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Physical, chemical, and antioxidant analysis of sorghum grain and flour from five hybrids to determine the drivers of liking of gluten-free sorghum breads

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ABSTRACT

Physical, chemical, and antioxidant analysis of grain and flour of five sorghum hybrids with different pericarp color (brown, red, and white) and endosperm texture were conducted to prepare gluten-free bread. Specific volume, texture, and acceptance were assessed in the breads. All characteristics were correlated to identify the drivers of liking. Only the brown BRS 305 and 1167048 hybrids presented pigmented testa layer with higher total phenolic contents (TPC) and total condensed tannins (TAN). The former stood out for antioxidants (1493 mg/100 g of TPC, 609.9 mg/100 g of TAN). The negative effect of antioxidants and fibers on bread acceptance was highlighted. Red sorghum BRS 332 presented higher acceptance, besides an interesting content of antioxidants (218 mg/100 g of TPC and 21.4 mg/100 g of TAN). Proteins, carbohydrates, and soluble starch were drivers of liking. Their contents could be adjusted with other ingredients to improve formulations of higher antioxidant sorghum breads.

1. Introduction

Bread is one of the essential foodstuffs consumed in different forms around the world. Recent evidence showed that it came before cereal domestication (Arranz-Otaegui, Gonzalez Carretero, Ramsey, Fuller, & Richter, 2018). Bread keeps on being unanimous by having an average worldwide consumption of around 24.5 kg by a person in 2020 (www.statista.com). Gluten-free bread (GFB) is destined for coeliac and adepts of the gluten-free diet. GFB usually uses rice (*Oryza sativa* L.), potato (*Solanum tuberosum* L.), and maize (*Zea mays* L) flours, as ingredients rich in starch. The two first crops have low contents of protein, fiber, and bioactive compounds. These three crops are among the most produced and demanded by different sectors like food, feed, fuel, and beverages. This brings inflation to these crops, while others could have a higher protagonism, thus diversifying our agriculture and enriching our diet.

In this context, sorghum (*Sorghum bicolor* L.) is a gluten-free cereal (Pontieri et al., 2013) with a high starch level. It has a higher content of protein (Raemaekers, 2001) than rice, potato, and cassava (*Manihot esculenta* Crantz), besides bioactive compounds (Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2017). Unlike maize flour, sorghum does not present odor (Ciacci et al., 2007), which is desirable for bakery products. Besides gluten-free diets, it can be suitable to other specific diets, such as those for people with diabetes, obesity, overweight, or high-triglycerides (Cardoso et al., 2017).

Sorghum is the 5th most-produced cereal worldwide. It makes part of secondary and coarse crops expected by the international community to have a greater role, aiming to diversify agriculture, food, and inputs of production. Sorghum has a C4 photosynthetic metabolism and grows well in semi-arid, dry, and hot environments. These agronomic characteristics make it strategic for food security in Africa and Asia, particularly in arid regions with little water availability. In Sudan, which has many desert territories, 52% of the cereals consumed per capita are sorghums (FAO, 2020). It is also an excellent option for sequential cropping in the tropics when environmental and season conditions are not favorable for the main commodities crops.

Although the gluten-free market is booming, leading to the

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increasing demand for gluten-free starch, sorghum and other potential species present some limitations to the bakery beyond the absence of a gluten protein network. Although healthy, sorghum flours showed to affect the bread quality, mainly the specific volume and crumb structure. This is suggested to happen due to kernel hardness, pericarp composition, starch properties, kafirin properties, and phenolic compounds (Goodall, Campanella, Ejeta, & Hamaker, 2012; Schober, Messerschmidt, Bean, Park, & Arendt, 2005; Taylor, Schober, & Bean, 2006). On the other hand, most of the studies involving sorghum bread use commercial flour with scarce characteristics of the sorghum material used. To the best of our knowledge, the work of Schober et al. (2005) was one of the few to explore the diversity from nine decorticated sorghum grain hybrids, seven white and two red.

In the same way, texture analysis and acceptance of sorghum bread are also limited. This kind of data is essential to new product development based on alternative starch gluten-free sources in the bakery sector, dominated by wheat gluten starch. Aguiar, Rodrigues, Queiroz, Melo, & Pineli (2020) performed the sensory analyses of six accessions of sorghum, four of them present in our study. However, they did not associate the physical and chemical characteristics of the genotypes with the sensory outcomes. This reinforces new studies to explore the large sorghum diversity found in thousands of cultivated accessions to develop GFB and other gluten-free products. This work aimed to exploit the sorghum grain diversity of five hybrids with different pericarp colors (brown, red, and white), endosperm texture (ET - floury and vitreous), presence or absence of pigmented testa layer (PTL), and TAN; looking forward to developing GFB. Grain physical traits, chemical composition and antioxidant analysis of grain flours were correlated to identify the drivers of liking and disliking, with the intent of future formulations adjustments.

2. Material and methods

2.1. Sorghum grains

Grains of five sorghum hybrids were provided by the Brazilian Agricultural Research Corporation (Embrapa) Maize and Sorghum, located in Sete Lagoas, MG, Brazil. A hybrid with white pericarp without tannins (CMSXS 180), two hybrids with brown pericarp with tannins (1167048 and BRS 305), and two ones with red pericarp without tannins (BRS 330 and BRS 332). Embrapa Maize and Sorghum developed these five hybrids of sorghum in Brazil with good performance in the yield.

2.2. Physical traits

ET is a visual analysis of the proportion of vitreous and floury endosperm in a longitudinal cross-section of 10 random grains assessed by three evaluators. Each grain was scored based on a scale from 1 to 5 (±0.5). Scale 1 corresponded to a completely vitreous endosperm and scale 5 to completely floury endosperm. The PTL was also visually identified in the grain longitudinal cross-section, indicating the presence of tannin. Kernel weight (KW) is a relative way to measure the grain size. Thus, a hundred randomly cleaned grains (air jet), unbroken, and uniform were weighted. For each hybrid, three independent replicates were evaluated.

2.3. Chemical composition

Sorghum grains, previously cleaned by air jet in a sieve, were milled in a HAWOS mill, model Muhle 1. The chemical analysis used these grain flours for. The results (g/100 g) were expressed on a dry basis (db), except for moisture.

2.3.1. Centesimal and starch analyses

The moisture content was evaluated at 105 °C, up to constant weight. According to method 945.45 (AOAC, 2005), determined ash content at 600 °C for 240 min. Total lipid content (LI) was determined by the Am 5-04 method (AOCS, 2005), using an Ankom Technology XT15 Extractor (Ankom Technology, New York, NY, USA). The protein content (PR) was assessed through method 991.22 (AOAC, 2005), using the conversion factor of 6.25. The soluble and insoluble fibers (SF and IF) were determined by the enzymatic method, according to method 985.29 (AOAC, 2005). The carbohydrate content was calculated by subtracting PR, LI, ash content, SF, IF from 100, according to method 986.25 (AOAC, 2005). The contents of resistant and soluble starches (RS and SS) were determined using the K-SRTAR 09/14 kit (Megazyme International Ireland Ltd., Wicklow, Ireland), method 2002.02 (AOAC, 2005). Samples of 100 mg of each grain flour were exposed simultaneously to the action of pancreatic α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL), at 37 °C, for 16 h, in a water bath under agitation. Then, SS was separated by centrifugation. RS was recovered as a pellet after centrifugation. The pellets were solubilized with KOH 2 mol/L. The RS and SS concentrations were measured at 510 nm in UV–Visible spectrophotometer (Evolution 201, Thermo Scientific).

The amylose content (AM) was determined using the K-AMYL 09/14 kit (Megazyme International Ireland Ltd.), based on the method using concanavalin A, according to the manufacturer's specification. Initially, the starch's complete dispersion was carried out by adding dimethylsulphoxide in a boiling water bath. Amylopectin was precipitated with concanavalin A and removed by centrifugation. The enzymatic hydrolysis of amylose was carried out with the addition of amyloglucosidase/α-amylase solution. For the determination of glucose, an enzyme reagent containing glucosidase and peroxidase was added and, subsequently, the absorbance was measured at 510 nm.

2.4. Antioxidant content and activity

Extracts were previously prepared to assess the TPC, TAN, and antioxidant capacity. Lyophilized and crushed flour samples (0.5 g) were added to 10 mL of a 70% methanol (v/v), acidified with 1.0% HCl (v/v) solution. This solution was stirred for 2 h on a shaking table. After, the samples were left to stand for 12 h. Then, the samples were centrifuged at 4000 rpm for 10 min, and the supernatant was collected in 50 mL Falcon. The collected volume was completed with distilled water in a 50 mL volumetric flask and filtered with glass wool. The samples were placed in 50 mL amber glass. The extracts were kept at −80 °C until the time of analysis. The extracts were prepared and analyzed sheltered from the light. TPC (mg GAE/100 g) was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965). An aliquot of one mL of extract was added to 1.0 mL of Folin-Ciocalteu solution (1:3). After 1 min of rest, 2.0 mL of sodium carbonate (20%, w/v) and 2.0 mL of distilled water were added. The sample was homogenized and incubated for 30 min, at room temperature, and protected from light. Then, the absorbance was read at 700 nm. The quantification of TAN (mg/100 g) was performed according to the 4-dimethylaminocinnamaldehyde (DMAC) method by adding 210 μL of DMAC solution (0.1%, w/v) at 70 μL of each extract, followed by resting for 25 min (Brand-Willians, Cuvelier, & Berset, 1995). Then, the absorbance was read at 630 nm. TPC and TAN were expressed in fresh weight (FW). 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method was evaluated based on the reduction of absorbance measured at 515 nm of the DPPH radical antioxidants (Brand-Williams et al., 1995). The antioxidant capacity was expressed by DPPH, obtaining the inhibitory concentration (IC50), with the results expressed in mg/mL (FW). For ferric reducing ability power (FRAP μM TE/g, FW) analysis, 270 μL of distilled water and 2.7 mL of FRAP reagent are added to 90 μL of each extract, keeping the mixture in a water bath for 30 min. Then, the absorbance was read at 595 nm (Pulido, Bravo, & Saura-Calixto, 2000).

2.5. Bread processing and analyses

2.5.1. Preparation of the breads

Bread formulation was made considering the replacement of wheat flour by sorghum flour (Pineli, Zandonadi, Botelho, de Oliveira, & de Alencar Figueiredo, 2015). Bread preparation (Supplementary Table 1) differed only by the sorghum hybrid used, maintaining the proportion (g/100 g flour mix) of the other ingredients (Aguiar et al., 2020). According to Supplementary Table 1, sorghum flour represented 22% of all ingredients and 38.4% of the formulation without water, and 61% of total starch flours.

Starch flours (sorghum, potato, and cassava), xanthan gum, salt and sugar were mixed in a food processor (model RI7782-01, Philips Walita, China). Water and oil were added, and the mixture was homogenized. Whole egg and egg white were added to the dough and mixed. Biological yeast was pre-activated in water and sugar for 10 min at 37–43 °C. Then it was added to the dough, which was placed in a previously greased aluminum form, remaining for 25 min to ferment. The breads were baked in a preheated oven at 190 °C for 45 min (Brastemp, Brazil). All breads were cooled to room temperature and then portioned for analysis.

2.5.2. Specific volume and texture analysis

The specific bread volume was determined by the millet seed displacement method (AACC, 2000). The texture analysis of the breads was made with method 74–09 (AACC, 2000), with adaptations in a TA. XT plus texturometer and the Software Exponent v. 6.1.4. The breads were cut into slices of 25 mm thickness, with a deformation level of 40%. A 36 mm D aluminum cylindrical probe and a 5 g trigger load were used, and the test speed was 1.7 mm/s, 2 counting cycles, and 2s recovery time. The samples were analyzed on the same day of manufacture, being three repetitions analyzed in triplicate. The following variables were analyzed: hardness (N), cohesiveness (%), adhesiveness (mJ), elasticity (%), and chewiness (N).

2.5.3. Sensory evaluation

Acceptance test was performed using an unstructured 9 cm-hedonic scale for overall liking, appearance, flavor, aroma, and texture attributes. Eighty-six untrained consumers of regular bread (at least once a week) were recruited for the test. Each evaluator received five samples of a loaf of bread, 1.0 cm thick. The samples were served monadically and in a randomized and balanced order. All samples were made and baked on the same day of sensory evaluation and rested for 3 h to cool before being cut. The Research Ethics Committee approved Protocols used in this study by the UnB (nº 1.331.651).

2.6. Experimental design and statistical analysis

A completely randomized design was adopted, with grain flours from five sorghum hybrids. Three repetitions were used for the physical and chemical analyzes of grains, flours, and breads. The data were submitted to analysis of variance (ANOVA), and if significant, Tukey test at 5% probability was applied. The acceptance data were analyzed by two-way ANOVA, being the sources of variation between the samples and the judges. ANOVA was followed by multiple comparisons with the Fisher test ($p < 0.05$), if ANOVA was significant. Multivariate regression was also obtained by partial least square (PLS) based on covariance, considering sensory acceptances as dependent variables. The physical and chemical variables of sorghum grains and their flour~~,~~ and the texture bread measures were considered independent or explanatory variables in the PLS model. Jackknife (LOO) cross-validation was applied. Statistical analyzes used the XLSTAT 2015 Program (Addinsoft, Paris, France).

3. Results and discussion

Grains and grain flours of five sorghum hybrids: a white pericarp without tannins (CMSXS 180), two red pericarps without tannins (BRS 330 and BRS 332), and two brown pericarps with tannins (1167048 and BRS 305) were analyzed in this study aiming to develop sorghum GFB. Sorghum flour was majorly used (61%) as the starch source of the dough, complemented by potato (28%) and cassava (11%) flour starches. More than exploring the variations and quantities of ingredients of sorghum GFB preparations (Akin, Miller, Jaffe, Koppel, & Ehmke, 2019; Bize, Smith, Aramouni, & Bean, 2017; Monthe et al., 2019; Onyango, Mutungi, Unbehend, & Lindhauer, 2011), we exploited the potential grain and flour diversity as in few studies (Schober et al., 2005).

The diversity of these five sorghum cultivated hybrids is based on grain physical, chemical, and antioxidant traits. However, few data about these traits are available for these five sorghum hybrids. Overall, these hybrids have been genetically improved, aiming to meet a sequential cropping demand looking high yield and adaptability. The brown genotype BRS 305 has been drawn attention (Lopes et al., 2018; do Prado et al., 2019) because of its high antioxidant content; thus, some grain and flour trait data are available (Antunes et al., 2007; Martino et al., 2012; Teixeira et al., 2016).

3.1. Grain analysis

Only the two brown sorghum grain hybrids (Table 1, Supplementary Figure 1) had a PTL, which was an easy and fast visual way to indicate the tannin's presence. Meanwhile, the white free-tannin hybrid (CMSXS 180) was significantly harder (ET) than the red and brown ones (around 2 times, Table 1). The red free-tannin grains had significantly lower values of KW, suggesting they had a lower grain size (Table 1).

These four sorghum grain traits (ET, color, KW, and PTL) analyzed here play an important role in sorghum human food in different preparations (Kayode, Adegbidi, Hounhouigan, Linnemann, & Nout, 2005). For *injera*, an Ethiopian leavened flatbread, tannin brings astringent taste and poor eye color due to its darkness, which is undesirable for consumers (Yetneberk, Kock, Rooney, & Taylor, 2004). On the other hand, for the healthy bread and gluten-free market, these undesirable color characteristics are valuable because they contain proteins at aleurone and the bioactive and antioxidant compounds from the pericarp layer. Even preventing proper dough fermentation due to protein binding to tannin, a higher effort to develop sorghum bread combined with other starch sources promises success in many diets. Both brown-tannin sorghum hybrids had a great advantage of not being hard (ET from 4.1 to 4.4, Table 1), recommended succeed in developing sorghum bread (Schober et al., 2005; Taylor et al., 2006). Usually, hard and tannin grains are less susceptible to mold and storage pests (Chandrashekar & Satyanarayana, 2006) and lower digestibility (Austin, Turner, McDonough, & Rooney, 2012). The ET and KW found here ranged in line with other sorghum studies (de Alencar Figueiredo et al.,

Table 1

Endosperm texture and kernel mass of 100 grains of sorghum genotypes.

Genotype	Pigmented Testa layer (PTL)	Endosperm texture (ET) (1.0–5.0, ±0.5)	Kernel weight (KW) (g)
Brown 1167048	Yes	$4.4^{a} \pm 7.35$	$2.2^{b} \pm 0.03$
Brown BRS 305	Yes	$4.1^{ab} \pm 4.88$	$2.3^{ab} \pm 0.02$
Red BRS 330	No	$3.8^{ab} \pm 9.04$	$1.5^{d} \pm 0.01$
Red BRS 332	No	$3.5^{b} \pm 7.21$	$1.8^{c} \pm 0.03$
White CMSXS 180	No	$2.0^{c} \pm 13.04$	$2.4^{a} \pm 0.01$

Averages followed by the same lowercase letter in the columns do not differ statistically by the Tukey test at 5% probability. $n = 3$ sample replicates, triplicate analyses.

2010; Hill, Slade Lee, & Henry, 2012), mainly for BRS 305 (Antunes et al., 2007).

3.2. Flour analysis

The centesimal composition of sorghum grain flour in this study (Table 2) ranged according to other studies with larger sampling aiming the use of sorghum for human feeding (de Alencar Figueiredo et al., 2010; Queiroz et al., 2015) and sorghum bread (Schober et al., 2005). Moisture values (from 12.01 to 14.45%) showed that grains were well stored and in good conditions for this study. The brown genotype BRS 305 showed the highest significant ash content (1.79%). It stood out for fiber (19.78%), suggesting that it is the best mineral and dietary fiber source, associated with several health benefits. However, for the baking properties using sorghum flour, lower ash and fiber contents are desirable (Trappey, Khouryieh, Aramouni, & Herald, 2015). On the other hand, the other four sorghums hybrids had less ash (from 1.19 to 1.50 times) and less fiber (from 1.26 to 1.47 times) than BRS 305, but they had the drawback of having high LI (3.6–5.43%), it means from 1.37 to 2.18 times than the BRS 305 (2.49%). Sorghum flours from decorticated grains are more indicated to develop sorghum bread (Trappey et al., 2015). Still, most of the bioactive compounds found outside the endosperm are lost, including PR, SF, IS, and antioxidants (Taylor and Duodu, 2015).

The protein content of the five hybrids (from 9.57 to 11.135) was placed in the inferior limit related to sorghum studies (de Alencar Figueiredo et al., 2010; Hill et al., 2012; Queiroz et al., 2015; Schober et al., 2005). While the whole grain is the main source of protein in Africa (Belton & Taylor, 2004), sorghum bread from decorticated grains (with less protein and bioactive compounds) keep being the best formulation for breads (Schober et al., 2005; Trappey et al., 2015; Yetneberk et al., 2004). As a matter of fact, the highest challenge for developing bread and other gluten-free products is because none of the proteins from gluten-free cereals was able to replace the gluten protein found on wheat and others with gluten protein cereals (de Mesa-Stonestreet, Alavi, & Bean, 2010). On the other hand, sorghum has unique nutritional properties, gluten-free, and suitable for coeliac disease people, despite the relatively low digestibility of protein (Cardoso et al., 2017; Taylor et al., 2006).

Brown BRS 305 sorghum flour had the lowest soluble starch content (20.32%) compared to other flours hybrids (around 3 times lower, Table 2). Its carbohydrates were predominantly resistant starches (41%), while the other four hybrids showed absent or shallow RS values (from 0.0 to 1.0). This high content of RS of the brown BRS 305 is close to the study of Teixeira et al. (2016) with the same genotype BRS 305, in which they measured 50% of RS. The starch not digested by amylases in the small intestine has beneficial health effects like those attributed to dietary fibers (Birt et al., 2013), but not nutritional function.

AM of the studied hybrids is comprised by other studies (de Alencar Figueiredo et al., 2010; Hill et al., 2012). Three of them (CMSXS 180, BRS 305, and BRS 332) with different pericarp colors showed the lowest AM values (from 11.5 to 17.0%, Table 2). Thus, they could be considered heterowaxy (Sang, Bean, Seib, Pedersen, & Shi, 2008) or even waxy (Wong et al., 2010). Sorghum heterowaxy with around half AM than the normal sorghum is characterized by the high content of RS (Sang et al., 2008), which is the case of BRS 305, but not for the other two hybrids. In Taylor et al. (2006)'s review, sorghum breads made with normal sorghum (nonwaxy) followed by heterowaxy had the best results, while waxy sorghum produces unacceptable sorghum breads.

Unlike conventional cereals such as wheat, maize, and rice; sorghum grains are a source of antioxidants due to phenolic compounds, mainly condensed tannins in some sorghum accessions (Awika, Yang, Browning, & Faraj, 2009; Gülçin, Huyut, Elmastaş, & Aboul-Enein, 2010). In this study, brown tannin grains have a superior TPC (from 3 to 6 times) and TCT (from 15 to 30 times) than red and white grains. TPC in sorghum grains flours ranged from 173 to about 1493 mg GAE/100 g, while TCT ranged from 16 to 610 (Table 2). Ragaee, Abdelaal, & Noaman (2006) recorded a TPC of 412.8 mg/100 g for whole-grain sorghum, an intermediary between our study of red and brown sorghum grains. The value of TPC in brown BRS 305 is about 50% higher than those found for the two brown sorghums with tannins (inbred line TX 430 and accession SC 319/PI533833) studied by (Oliveira et al., 2017). On the other hand, they were closer to the value of TPC presented by brown hybrid 1167048. TPC of brown BRS 305 sorghum flour was about 80% higher than in its counterpart brown 1167048. This latter hybrid presented TPC about 5, 6, and 9-fold higher than red BRS 330, red BRS 332, and white CMSXS 180 suggesting an association between a pericarp brown color with high phenolic compounds in sorghum. Comparing different sorghum pericarp colors (Rao et al., 2018) to this study, brown BRS 305 presented content of TPC (1493 mg GAE/100 g) from 1.29 to 4.17-fold higher than the black (Shawaya short 1) and brown (IS 13116) sorghums (11.50 and 3.58 mg GAE/g, respectively). The red sorghum hybrids of

Table 2

Chemical composition of antioxidant content and activity of grain flours obtained from different sorghum genotypes.

Genotypes	Chemical composition									
	Moisture (g/100 g)^{FW}	Ashes (g/100 g)^{DB}	Proteins (g/100 g)^{DB}	Lipids (g/100 g)^{DB}	Fibers (g/100 g)^{DB}		Carbohydrates (g/100 g)^{DB}	Starch (g/100 g) ^{DB}		Amylose (g/100 g)^{DB}
					Unsoluble	Soluble		Resistant	Soluble	
Brown 1167048	$12.01^{d} \pm 0.08$	$1.49^{b} \pm 0.09$	$9.57^{b} \pm 0.23$	$3.87^{b} \pm 0.22$	$14.17^{b} \pm 0.31$	$1.56^{b} \pm 0.15$	$69.33^{a} \pm 0.38$	$0.39^{d} \pm 0.04$	$65.29^{c} \pm 1.26$	$22.75^{a} \pm 0.08$
Brown BRS 305	$14.21^{b} \pm 0.00$	$1.78^{a} \pm 0.03$	$10.90^{a} \pm 0.07$	$2.49^{c} \pm 0.19$	$17.07^{a} \pm 0.54$	$2.71^{a} \pm 0.42$	$65.06^{b} \pm 0.15$	$41.35\ ^{a} \pm 0.12$	$20.32^{d} \pm 0.29$	$13.25^{d} \pm 0.30$
Red BRS 330	$13.69^{c} \pm 0.00$	$1.41^{bc} \pm 0.06$	$11.06^{a} \pm 0.19$	$3.60^{b} \pm 0.20$	$14.09^{b} \pm 0.01$	$0.32^{c} \pm 0.03$	$69.54^{a} \pm 0{,}09$	$1.01^{b} \pm 0.06$	$68.31^{b} \pm 0.12$	$19.25^{b} \pm 0.11$
Red BRS 332	$14.45^{a} \pm 0.05$	$1.55^{b} \pm 0.15$	$11.13^{a} \pm 0.20$	$3.97^{b} \pm 0.06$	$12.29^{c} \pm 0.23$	$1.16^{b} \pm 0.00$	$69.90\ ^{a} \pm 0.33$	$0.77^{c} \pm 0.03$	$66.23^{c} \pm 0.70$	$16.97^{c} \pm 0.44$
White CMSXS 180	$14.26^{ab} \pm 0.18$	$1.19^{c} \pm 0.02$	$10.10^{b} \pm 0.13$	$5.43^{a} \pm 0.14$	$12.70^{c} \pm 0.07$	$1.21^{b} \pm 0.12$	$69.30\ ^{a} \pm 0.17$	$0.00^{e} \pm 0.00$	$71.74^{a} \pm 0.64$	$11.50^{e} \pm 0.49$

	Antioxidant contents		Antioxidant activity	
	Total Phenolic Compounds (mg GAE/100 g)^{FW}	Total Condensed Tannins (mg PE/100 g)^{FW}	DPPH IC_{50} (mg/mL)^{FW}	FRAP (μM TE/g)^{FW}
Brown 1167048	$824.0^{b} \pm 17.0$	$350.5^{b} \pm 10.2$	$6.05^{b} \pm 0.11$	$400.1^{b} \pm 45.7$
Brown BRS 305	$1493.0^{a} \pm 11.7$	$609.9^{a} \pm 1.3$	$4.40^{b} \pm 0.18$	$802.0^{a} \pm 12.3$
Red BRS 330	$239.0^{c} \pm 14.9$	$20.7^{c} \pm 1.3$	$21.8^{a} \pm 2.87$	$121.7^{c} \pm 11.3$
Red BRS 332	$218.0^{c} \pm 2.8$	$21.4^{c} \pm 0.0$	$20.3^{a} \pm 3.96$	$113.6^{c} \pm 16.7$
White CMSXS 180	$173.0^{c} \pm 4.8$	$16.0^{c} \pm 0.9$	$17.6^{a} \pm 0.82$	$62.6^{d} \pm 5.0$

Averages followed by the same lowercase letter in the columns do not differ statistically by the Tukey test at 5% probability. n = 3 samples. Analyzed in triplicate. DB: Expressed on dry basis. IC_{50} - half-inhibitory concentration.

this study presented around 3-fold more TPC (218 and 239 mg GAE/100 g) than the red varieties (from 0.66 to 0.88 mg GAE/g - QL33/QL36, B923296, and QL33). Additionally, white sorghum (CMSXS 180) presented TPC (173 mg GAE/100 g) 7-fold higher than the white one (0.24 mg GAE/g QL12).

TCT contents, in a range of 16–623 mg/100 g, followed the results of TPC, as brown BRS 305 sorghum flour showed about 74% more TCT than brown 1167048 sample (Table 2). Red and white samples did not differ, and brown 1167048 flour had TCT content 16 to 22-fold higher than in red and white sorghum flours. Moraes et al. (2015) found 863 mg catechin eq/100 g of condensed tannins in brown sorghum flour SC 21 accession (PI 534127), which is about 40% higher than tannins found in BRS 305 sorghum flour. The highest content of tannins in red wine presented in the USDA database (http://www.nal.usda.gov/fnic/foodcomp/Data/PA) is about 30 mg/100 g, which is closer to the contents of red sorghum flour from our study and about 12 and 20-fold lower than tannins found in brown 1167048 and BRS 305 sorghum flours, respectively.

DPPH IC_{50} (from 4.40 to 21.8 mg/mL) revealed a higher antioxidant capacity of brown sorghum flours (about 4-fold) in comparison to the other samples, and no difference among all red and white flours. FRAP method was more discriminative as the antioxidant capacity of brown BRS 305 sorghum flour (800 μM TE/g) was twice the capacity of brown 1167048 sorghum flour (402 μM TE/g). The red sorghum flours presented FRAP (113 and 121 μM TE/g) antioxidant capacity about 2-fold higher than those of white sorghum flour (62 μM TE/g). The brown pericarp of accession SC 21 (PI 534127) from whole sorghum flour had an antioxidant capacity of 90.74 μM Trolox/g, by FRAP method (Moraes et al., 2015). This value is closer to our study's FRAP antioxidant capacity's red whole sorghum flours and between 4 and 8 fold lower than the brown sorghums. Rao et al. (2018) found FRAP values between 9.23 (white QL 12 sorghum) and 85.58 μM TE/g (Shawaya short black 1), converging with the lower contents of TPC found for their six studied varieties.

3.3. Bread analysis

The instrumental analysis and sensory acceptance of sorghum breads are described in Table 3. There was no difference among the five hybrids for elasticity and adhesiveness of sorghum breads, concerning instrumental analyses. The specific volume was the same statically for one brown (1167048), one red (BRS 330), and the white (CMSXS 180) genotype (from 3.19 to 3.49 cm^3/g). Sorghum breads using 70% of sorghum flour (Bize et al., 2017) showed the specific volume (2.11 and 2.95 cm^3/g) close to the lower levels of this study (Table 3). The use of l4% of sorghum flour (Schober et al., 2005) kept showing the lowest specific volume than our results (Table 3).

Hardness (HAR) varied from 6.39 to 18.96 N, a difference of about 3-fold, thus emphasizing the effect of hybrid on this texture variable (Table 3). This large variability (from 7.5 to 21.6 N) was also recorded for the crumb hardness of sorghum breads using nine hybrids (Schober et al., 2005). The red BRS 330 bread had the highest hardness, whereas the other red BRS 332 and white sorghum breads showed the lowest hardness, which indicates a lack of clear relation between sorghum color and hardness, the same observed for the specific volume. Hardness between 5.0 and 6.5 N in breads made with white sorghum flour (Bize et al., 2017) was similar to the values presented in this study for white CMSXS 180 and red BRS 332 breads. On the other hand, the use of only 20% of sorghum flour (Monthe et al., 2019) achieved a hardness of 2.70 N. It means 2.4-fold softer than our softest bread, which used 61% of sorghum flour. Higher cohesiveness was found for red BRS 332 and white sorghum breads, with no difference among the other hybrids. As for hardness, the red BRS 330 and the brown 1167048 breads presented higher chewiness. Regarding cohesiveness and chewiness, similar results were obtained to those presented in Table 3 for breads made with 50% of sorghum and 50% of potato or maize starch (Onyango et al., 2011). However, these authors found elasticity values between 0.90 and 1.00, slightly lower or close to those observed in the present study, which remained at or above 0.98 (Table 3).

GFB is generally less accepted than regular breads as they are not part of regular bread consumers' eating habits. The averages of acceptance regarding all evaluated attributes (Table 3) were close to five on the 9-point scale. It may be influenced by consumers and references of regular bread quality. However, the comparison between the averages of acceptance makes it possible to evaluate which hybrid has a higher potential in bread making and other baking products. With concerns to overall liking, red (BRS 330) and white (CMSXS 180) sorghum breads showed higher acceptance (5.5). Still, only red BRS 332 (5.9) was significantly ($p \leq 0.05$) more accepted than brown sorghum breads (BRS 305 and 1167048, 5.0 and 5.1, respectively). Red sorghum breads presented the highest appearance acceptances (6.0 and 6.3), with an average in the slight/moderate liking of the scale. In contrast, consumers were indifferent to the appearance of brown and white sorghum breads (from 4.6 to 5.1). For odor and texture acceptances, red BRS 332 bread presented averages (5.8 and 6.1, respectively) significantly higher than brown sorghum breads (from 4.6 to 4.7 and 5.1, respectively). Instead, red BRS 330 and white sorghum breads did not differ from any other breads, being considered intermediates. On the other hand, flavor acceptance discriminated the most accepted red BRS 332 sorghum bread (5.8) from the least accepted brown BRS 305 sorghum bread (4.8). Only few studies performed sensory studies on gluten-free sorghum breads. The flat sorghum breads made with commercial white and red sorghum flours achieved acceptance averages around 5 (Yousif, Nhepera, & Johnson, 2012). Aguiar et al. (2020) work with six sorghum accessions, including four hybrids of these study (reds BRS 330 and BRS 332; brown BRS 305 and 1167048) reached a slightly higher acceptance for some

Table 3
Texture instrumental analyses and sensory acceptance of sorghum breads.

Analysis	Descriptors	Brown 1167048	Brown BRS 305	Red BRS 330	Red BRS 332	White CMSXS 180
Instrumental	Specific volume (cm^3/g)	$3.34^{a} \pm 0.01$	$2.71^{b} \pm 0.07$	$3.49^{a} \pm 0.06$	$2.61^{b} \pm 0.35$	$3.19^{a} \pm 0.13$
	Hardness (N)	$14.64^{b} \pm 0.65$	$10.88^{c} \pm 1.29$	$18.96^{a} \pm 1.92$	$6.39^{d} \pm 1.42$	$5.38^{d} \pm 0.45$
	Cohesiveness*	$0.46^{b} \pm 0.03$	$0.45^{b} \pm 0.03$	$0.44^{b} \pm 0.03$	$0.53^{a} \pm 0.04$	$0.54^{a} \pm 0.02$
	Elasticity*	$0.99^{a} \pm 0.02$	$0.98^{a} \pm 0.01$	$0.98^{a} \pm 0.01$	$0.99^{a} \pm 0.01$	$0.99^{a} \pm 0.01$
	Adhesiveness (mJ)	$0.04^{a} \pm 0.02$	$0.05^{a} \pm 0.02$	$0.08^{a} \pm 0.02$	$0.04^{a} \pm 0.01$	$0.05^{a} \pm 0.01$
	Chewiness (N)	$6.62^{a} \pm 0.46$	$4.77^{b} \pm 0.68$	$8.17^{a} \pm 0.89$	$3.36^{bc} \pm 0.82$	$2.93^{c} \pm 0.24$
Sensory acceptance	Overall	$5.1^{bc} \pm 2.1$	$5.0^{c} \pm 2.1$	$5.5^{ab} \pm 2.0$	$5.9^{a} \pm 1.8$	$5.5^{abc} \pm 1.9$
	Appearance	$4.6^{b} \pm 2.4$	$5.0^{b} \pm 2.3$	$6.0^{a} \pm 1.9$	$6.3^{a} \pm 1.8$	$5.1^{b} \pm 2.3$
	Odor	$4.6^{b} \pm 2.3$	$4.7^{b} \pm 2.3$	$5.1^{ab} \pm 2.3$	$5.4^{a} \pm 2.1$	$5.0^{ab} \pm 2.1$
	Flavor	$5.0^{ab} \pm 2.3$	$4.8^{b} \pm 2.2$	$5.4^{ab} \pm 2.3$	$5.8^{a} \pm 2.0$	$5.4^{ab} \pm 2.2$
	Texture	$5.1^{b} \pm 2.1$	$5.1^{b} \pm 2.2$	$5.7^{ab} \pm 1.8$	$6.1^{a} \pm 1.8$	$5.6^{ab} \pm 2.1$

* Dimensionless terms.

Values constitute means ± standard deviations ($n = 3$). In the same line, means with equal letters do not show significant differences by the Tukey test for instrumental analysis and the Fisher LSD test for sensory analysis ($p \leq 0.05$).

hybrids. However, in this latter study, cluster analysis showed that overall liking differed among two groups of consumers, which acceptance was closer to 7 in the group with a higher proportion of older consumers (above 56 years old). In the group with a higher proportion of younger people (between 18 and 55 years old), acceptance averages were closer to our study, around 5. These authors also found a significantly higher acceptance for red BRS 332 sorghum bread in the cluster with a higher proportion of aged consumers. For younger consumers, the higher overall acceptance was for red (BRS 330 and BRS 332) and brown (BRS 305 and 1167048) sorghum breads. For all consumers (n = 124), BRS 332 had a higher acceptance (7.3), being significantly different for brown breads but not significant for white (BRS 501).

Partial least squares (PLS) regression is a technique with the goal to predict a set of dependent variables from a set of independent variables or predictors, by combining features from and generalizes principal component analysis (PCA) and multiple linear regression. The quality of the prediction obtained from a PLS regression model is evaluated with cross-validation techniques such as the jackknife (Abdi, 2010). PLS model analyzed the variable importance in the projection (VIP) with the standardized coefficients at the PLS map. VIP (Fig. 1) allowed to identify the explanatory variables that most contributed to the models. In the first component, which explained around 70% of the variation, in decreasing order, significant VIP traits were TAN, TPC, IF, KW, SF, carbohydrates, SS, RS, cohesivity, ET, and LI. In the second component, which explained with the first component 90% of the variation, the significant traits in decreasing order were PR, TAN, TPC, KW, IF, moisture, SF, carbohydrates, SS, RS, LI, ashes, specific volume, cohesivity, ET, and elasticity. It is worth noticing that hardness, chewiness, adhesivity, and AM were not significant in any component.

The PLS model's standardized coefficients for each dependent variable (the sensory acceptance attributes) were presented in Fig. 2. For all acceptance attributes, the explanatory variables KW, IF, SF, TAN, and TPC were disliking drivers, whereas PR and moisture were drivers of liking of all attributes. Carbohydrates presented a significant positive influence on all attributes, except for appearance acceptance, whereas SS presented a positive impact on flavor and overall liking. Considering only the significant VIP and standardized coefficients, the PLS map (Fig. 3) revealed that all acceptance attributes were associated with red sorghum breads and mainly to BRS 332 hybrid in both dimensions. The explanatory variables PR and moisture were located in the same quadrant, showing a positive association to acceptance and the red breads. The white bread was associated with acceptance only in the first dimension of the map and closer to carbohydrates and SS variables, LI, specific volume, and cohesivity. Brown sorghum breads were in the opposite quadrants of acceptance. In the first dimension, the BRS 305 hybrid was more distant from acceptance attributes and closer to fibers, TAN, TPC, ET, and RS. By contrast, the brown hybrid 1167048 was orthogonally opposite to acceptance and associated with the variable KW.

PLS model studies to determine the drivers of (dis)liking of GFB are scarce. However, they correlate acceptance data to sensory descriptive analysis, being essential to develop gluten-free products from starch sources of secondary and orphan crops. Apparent softness, traditional bread aroma, sweetness, and crumb colors were raised as drivers of liking of prebiotic GFB made with rice, potato, cassava starch sources (Morais, Cruz, Faria, & Bolini, 2014). Our team's recent work (Aguiar et al., 2020) determined the positive and negative sensory attributes to acceptance regarding six gluten-free sorghum breads, comprising the same red and brown hybrids current work and the other two white sorghums. These two studies had two descriptors in common, hardness and chewiness. The former study identified traditional bread aroma as a driver of liking, along with the appearance of whole flour breads, uniform alveoli, neutral flavor, soft aroma, crumb color, crust color, and spots. To the best of our knowledge, the current study is the first that apply PLS model analysis correlating acceptance data to instrumental and composition measures, which is an advantageous approach to food development and research. Unlike those studies, the sorghum bread drivers of liking (PR, CHO, and SS) and disliking (Fibers, TPC, and TAN) are grain traits with a broad variability. Thus, the improvement for greater acceptance of sorghum bread goes through a search in the accessions described and the characterization of several others little studied. This procedure generates a more natural product with less manipulation of ingredients. Additionally, instrumental data are informative and more straightforward to collect than sensory descriptive analysis.

The brown BRS 305 hybrid showed a high presence of TAN, TPC, antioxidant activity, and RS found in this work (Tables 2 and 3) and other studies (Moraes et al., 2012; Teixeira et al., 2016). Nutritionally,

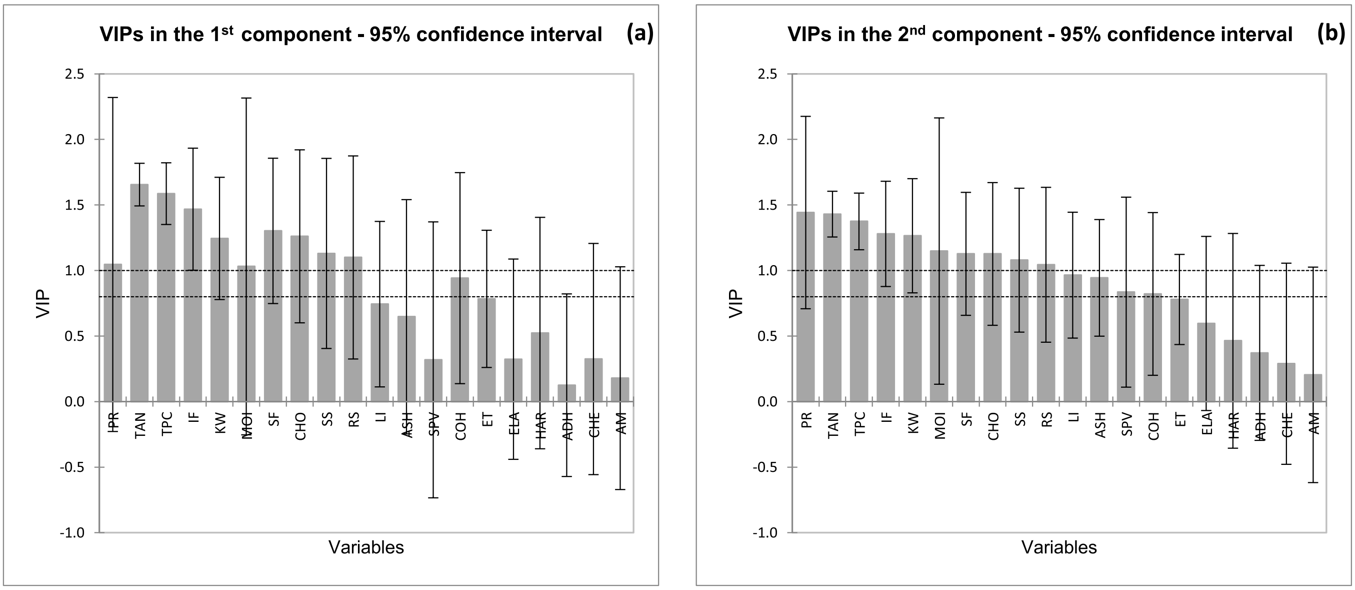
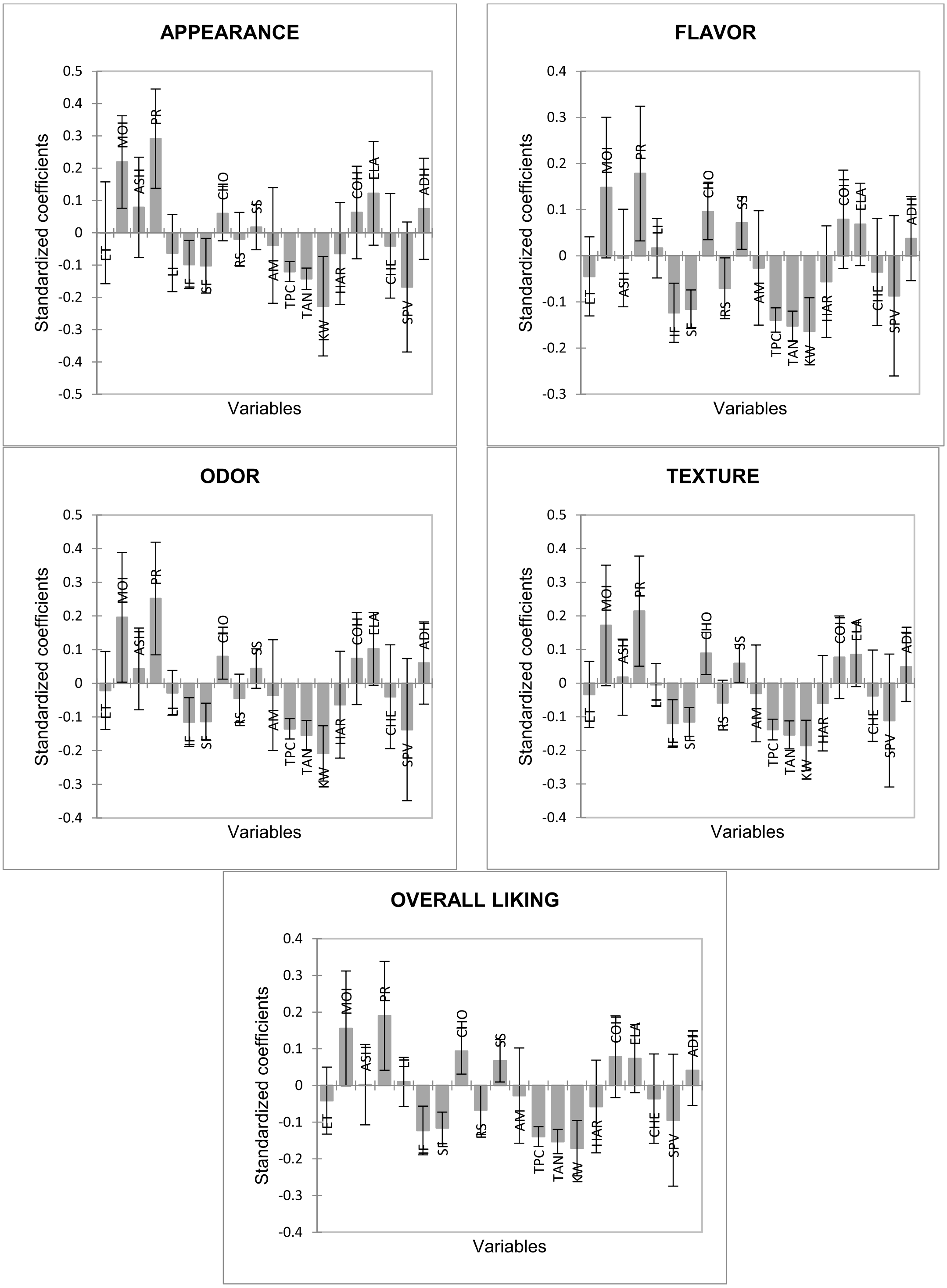


Fig. 1. Variable importance in the projection (VIP) obtained in PLS regression. Attributes are: ET (endosperm texture), KW (kernel weight) of the grains, ASH (ashes), MOI (moisture), PR (protein content), LI (lipid content), CHO (carbohydrates), IF (insoluble fiber), SF (soluble fiber), RS (resistant starch), SS (soluble starch), AM (amylose content), TPC (total phenolics content), TAN (condensed tannins of the flours), HAR (hardness), COH (cohesivity), ELA (elasticity), CHE (chewiness), SPV (specific volume), and ADH (adhesivity of the breads).


APPEARANCE

FLAVOR

ODOR

TEXTURE

OVERALL LIKING

Fig. 2. Standardized coefficients of the model for each dependent variable: overall liking (OVAL), and attribute likings: APP (appearance), FLV (flavor), ODR (odor), and TEXT (texture). Attributes are: ET (endosperm texture), KW (kernel weight) of the grains, ASH (ashes), MOI (moisture), PR (protein content), LI (lipid content), CHO (carbohydrates), IF (insoluble fiber), SF (soluble fiber), RS (resistant starch), SS (soluble starch), AM (amylose content), TPC (total phenolics content), TAN (condensed tannins of the flours), HAR (hardness), COH (cohesivity), ELA (elasticity), CHE (chewiness), SPV (specific volume), and ADH (adhesivity of the breads).

all these attributes are beneficial to health and desired in many diets. Thus, the BRS 305 has been receiving special attention for some food products like extruded breakfast (Lopes et al., 2018), beef burgers (do Prado et al., 2019), and cereal bars (Verma, Khetrapaul, & Verma, 2018). Agriculturally, sorghum with high antioxidant activity has a role in avoiding the attack of birds. Few commercial sorghums with tannin are cultivated in Brazil. They are planted in specifics areas, exactly for this grain protection role. However, in our sensory acceptance analysis for GFB, it had the worst performance. On the other hand, the red BRS 332 without tannin was the recommended hybrid for sorghum GFB production regarding sensory acceptance and the intermediary content of antioxidants (Tables 2 and 3).

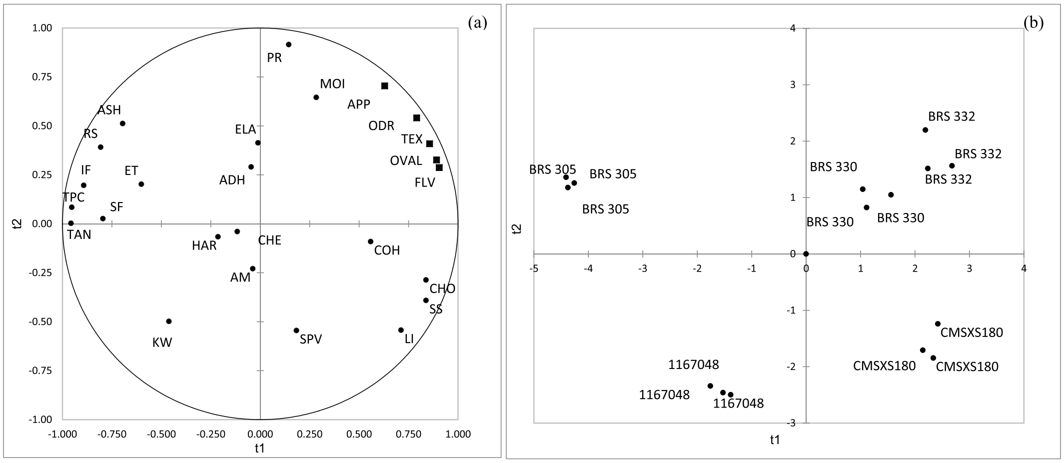


Fig. 3. Relationship between Overall liking (OVAL), attribute likings (APP – appearance, FLV (flavor), ODR (odor), and TEX (texture) and instrumental data (a) and the positions of the samples (b). Treatments breads made with different genotypes of brown (1167048 and BRS 305), red (BRS 330 and BRS 332) and white (CMSXS 180) sorghums. Attributes are: ET (endosperm texture), KW (kernel weight), ASH (ashes), MOI (moisture), PR (protein content), LI (lipid content), CHO (carbohydrates), IF (insoluble fiber), SF (soluble fiber), RS (resistant starch), SS (soluble starch), AM (amylose content), TPC (total phenolics content), TAN (condensed tannins of the flours), HAR (hardness), COH (cohesivity), ELA (elasticity), CHE (chewiness), SPV (specific volume), and ADH (adhesivity of the breads).

Cultivated sorghum has a large diversity found on thousands of accessions used for human food. Certainly, other sorghums are more recommended to produce commercial GFB competitively than the five hybrids used in this and other studies. Thus, more studies should explore these possibilities. To happen it, should boost the seed market to distribute these selfing cultivated sorghums with smallholder farmers. These results will check the finds and potential of many sorghum accessions in many studies for human food. The rising demand with the high value-added of different gluten-free starches for coeliac, gluten-free and other diets is blowing. This could be an excellent opportunity for different research areas and extension services to work directly with smallholder farmers. Thus, sorghum and other climate-resilient crops will be better commercialized with the natural and gluten-free product market.

Traditionally, a long time ago, black and color bread used coarse cereals were consumed by low-income people, while middle-class and wealthy people consumed wheat white bread. The expansion of wheat production democratized and established white bread in the world. Healthy consumers have inversed and invested in black and colors breads in the last decades due to consciousness of antioxidants and bioactive compounds' consumption. This goes to meet sorghum pericarp with and without tannin. The drivers of liking of sorghum gluten-free bread identified in this work based on grain traits, chemical and antioxidant flour and bread analysis, and the sensory acceptance will pave the way for more studies to identify sorghum accessions suitable for being used in higher proportions and potentially alone. New variations and quantities of ingredients in dough preparation will also be essential on GFB of sorghum.

4. Conclusions

This study has shown the influence of grain and flour of five sorghum hybrids and their different physical and chemical characteristics on GFB quality. Brown sorghum BRS 305 stood out for its antioxidant properties and therefore attended to the appeal of food enriched in polyphenolic compounds. However, the negative effect of tannins and other phenolics on bread acceptance was highlighted, along with the impairment associated with sorghum fibers' contents. Identifying protein and carbohydrates/soluble starch as drivers of liking could guide future bread formulations with hybrids' high antioxidant properties, aiming for healthy bread. Red sorghum BRS 332 stood out for the higher acceptance besides an interesting content of antioxidants among the studied hybrids. It should be considered a good choice for GFB.

CRediT authorship contribution statement

Lívia de Lacerda de Oliveira: Supervision, Conceptualization, Investigation, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Guilherme Theodoro de Oliveira:** Conceptualization, Investigation, Methodology, Formal analysis, Investigation. **Ernandes Rodrigues de Alencar:** Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Valeria Aparecida Vieira Queiroz:** Conceptualization, Methodology, Formal analysis. **Lúcio Flávio de Alencar Figueiredo:** Supervision, Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Authors declare that there is no conflict of interest regarding the publication of this article. Declarations of interest: none.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112407.

Author declaration

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us.

I confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

I further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

I understand that the Corresponding Author (Prof. Dra. Lívia de Lacerda de Oliveira) is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. I confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

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