity (C). Means followed by the same letter, in the same graph (A or B), do not differ by SNK test at 5% probability. AIN-93M, standard diet; AIN-93M + P, Standard diet + paracetamol; ST + P, toasted sorghum flour with tannin + paracetamol; SW + P, toasted sorghum flour without tannin + paracetamol.

containing high tannin contents have phenolic acids, flavonoids, and condensed tannins (Hahn et al., 1983; Rao et al., 2018).

In this sense, toasted sorghum flour with tannins showed excellent results due to the presence of phenolic acids, flavonoids, and possibly low-molecular weight condensed tannins (Barros, Awika, & Rooney, 2014). Despite the predominance of polymeric proanthocyanidins, sorghum with tannins also has catechins and low-molecular weight proanthocyanidins which are more absorbed in the body (Barros et al., 2014). In addition, thermal processing of sorghum can alter the content and molecular weight of proanthocyanidins, facilitating their absorption in the body (Awika, Dykes, Gu, Rooney, & Prior, 2003). Sousa et al. (2019) demonstrated the protective effect of extruded sorghum flour on the modulation of intestinal microbiota, inflammation, and oxidative stress in obese rats on a high-fat diet. The effects were observed when the extruded sorghum flour replaced 50% and 100% of the dietary fiber, contributing to the reduced production of reactive oxygen species, increased expression of antioxidant enzymes, such as SOD, as well as improvement in intestinal dysbiosis. In the present work, the mild heat treatment applied did not promote drastic changes in the chemical composition of the toasted flours, in fact the bioactive compounds were maintained. This result is in agreement with Cardoso et al. (2014) who found that dry heat stood out for preserving a higher proportion of antioxidants, such as phenolic compounds, compared to the moist heat method.

On the other hand, white sorghum showed similar efficacy as tannin sorghum, although it had lower total phenolic content and antioxidant capacity. This fact can be attributed to the higher bioavailability of their phenolic acids when compared to condensed tannins (Scalbert & Williamson, 2000). This is because the chemical structure of polyphenols and possible interactions with other compounds determine their rate and extent of intestinal absorption. Barros et al. (2014) evaluated the effects of phenolic extracts of sorghum containing proanthocyanidins of different molecular weights on the formation of resistant starch. The authors demonstrated that the higher the degree of polymerization, the greater the interaction of proanthocyanidins with starch. Thus, the high molecular weight of the condensed tannins present in sorghum implies they are not absorbed in the gastrointestinal tract. Therefore, although white sorghum does not contain condensed tannins in its composition, it contains other bioactive compounds, such as phenolic acids, which may have beneficial effects on health (Hahn et al., 1983).

In the groups that received toasted sorghum flours with and without tannins, a significant correlation ($r = -0.852$) ($P = 0.001$) was observed between the consumption of total phenolics and SOD levels (Figure 2C), showing that the higher the consumption

Table 7–Cellular components in hepatic tissue of the experimental animals after the treatments with toasted sorghum flours, for 4 weeks.

Cellular components (%)	AIN-93M	AIN-93M + P	ST + P	SW + P
Nucleus	9.59 ± 2.88a	10.57 ± 3.45a	8.76 ± 2.05a	9.86 ± 1.65a
Cytoplasm	75.36 ± 3.63a	80.59 ± 3.64a	80.10 ± 2.13a	80.00 ± 1.35a
Fat	2.59 ± 2.52a	0.48 ± 0.53a	0.52 ± 0.89a	0.34 ± 0.65a
Macrophage	0.88 ± 0.65a	0.48 ± 0.24a	0.50 ± 0.39a	0.77 ± 0.45a
Blood vessel	11.59 ± 2.82a	7.88 ± 1.50a	10.11 ± 1.12a	9.02 ± 2.35a

AIN-93M, standard diet.
AIN-93M+P, Standard diet + paracetamol.
ST+P, toasted sorghum flour with tannins + paracetamol.
SW+P, toasted sorghum flour without tannins + paracetamol.
Data are expressed as means values ± standard deviation; same letters on the line do not differ by SNK test at 5% probability.

of total phenolics, the lower the increase in SOD levels. Moreover, in the group that received sorghum flour with tannins, the consumption of condensed tannins did not show significant correlation with SOD ($r = 0.466$) ($P = 0.429$). Our results demonstrate that other phenolic/bioactive compounds are acting more effectively in the elimination of reactive oxygen species, proving once again that white sorghum showed similar efficacy to sorghum with tannins.

3.2.3 Histology. No significant differences in liver tissue cell components were observed between the experimental groups during the 4-week treatment (Table 7). Our results suggest that although paracetamol promotes oxidative stress, its extent did not differ from control, and the sorghum flour diet maintained this balance. This fact may be due to the high fiber content of the sorghum varieties as well as their bioactive compounds. Although the present study did not evaluate the histology of adipose tissue, our results suggest a beneficial effect of toasted sorghum flour in the modulation of lipid metabolism, in view of the improvement in lipid profile compared to the test groups.

In addition, several studies have demonstrated the hepatoprotective effect of phenolic compounds in the prevention of paracetamol-induced oxidative stress (Girish et al., 2009; Liu et al., 2011). Pang et al. (2016) demonstrated the protective effect of caffeic acid on paracetamol-induced liver injury in mice. The authors observed that caffeic acid reverses severe liver injury, indicated by intrahepatic hemorrhage, lymphocyte infiltration, and destruction of liver structure.

4. CONCLUSIONS

Toasted sorghum flours with and without tannins are a good source of dietary fiber and bioactive compounds. Their consumption by male SHRs treated with paracetamol promoted significant improvement of lipid profile, through an increase in HDLc and reduction in the oxidative stress. In addition, consumption of sorghum phenolic compounds was associated with a lower SOD activity, which demonstrates that they act directly on the elimination of reactive oxygen species. Therefore, toasted sorghum flours with or without tannins can be used as promising ready-to-eat foods and as ingredients for the development of sorghum-based products, demonstrating that the use of sorghum for human consumption should be encouraged.

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AUTHOR CONTRIBUTIONS

T. Silva designed the study, performed the experiments, and prepared the manuscript; U. Lacerda was involved in the chemical analyses and assisted with the *in vivo* experiment; S. da Matta was involved in the histomorphometric and histopathologic analysis; V. Queiroz and P. Stringheta revised and provided valuable comments to improve the manuscript; H. Martino and F. Barros were involved in the project administration, helped to improve the research plan, provided guidance on experimental techniques and revised the manuscript. All authors revised and approved the final article.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

AACC. (2000). *American association for clinical chemistry. International approved methods of analysis*. Washington, D. C.: AACC.

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Antunes, R. C., Rodriguez, N. M., Gonçalves, L. C., Rodrigues, J. A. S., Borges, I., Borges, A. L. C. C., & Saliba, E. O. S. (2007). Composição bromatológica e parâmetros físicos de grãos de sorgo com diferentes texturas do endosperma. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *59*(5), 1351–1354. https://doi.org/10.1590/S0102-09352007000500042

Anunciação, P. C., Cardoso, L. M., Alfenas, R. C. G., Queiroz, V. A. V., Carvalho, C. W. P., Martino, H. S. D., & Pinheiro-Sant`Ana, H. M. (2019). Extruded sorghum consumption associated with caloric restricted diet reduces body fat in overweight men: A randomized controlled trial. *Food Research International*, *119*, 693–700.

AOAC. (1992). *Official methods of analysis of the Association of Official Analytical Chemists*. Washington, D. C.: AOAC.

AOAC. (1998). *Association of official analytical chemists. Official methods of analysis of the association of official analytical chemists*. Washington, D. C.: AOAC.

Arbex, P. M., Moreira, M. E. de C., Toledo, R. C. L., Cardoso, L. de M., Pinheiro-Sant'ana, H. M., Benjamin, L. dos A., . . . Martino, H. S. D. (2018). Extruded sorghum flour (*Sorghum bicolor* L.) modulate adiposity and inflammation in high fat diet-induced obese rats. *Journal of Functional Foods*, *42*, 346–355. https://doi.org/10.1016/j.jff.2018.01.010

Arnaiz, S. L., Llesuy, S., Cutrin, J. C., & Boveris, A. (1995). Oxidative stress by acute acetaminophen administration in mouse liver. *Free Radical Biology and Medicine*, *19*(3), 303–310.

Asif, M., Rooney, L. W., Acosta-Sanchez, D., Mack, C. A., & Riaz, M. N. (2010). Uses of sorghum grain in gluten-free products. *Cereal Food World*, *55*, 285–291. Retrieved from https://www.pioneer.com/home/site/us/agronomy/library/sorghum-grain-color/

Awika, J. M., Dykes, L., Gu, L., Rooney, L. W., & Prior, R. L. (2003). Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. *Journal of Agricultural and Food Chemistry*, *51*(18), 5516–5521. https://doi.org/10.1021/jf0343128

Awika, J. M., & Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, *65*, 1199–1221. https://doi.org/10.1007/978-1-4939-9039-9_9

Awika, J. M., Yang, L., Browning, J. D., & Faraj, A. (2009). Comparative antioxidant, antiproliferative and phase II enzyme inducing potential of sorghum (*Sorghum bicolor*) varieties. *LWT—Food Science and Technology*, *42*(6), 1041–1046. https://doi.org/10.1016/j.lwt.2009.02.003

Barros, F. A. R., Awika, J. M., & Rooney, L. W. (2012). Interaction of tannins and other sorghum phenolic compounds with starch and effects on in vitro starch digestibility. *Journal of Agricultural and Food Chemistry*, *60*, 11609–11617.

Barros, F. A. R., Awika, J., & Rooney, L. W. (2014). Effect of molecular weight profile of sorghum proanthocyanidins on resistant starch formation. *Journal of the Science of Food and Agriculture*, *94*(6), 1212–1217.

Bellassoued, K., Ghrab, F., Makni-ayadi, F., Pelt, J. Van, Elfeki, A., & Ammar, E. (2015). Protective effect of kombucha on rats fed a hypercholesterolemic diet is mediated by its antioxidant activity. *Pharmaceutical Biology*, *53*(11), 1–11. https://doi.org/10.3109/13880209.2014.1001408

Binda, D., Nicod, L., Viollon-Abadie, C., Rodriguez, S., Berthelot, A., Coassolo, P., & Richert L. (2001). Strain difference (WKY, SPRD) in the hepatic antioxidant status in rat and effect of hypertension (SHR, DOCA). Ex vivo and in vitro data. *Molecular and Cellular Biochemistry*, *218*, 139–46. https://doi.org/10.1023/a:1007268825721

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, *52*, 302–310.

Cardoso, L. de M., Montini, T. A., Pinheiro, S. S., Pinheiro-Sant'Ana, H. M., Martino, H. S. D., & Moreira, A. V. B. (2014). Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum. *Food Chemistry*, *152*, 210–217. https://doi.org/10.1016/j.foodchem.2013.11.106

Cardoso, L. de M., Pinheiro, S. S., Martino, H. S. D., & Pinheiro-Sant'Ana, H. M. (2017). Sorghum (*Sorghum bicolor* L.): nutrients, bioactive compounds, and potential impact on human health. *Critical Reviews in Food Science and Nutrition*, *57*(2), 372–390. https://doi.org/10.1080/10408398.2014.887057

Girish, C., Koner, B. C., Jayanthi, S., Ramachandra Rao, K., Rajesh, B., & Pradhan, S. C. (2009). Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. *Fundamental and Clinical Pharmacology*, *23*(6), 735–745. https://doi.org/10.1111/j.1472-8206.2009.00722.x

Griess, P. (1879). Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt, Ueber einige Azoverbindungen. *Berichte Der Deutschen Chemischen Gesellschaft*, *12*(1), 426–428. https://doi.org/10.1002/cber.187901201117

Hahn, D. H., Faubion, J. M., & Rooney, L. W. (1983). Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. *Cereal Chemistry*, *60*(4), 255–259.

Infante, R. A., Natal, D. I. G., Moreira, M. E. de C., Bastiani, M. I. D., Chagas, C. G. O., Nutti, M. R., . . . Martino, H. S. D. (2017). Enriched sorghum cookies with biofortified sweet potato carotenoids have good acceptance and high iron bioavailability. *Journal of Functional Foods*, *38*, 89–99. https://doi.org/10.1016/j.jff.2017.08.044

Kelava, T., Ćavar, I., & Čulo, F. (2010). Influence of small doses of various drug vehicles on acetaminophen-induced liver injury. *Canadian Journal of Physiology and Pharmacology*, *88*(10), 960–967. https://doi.org/10.1139/y10-065

Kim, D. O., Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry*, *50*(13), 3713–3717. https://doi.org/10.1021/jf0120071c

Kohn, H. I., & Liversedge, M. (1944). On a new aerobic metabolite whose production by brain is inhibited by morphine, emetine, ergotamine, epinephrine, and menadione. *Journal of Pharmacology and Experimental Therapeutics*, *82*(3), 292–300.

Liu, Y. T., Lu, B. N., & Peng, J. Y. (2011). Hepatoprotective activity of the total flavonoids from *Rosa laevigata Michx* fruit in mice treated by paracetamol. *Food Chemistry*, *125*(2), 719–725. https://doi.org/10.1016/j.foodchem.2010.09.080

Lopes, R. C. S. O., Lima, S. L. S., Silva, B. P., Toledo, R. C. L., Moreira, M. E. de C., Anunciação, P. C., . . . Martino, H. S. D. (2018). Evaluation of the health benefits of consumption of extruded tannin sorghum with unfermented probiotic milk in individuals with chronic kidney disease. *Food Research International*, *107*, 629–638. https://doi.org/10.1016/j.foodres.2018.03.004

Marklund, S. (1985). Pyrogallol autoxidation. In R. A. Greenwald (Ed.), *Handbook of methods for oxygen radical research*. Boca Raton, FL: CRC Press. https://doi.org/10.1201/9781351072922

Martino, H. S. D., Tomaz, P. A., Moraes, É. A., Conceição, L. L., Oliveira, D. da S., Queiroz, V. A. V., . . . Ribeiro, S. M. R. (2012). Chemical characterization and size distribution of sorghum genotypes for human consumption. *Revista Do Instituto Adolfo Lutz*, *71*(2), 337–344.

Moraes, É. A., Marineli, R. da S., Lenquiste, S. A., Queiroz, V. A. V., Camargo, R. L., Borck, P. C., . . . Maróstica Júnior, M. R. (2018). Whole sorghum flour improves glucose tolerance, insulin resistance and preserved pancreatic islets function in obesity diet-induced rats. *Journal of Functional Foods*, *45*, 530–540. https://doi.org/10.1016/j.jff.2017.03.047

Moraes, É. A., Marineli, R. D. S., Lenquiste, S. A., Steel, C. J., Menezes, C. B. De, Queiroz, V. A. V., & Maróstica Júnior, M. R. (2015). Sorghum flour fractions: Correlations among polysaccharides, phenolic compounds, antioxidant activity and glycemic index. *Food Chemistry*, *180*, 116–123. https://doi.org/10.1016/j.foodchem.2015.02.023

Moraes, É. A., Natal, D. I. G., Queiroz, V. A. V., Schaffert, R. E., Cecon, P. R., De Paula, S. O., . . . Martino, H. S. D. (2012). Sorghum genotype may reduce low-grade inflammatory response and oxidative stress and maintains jejunum morphology of rats fed a hyperlipidic diet. *Food Research International*, *49*, 553–559. https://doi.org/10.1016/j.foodres.2012.07.029

Niroumand, S., Khajedaluee, M., & Khadem-rezaiyan, M. (2015). Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. *Medical Journal of the Islamic Republic Iran*, *29*, 240.

Noh, J. R., Kim, Y. H., Hwang, J. H., Choi, D. H., Kim, K. S., Oh, W. K., & Lee, C. H. (2015). Sulforaphane protects against acetaminophen-induced hepatotoxicity. *Food and Chemical Toxicology*, *80*, 193–200. https://doi.org/10.1016/j.fct.2015.03.020

Oliveira, K. G., Queiroz, V. A. V., Carlos, L. A., Cardoso, L. M., Pinheiro-Sant'Ana, H. M., Anunciação, P. C., . . . Barros, F. A. R. (2017). Effect of the storage time and temperature on phenolic compounds of sorghum grain and flour. *Food Chemistry*, *216*, 390–398. https://doi.org/10.1016/j.foodchem.2016.08.047

Paiva, C. L., Queiroz, V. A. V., & Garcia, M. A. V. T. (2019). Características tecnológicas, sensoriais e químicas de massas secas sem glúten à base de farinhas de sorgo e milho. *Brazilian Journal of Food Technology*, *22*, 1–9. https://doi.org/10.1590/1981-6723.09518

Palavecino, P. M., Ribotta, P. D., León, A. E., & Bustos, M. C. (2019). Gluten-free sorghum pasta: Starch digestibility and antioxidant capacity compared with commercial products. *Journal of the Science of Food and Agriculture*, *99*, 1351–1357. https://doi.org/10.1002/jsfa.9310

Pang, C., Zheng, Z., Shi, L., Sheng, Y., Wei, H., Wang, Z., & Ji, L. (2016). Caffeic acid prevents acetaminophen-induced liver injury by activating the Keap1-Nrf2 antioxidative defense system. *Free Radical Biology and Medicine*, *91*, 236–246. https://doi.org/10.1016/j.freeradbiomed.2015.12.024

Price, M. L., Scoyoc, S. V., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, *26*(5), 1214–1218. https://doi.org/10.1021/jf60219a031

Pyles, L. A., Stejskal, E. J., & Einzig, S. (1993). Spectrophotometric measurement of plasma 2-thiobarbituric acid-reactive substances in the presence of hemoglobin and bilirubin interference. *Society for Experimental Biology and Medicine*, *202*(4), 407–419. https://doi.org/10.3181/00379727-202-43552

Ragaee, S., Abdel-Aal, E. S. M., & Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, *98*(1), 32–38. https://doi.org/10.1016/j.foodchem.2005.04.039

Rao, S., Santhakumar, A. B., Chinkwo, K. A., Wu, G., Johnson, S. K., & Blanchard, C. L. (2018). Characterization of phenolic compounds and antioxidant activity in sorghum grains. *Journal of Cereal Science*, *84*, 103–111. https://doi.org/10.1016/j.jcs.2018.07.013

Re, R., Pellegrini, N., Proteggenete, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(98), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Reeves, P. G., Nielsen, F. H., & Fahey, G. C. J. (1993). Committee Report AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition*, 1939–1951.

Ritchie, L., Taddeo, S., Weeks, B., Carroll, R., Dykes, L., Rooney, L., & Turner, N. (2017). Impact of novel sorghum bran diets on DSS-induced colitis. *Nutrients*, *9*(4), 330. https://doi.org/10.3390/nu9040330

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *American Society for Nutritional Sciences*, *130*(8), 2073–2085.

Silva, M. E. T., Correa, K. O., Martins, M. A., Matta, S. L. P., Martino, H. S. D., & Coimbra, J. S. R. (2020). Food safety, hypolipidemic and hypoglycemic activities, and in vivo protein quality of microalga *Scenedesmus obliquus* in Wistar rats. *Journal of Functional Foods*, *65*, 103711. https://doi.org/10.1016/j.jff.2019.103711

Singleton, V. L., & Rossi, J. A. J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(33), 144–158.

Sousa, A. R., Castro Moreira, M. E., Grancieri, M., Toledo, R. C. L., Araújo, F. O., Mantovani, H. C., . . . Martino, H. S. D. (2019). Extruded sorghum (*Sorghum bicolor* L.) improves gut microbiota, reduces inflammation, and oxidative stress in obese rats fed a high-fat diet. *Journal of Functional Foods*, *58*, 282–291. https://doi.org/10.1016/j.jff.2019.05.009

Sousa, A. R., Moreira, M. E. C., Toledo, R. C. L., Benjamin, L. A., Queiroz, V. A. V., Veloso, M. P., . . . Martino, H. S. D. (2018). Extruded sorghum (*Sorghum bicolor* L.) reduces metabolic risk of hepatic steatosis in obese rats consuming a high fat diet. *Food Research International*, *112*, 48–55. https://doi.org/10.1016/j.foodres.2018.06.004

Teixeira, N. D. C., Queiroz, V. A. V., Rocha, M. C., Amorim, A. C. P., Soares, T. O., Monteiro, M. A. M., . . . Junqueira, R. G. (2016). Resistant starch content among several sorghum (*Sorghum bicolor*) genotypes and the effect of heat treatment on resistant starch retention in two genotypes. *Food Chemistry*, *197*, 291–296. https://doi.org/10.1016/j.foodchem.2015.10.099

Wu, W., Qiu, J., Wang, A., & Li, Z. (2020). Impact of whole cereals and processing on type 2 diabetes mellitus: A review. *Critical Reviews in Food Science and Nutrition*, *60*, 1447–1474. https://doi.org/10.1080/10408398.2019.1574708