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# Physicochemical and sensorial characteristics of beef burgers with added tannin and tannin-free whole sorghum flours as isolated soy protein replacer



MEAT SCIENCE

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#### ABSTRACT

The physicochemical and sensorial characterization of beef burgers with added sorghum flours as replacer for the isolated soy protein (ISP) usually used in the conventional formulations was performed. Three formulations were prepared: one conventional (CN) with 3% ISP and two with 3% tannin (BRS 305) and tannin-free (BR 501) whole sorghum flour (WSF) of BRS 305 and BR 501 genotypes. There was no difference among the formulations for most of the physicochemical characteristics. The moisture retention was higher in BRS 305 (P < .05). The added WSF influenced the color of the raw beef burger; and the proximate composition and the antioxidant characteristics of the raw and cooked formulations (P < .05). The purchase intention and flavor, texture and overall acceptability scores were higher for the sorghum products than CN (P < .05). Therefore, the replacing of ISP by WSF in beef burger, especially by the BRS 305 genotype, might be a technologically, nutritionally and sensorially viable option.

# 1. Introduction

The accelerated urban pace, with its consequent meal preparation time reduction, has brought significant changes in the nutritional habits of a large part of the population. Thus, foods with practical, sensorial pleasant and low cost characteristics, like beef burgers, have become a major attraction in the supermarkets, fast foods and restaurants worldwide (Oliveira et al., 2014). A one single fast food network reported selling > 75 hamburgers per second, of every minute, of every hour, of every day of the year ( $\approx$ 100 billions per year worldwide) (Spencer, Frank, & McIntosh, 2005).

The beef burger is a very popular product made with processed meat, which has high biological value proteins, essential fatty acids, vitamins and minerals (Angiolillo, Conte, & Del Nobile, 2015; Bastos et al., 2014). On the other hand, it contains high levels of saturated fat, cholesterol and sodium, all of which are associated with increased mortality rate and the risk of developing several non-communicable chronic diseases (CNCDs) when consumed in excess (Abete, Romaguera, Vieira, Lopez De Munain, & Norat, 2014; Claro et al., 2015).

Furthermore, these products often contain allergens, such as soy protein, which limits the choices of consumers who are alegeric. Soy is one of the most relevant foods involved in allergic reactions, being included in the World Health Organization list among the "big 8" allergens which comprises those foods that account for 90% for of all documented food allergies in the U.S (WHO, 2004). In Brazil, it is possible to add up to 4% soy protein in processed meat products (Brasil, 2000), and in the United States up to 12% (USDA, 2005). For the industrial sector this addition is of great value, since it reduces formulation costs, improves technological characteristics, delays lipid oxidation and acts as a partial meat and fat replacer (Brewer, 2012; Hayes, Bookwalter, & Bagley, 1977).

In this context, sorghum may be a viable and safe option as a substitute for soy in processed meat production because it is less allergenic. This cereal is a natural source of various bioactive compounds (phenolic acids, flavonoids and condensed tannins), soluble and insoluble fibers,

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various minerals, phytosterols, policosanols and resistant starch (Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2017; Lemlioglu-Austin, Turner, McDonough, & Rooney, 2012; Teixeira et al., 2016; Paiva et al., 2017). In addition, scientific evidence has shown an association between intake of this cereal, whether in the form of grain, flour or extruded, and prevention of several CNCDs (Lemlioglu-Austin et al., 2012; Moraes et al., 2015).

From a technological point of view, sorghum also presents desirable characteristics for processed meat products, such as good water and fat retention ability and natural antioxidant presence (Devatkal, Kadam, Naik, & Sahoo, 2011; Huang, Zayas, & Bowers, 1999; Kumar & Sharma, 2005; Malav, Sharma, Talukder, Mendiratta, & Kumar, 2015); besides having a neutral flavor, and the possibility of lower production cost than other cereals, such as corn (Lopes, 2004).

However, there are no studies evaluating the effects of adding different sorghum cultivars in the processed meat industry. It is known that genotypes have marked differences in their chemical and nutritional composition (Cardoso et al., 2015; Martino et al., 2012), which can lead to technological and sensorial changes in the product upon its addition.

Dykes, Rooney, Waniska, and Rooney (2005) reported that all sorghum genotypes contain phenolic compounds, and some may contain higher levels of condensed tannins and anthocyanins. It is known that the presence of tannins in foods can reduce the bioavailability of carbohydrates, proteins, and minerals (Barros, Awika, & Rooney, 2012). However, tannin presence in meat products has shown to be an alternative to delay lipid and protein oxidation, as well as reducing the volatile compounds formation during storage, thus the tannins may contibute to increase shelf life (Al-Hijazeen, Lee, Mendonca, & Ahn, 2016).

Thus, this work aimed to perform the physicochemical and sensorial characterization of beef burgers with added tannin and tannin-free whole sorghum flours as a replacer for a conventional formulation with isolated soy protein.

# 2. Material and methods

The experiment was carried out in laboratories of the Food Engineering Department of the Federal University of São João del-Rei, (Sete Lagoas, Minas Gerais, Brazil) and at Embrapa Milho e Sorgo (Sete Lagoas, Minas Gerais, Brazil).

#### 2.1. Whole sorghum flours (WSF) preparation

The sorghum grains of cultivars BRS 305, with brown pericarp and pigmented testa (with tannin), and BR 501, with white pericarp and without pigmented testa (tannin-free) were planted and harvested under the same field conditions, at the experimental fields of Embrapa Milho e Sorgo, in Sete Lagoas, MG, Brazil, in the 2015/16 crop season. These cultivars were selected because of their differences in chemical as previously identified by Martino et al. (2012).

To obtain the WSF, approximately 1 kg of the grains of each sorghum cultivar were manually selected, passed through a sieve to remove dirt and impurities, and milled twice in a stone mill (Hawos; Model: Mill 1) to obtain particles with 0.5 mm. The flours were stored in polyethylene plastic bags, protected from light and stored under refrigeration (4  $\pm$  1 °C). The proximate composition and antioxidant properties were performed, in triplicate, on WSF, according to the methods described in 2.4.

#### 2.2. Beef burger manufacture

The meat (Nine kilograms of fresh beef Knuckle from male 2-yearold Nelore) was purchased (48 h post-mortem) at a butcher shop inspected by the Brazilian Health Agency, at Sete Lagoas city, Minas Gerais, Brazil, and transported to the laboratory in polytehylene packages in thermal box ( $\approx$ 15 min) at temperature below 4 °C. The other ingredients were also purchased in the local commerce of that city.

Three formulations were elaborated on the same day: 1) Conventional (CN): 3% isolated soy protein (ISP); 2) BR 501: 3% WSF of BR 501 cultivar; and 3) BRS 305: 3% WSF of BRS 305 cultivar. The 3% WSF addition is the maximum limit allowed in Brazil to the total carbohydrate content for this type of product (Brasil, 2000). The other ingredients were added in the same amount in all formulations: meat (66.79%), fat (pork fat / 15%), water (4°  $\pm$  1C / 12.8%), salt (1.8%), onion powder (0.25%), garlic powder (0.25%) and monosodium glutamate (0.11%).

Once arrived to the laboratory, the excess of subcutaneous and intramuscular fat and connective tissue were manually trimmed off from meat. The pork fat was ground in a grinder (5 mm openings in the grinder plate - Eccel Metalúrgica Ltda®). The meat was cut into cubes and ground two times in a grinder (Eccel Metalúrgica Ltda\*/ 5 mm openings in the grinder plate) to obtain good uniformity. Both were weighed and stored under refrigeration (4  $\pm$  1 °C) until the other ingredients were weighed. The other ingredients were added and mixed to the meat in the following order: (1) garlic and onion powder, monosodium glutamate, salt and 50% cold water (4 °C), (2) ISP to the CN or WSF to the BR 501 and BRS 305 formulations, (3) the cold water remainded (50% / 4 °C), (4) pork fat. The total process lasted 9 min and the mean internal final temperature of batches reached about 10.7 °C, which was determined using individual thermometer inserted into the geometric center. Each of the three formulations was prepared in three separated batches, all on the same day.

For the production of beef burgers, 60 g portions were packed in individual polytehylene packages, pressed by hand into a mold (9 cm diameter / 1.5 cm height), and stored under -18 °C, for a minimum of 24 h and a maximum of 2 weeks, until analyses. Part of the analyses was performed in raw and cooked burgers.

The beef burgers were cooked on an electrical grill (Family Grill Plus Fun Kitchen, Model SS 36P, power: 1300 W), at 190 °C for 7 min (4 min on one side and 3 min on the other). A digital thermometer (Brand: CE\*) was used to measure the temperature of geometric center (core of product), which should be around at least 74 °C (Brasil, 1999). After cooking, the burgers were stored in an oven (Edanca Prata\*) until the geometric center temperature reached 60 °C before continuing the analysis (Belk et al., 2015).

# 2.3. Physical characterization

All measurements were replicates three times for each batch, except for shear force that was performed in four replicates.

# 2.3.1. Cooking yield

The cooking yield was calculated according to Eq. (1):

%yield = (weight of cooked sample/weight of raw sample)  $\times$  100 (1)

## 2.3.2. Cooking loss

Cooking loss was determined for each sample by calculating the weight diferences before and after cooking using Eq. (2):

%Cooking loss = [(weight of raw sample (g)-weight of cooked sample (g)) /weight of raw sample (g)] × 100 (2)

#### 2.3.3. Diameter reduction

The beef burger diameters were obtained before and after the cooking of each sample; and the diameter reduction was calculated according to Eq. (3):

%Diameter reduction = [(raw sample diameter-cooked sample diameter) /raw sample diameter] × 100 (3)

# 2.3.4. Moisture retention

Moisture retention was calculated using Eq. (4).

%moisture retention = 
$$[(cooked sample weight (g))]$$

$$\times$$
 %moisture in cooked sample)  
/(raw sample weight (g)

#### 2.3.5. Shear force

The shear force (N) was measured in cooked beef burgers, in three samples of each treatment, using pieces of  $2.5 \times 2.0 \times 1.5$  cm<sup>3</sup>, which were taken from four different areas of each sample tested, and then placed in a texture analyzer (Stable Micro System, model TA.TXplus, Godalming, UK) attached to the software Texture Exponent Lite version 5.1.1.0, 2010 (Polar Engineering, Nikiski, AK 99635, EUA) and equipped with an operacional Warner-Bratzler cell. Trial specifications were as follows: pre-load of 0.3 N; load cell of 1KN; percent deformation of 40%; and crosshead speed constant of 200 mm/mim.

## 2.3.6. Color

The color coordinates L\*(lightness), a\* (redness) and b\* (yellowness) were analyzed according to the system of the International Comission of Illumination (CIE LAB Sytem). Instrumental color was measured at room temperature using a colorimeter (CR-410, Konica Minolta Sensing Inc., Osaka, Japan), a 10° observer angle and illuminant D65 calibrated against a white tile immediately before readings were taken. All measurements were taken in triplicate, with a 90° clockwise sample rotation between measurements. The measurements on raw samples were recorded at 30–40 min of blooming, while on the cooked samples, the measurements were recorded immediately after cooking.

#### 2.4. Chemical characterization

All analyses were performed, in triplicate, on the raw and cooked formulations.

# 2.4.1. Proximate composition and pH

The moisture, protein, ash and carbohydrate contents were determined in raw and cooked beef burgers, in triplicate, according to AOAC (2005). The lipid content followed the AOCS (2009) protocol.

The pH values were measured in the homogenate prepared with 1 g of burger and 9 mL of distilled water, using a pHmeter (MS Tecnopon<sup>®</sup>, mPa 210) (Angiolillo et al., 2015).

# 2.4.2. Antioxidant properties: total anthocyanins (TA), total phenols (TP), condensed tanins (CT) and antioxidant capacity (AC)

The method used for TA determination was that described by Fuleki and Francis (1968), and further detailed by Awika and Rooney (2004). The absorbance of samples was read at 480 nm in a spectrophotometer (Instrutherm® Modelo UV-2000 A). The concentrations of 3-deoxiantocianinas were calculated based on the absorbance of luteolinidin (480 nm) using the eq. C (mol/L) = A/  $\varepsilon$ , where C is sample concentration, A is the absorbance and  $\varepsilon$  is the molar extinction coefficiet, of luteolinidin. The molar extinction coefficient of luteolinidin used was 29.157 (Njongmeta, 2009). Results were expressed as mg luteolinidin equivalentes (LE)/g sample, on dry basis (db).

The TP content was performed using Folin Ciocalteau reagent, according to the method proposed by Kaluza, McGrath, Roberts, and Schroeder (1980) and further detailed by Dykes et al. (2005). Results were calculeted and expressed as mg gallic acid equivalent (GAE)/g sample, on a dry basis (db).

For CT determination, the Vanillin/HCl reaction method described by Price, Van Scoyoc, and Butler (1978) was used. Results were calculated and expressed as mg catechin equivalente (CE)/g sample, on a dry basis (db).

Beef burger AC was determined by the ABTS assay (2,2-azinobis- [3etil-benzotiazoline-6-sulfonate] radical cation (ABTS)) according to Awika, Rooney, Wu, Prior, and Cisneros-Zevallos (2003) method. The absorbance of samples was read at 734 nm in a spectrophotometer (Instrutherm<sup>®</sup> Modelo UV-2000 A). Results were expressed as  $\mu$ mol Trolox.g<sup>-1</sup>, on a dry basis (db).

# 2.5. Consumer study

The sensory analysis was performed after approval by the Ethics Committee of the Federal University of Minas Gerais, Brazil, under protocol number 039570/2012. One hundred untrained panelists (41.18% male and 58.82% female, age from 18 and 48 years) from the Federal University of São João del-Rei, Sete Lagoas, Minas Gerais, Brazil, evaluated samples ( $\approx 25 \text{ g}$ ) of each of the three formulatios tested, in monadic order, presented to each panelist randomly, in a single testing session. The panelist evaluated the atributes aroma, color, flavor, texture and overall acceptability, using a 9-point hedonic scale (9 = extremely like, 1 = extremely dislike). Each sample was coded with a randomly selected three-digit number, and served to each panelist. Sensory evaluations were performed by the panelist under white fluorescente lighting. A glass of water and unsalted crackers were provided to cleanse the palate between samples. The purchase intention was also evaluated using a 5-point scale (5 = certainly would buy the product and 1 = certainly would not buy the product).

## 2.6. Statistical analysis

Processing and instrumental data were analyzed using the software SAS version 9.0. The analytical data were reported as mean  $\pm$  standard error of independent measurements and were subjected to analysis of variance (ANOVA), which considered the formulations as a fixed effect, and the replications of the experiment as a random term. The Tukey test was used to determine significant diferences among formulations (P < .05). The diferences between raw and cooked samples were determinated by paired *t*-test (P < .05).

For sensory evaluation, ANOVA was conducted, with panelist and treatment as independent variables (random and fixed variables, respectively), and hedonic scores corresponding to an individual sensory attribute as the dependent variable. Tukey's test was used for post-hoc analyses (P < .05).

Multivariate method of principal component analysis (PCA) based on correlation matrix was employed to assess relationships among all variables measured in raw and cooked meat products, separately. The PCA analyzes were performed using the software Statistica<sup>®</sup> versão 13.

# 3. Results and discussion

# 3.1. Cooking yield, cooking loss, diameter reduction and moisture retention

The results of yield, cooking loss, diameter reduction, moisture retention and shear force of cooked beef burgers are presented in Table 1. The BRS 305 presented higher yield and lower cooking loss compared to the others formulations (P < .05). The diameter reduction of BRS 305 was higher than CN (P < .05), but not different from BR 501 (P > .05). The diameter reduction did not differ between BRS 305 and BR 501. These results suggest that the use of BRS 305 and BR 501 WSF as ISP replacer may be a viable option from a technological point of view.

The moisture retention of BRS 305 was significantly higher than BR

#### Table 1

Yield, cooking loss, diameter reduction, moisture retention and shear force (mean  $\pm$  standard error) of cooked beef burgers with isolate soy protein (CN) or whole sorghum flours of BR 501 and BRS 305 genotypes.

	CN	BR 501	BRS 305
Yield (%) Cooking loss (%) Diameter reduction (%) Moisture retention (%) Shear Force (N)	$\begin{array}{rrrr} 73.29 \ \pm \ 1.72^{\rm b} \\ 26.71 \ \pm \ 1.72^{\rm a} \\ 20.54 \ \pm \ 1.99^{\rm a} \\ 67.36 \ \pm \ 2.40^{\rm ab} \\ 40.99 \ \pm \ 4.25^{\rm a} \end{array}$	$\begin{array}{rrrr} 72.91 \ \pm \ 0.66^b \\ 27.09 \ \pm \ 0.67^a \\ 18.47 \ \pm \ 0.67^{ab} \\ 63.82 \ \pm \ 0.65^b \\ 36.24 \ \pm \ 5.09^a \end{array}$	$\begin{array}{rrrr} 78.49 \ \pm \ 0.49^a \\ 21.51 \ \pm \ 0.50^b \\ 13.74 \ \pm \ 1.65^b \\ 69.12 \ \pm \ 0.62^a \\ 41.55 \ \pm \ 4.74^a \end{array}$

Means with different letters, in the same line, are significantly different by the Tukey test (P < .05).

501 (P < .05), but not different from CN (P > .05), which might be due to the higher amount of fiber and resistant starch, lower grain hardness (farinaceous endosperm) and lower grain size of the BRS 305 genotype compared to BR 501 (Martino et al., 2012; Teixeira et al., 2016).

Mehta, Ahlawat, Sharma, and Dabur (2015) reported that higher dietary fiber content is able to strengthen the three-dimensional structure of the meat system (binding between water, protein and lipid). In addition, the presence of resistant starch, which is a starch that the small intestine cannot digest, and which behaves like soluble fiber, also strengthens the three-dimensional structure of the meat system. This is due to better hydration and oil binding properties (related to chemical struture, pH, ionic strength and particle size), which favors technological characteristics like higher water and fat retention, increase in emulsion stability, ability to decreased cooking loss and higher yield.

Martino et al. (2012) showed that the BRS 305 sorghum genotype grains had 11.43% total fiber, 11.28% being insoluble and 0.15% soluble; and the BR 501 genotype showed 11.18% of total fiber, 11.01% insoluble and 0.17% soluble. Teixeira et al. (2016) verified that the BRS 305 sorghum genotype presented a resistant starch content of 52.26  $\pm$  1.38 g / 100 g in its grains (without any heat treatment), higher than other evaluated genotypes.

This result has relevance for the food industry in the search for technological solutions to improve the water retention in meat products, since the water loss, besides reducing the yield, causes accumulation of liquid in the packages and changes product color, texture and acceptability (Bastos et al., 2014).

A study that evaluated beef patties with addition of sorghum flour (2, 4 and 6%) showed lower cooking loss, lower diameter reduction, higher water and fat retention capacity and higher yield compared to the control (Huang et al., 1999). Oliveira et al. (2014) found that the addition of linseed flour, in beef burgers, contributed to a higher moisture retention and consequently a higher product yield.

#### 3.2. Shear force

The shear force did not differ among the formulations (P > .05; Table 1). Huang et al. (1999) reported a lower shear force value (1.39 kgf = 13.63 N) for beef patties with 6% sorghum flour compared to no sorghum flour (4.99 kgf = 48.93 N). It is worth mentioning that differences between studies may be related to the muscle types and anatomical location, age and animal breed. Moreover, in the present study the CN formulation contained ISP, which has an important role in the meat industry improving product texture (Kassama, Ngadi, & Raghavan, 2003).

#### 3.3. Color and pH

The results the color and pH of raw and cooked beef burgers are presented in Table 2. The raw CN formulation showed higher a<sup>\*</sup> (redness) e b<sup>\*</sup> (yellowness) values (P < .05) compared to the other

formulations. This result might be due to the presence of starch and fiber in the formulations with WSF (BRS 305 and BR 501) and the consequent dilution of the myoglobin pigment. However, difference among raw samples did not remain after cooking (P > .05). Comparing the raw and cooked samples, all the formulations reduced the a\* value and increased of b\* after cooking, which is probably due to the effect of the Maillard and caramelization reactions that occurred during the heat treatment applied. It was also observed that addition of ISP in the CN significantly reduced the L\* (lightness) value after cooking (P < .05).

Devatkal et al. (2011) found lower a\* values in chicken nuggets with 5 and 10% sorghum flour compared to the control, but without significant difference for the b\* value. A similar result was shown in work with chicken nuggets with added soybean hulls and green banana flours, where the authors attributed the results to the higher level of white components contained in the flours added (Kumar, Biswas, Sahoo, Chatli, & Sivakumar, 2013). Also, beef burgers with added dietary fibers showed lower a\* values than the control, possibly due to a gel formation between the added fibers and the meat protein, thus reducing the red coloration (Angiolillo et al., 2015).

There was no difference in the pH value between raw and cooked formulations (P > .05). The pH values ranged from 6.07 to 6.11 for the raw samples, and 6.17 to 6.22 for the cooked samples. The pH is an indicator of the meat quality and its values should be between 5.8 and 6.2 for a cooked product. Cooked meats with pH values around 6.4 should be immediatly consumed and those with values above 6.4, indicating a deterioration process, should be discarded (Barros et al., 2012). Similar results were reported by Huang et al. (1999) in study where beef patty formulations with different sorghum flour contents (0%, 2%, 4% and 6%) presented pH values between 5.94 and 6.24 for raw samples and between 6.04 and 6.25 for cooked samples.

The pH increased significantly after cooking in BRS 305 (P < .05). This result was also observed in beef patties formulated with bambara groundnut, regardless of the added concentrations (Alakali, Irtwange, & Mzer, 2010). On the other hand, in chicken burgers with addition of amaranth and pumpkin seed, maintenance of the pH values after cooking was reported, probably due to a buffering effect of these ingredientes as reported by the authors (Longato et al., 2017). According to Mehta et al. (2015) changes in the meat's pH depend strongly on the pH of the fiber that makes up the added food.

#### 3.4. Proximate composition

The proximate composition of the raw and cooked beef burgers with addition of WSF and ISP are presented in Table 3. The raw beef burger samples differed in moisture and lipid contents, among the formulations (P < .05), and protein content of BR 501 and BRS 305 was lower than CN (P < .05), but not different between them (P > .05; Tabela 3). The ash and carbohydrate contents did differ between CN and BR501, but did not from BRS 305. After cooking the moisture differed among CN and the others formulations (P < .05). Higher protein and ash contents and lower carbohydrate values were verified for cooked CN, while lower lipid content was found for cooked BR 501 (P < .05).

The highest moisture was found for BRS 305, both in raw and cooked form, compared to the CN which is associated with the higher moisture retention verified for this formulation, due to the higher fiber and resitent starch content presented by this sorghum genotype (Martino et al., 2012; Teixeira et al., 2016). The lipid content did not differ bethween BRS 305 and CN P > .05), but diffent from BR 501 (P < .05).

Devatkal et al. (2011) observed that cooked nuggets with 5% added sorghum flour in substitution of wheat flour presented higher moisture than control and the formulation with 10% sorghum flour addition, but without differing in lipids, proteins, carbohydrates and ash content. This result suggests that there is a maximum addition percentage, which provides desired product characteristics. Danowska-Oziewicz (2014) reported that low-fat pork patties with 5% and 10% added ISP

#### Table 2

	CN		BR 501		BRS 305	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
pH L* a* b*	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 6.17 \ \pm \ 0.02^{aA} \\ 33.73 \ \pm \ 1.38^{aB} \\ 6.14 \ \pm \ 0.19^{aB} \\ 14.21 \ \pm \ 0.50^{aB} \end{array}$	$\begin{array}{rrrr} 6.11 \ \pm \ 0.01^{aA} \\ 45.02 \ \pm \ 1.13^{aA} \\ 11.66 \ \pm \ 0.41^{bA} \\ 9.12 \ \pm \ 0.54^{bA} \end{array}$	$\begin{array}{rrrr} 6.22 \ \pm \ 0.05^{aA} \\ 35.09 \ \pm \ 3.27^{aA} \\ 6.37 \ \pm \ 0.58^{aB} \\ 13.74 \ \pm \ 1.39^{aB} \end{array}$	$\begin{array}{rrrr} 6.10 \ \pm \ 0.02^{aA} \\ 44.50 \ \pm \ 1.80^{aA} \\ 11.03 \ \pm \ 0.12^{bA} \\ 8.28 \ \pm \ 0.10^{bA} \end{array}$	$\begin{array}{r} 6.22 \ \pm \ 0.00^{aB} \\ 40.38 \ \pm \ 3.05^{aA} \\ 4.72 \ \pm \ 0.25^{aB} \\ 12.52 \ \pm \ 0.50^{aB} \end{array}$

Color and pH (mean  $\pm$  standard error) of raw and cooked beef burgers with isolate soy protein (CN) or whole sorghum flours of BR 501 and BRS 305 genotypes.

Means with different lowercase letters, in the same line, are significantly different by the Tukey test (P < .05) among the raw or cooked samples. Means with different capital letters, in the same line, are significantly different by the paired t-test (P < .05) comparing the raw and cooked samples.

showed higher protein content, but the patties with 2% ISP presented no difference from the control.

Comparing the raw and cooked samples it was found that the moisture was reduced, while the protein content increased significantly in all formulations (P < .05). There was also an increase in lipid content in BRS 305 and BR 501after cooking, and carbohydrate content only increased in BRS 305 (P < .05). The ash content increased in CN and BRS 305 after cooking (P < .05). Alterations in the proximate composition may be a result of changes in the total beef burger mass, due to water loss via dripping and evaporation, or due to higher retention water during cooking, associated with the type of ingredient added to the formulation.

# 3.5. Antioxidant properties

Table 3, also, shows the antioxidant properties of beef burgers with added WSF and ISP. There were significant diferences in the total anthocyanin (TA) contents, both in raw and cooked formulations, with higher values for BRS 305 (P < .05). In addition, higher condensed tannins (CT), total phenol (TP) contents and higher antioxidant capacity (AC) for BRS 305 (raw) compared to the other formulations were verified (P < .05). There is association between TP and AC in foods, since the phenolic compounds are mainly responsible for AC, being found in higher quantity in the sorghum genotypes with pigmented testa and CT (Awika et al., 2003), as the genotype BRS 305.

Tannic acid is a potent antioxidant because its chemical structure is able to inhibit the formation of hydroxyl radicals from the Fenton reaction by complexing ferrous ions (Lopes, Schulman, & Hermes-Lima, 1999), thereby retarding lipid and protein oxidation in meat. Therefore, the presence of CT in meat products might be beneficial from the technological point of view, since it may allow longer maintenance of the red color and flavor.

In the raw formulations, the BR 501 did not differ from CN (P < .05), probably due to the characteristics of this sorghum genotype, which has white pericarp and does not have CT (Martino et al., 2012). Consequently the BR 501 has fewer antioxidants, similar to that verified in the CN, with added ISP. Longato et al. (2017), in a study that evaluated the antioxidant properties of raw and cooked chicken burgers with 1 to 2% added amaranth or pumpkin seed, reported lower AC values for these formulations using the same determination method (ABTS)

In cooked formulations, there was no significant difference in CT content (P > .05). However, AC and TP remained higher in cooked BRS 305 (P < .05), probably due to the synergistic effect of higher TA (3-deoxyanthocyanine) (P < .05) and CT (P > .05) contents.

When comparing the antioxidant properties between the raw and cooked samples, reductions in the AC and TP were noticed in all the formulations, while the TA content was reduced only in BR 501 and BRS 305 (P < .05). The CT content did not differ from raw after cooking in all formulations evaluated (P > .05). During cooking, some compounds can form insoluble complexes with proteins, starches (amylose and amylopectin) and minerals (e.g. iron) through hydrogen bonding, polar bonding and hydrophobic interactions, thus reducing extraction and the detection of such compounds (Barros, Costa, et al., 2012). Furthermore, it is known that many phenolic compounds are sensitive to high temperatures as that used in the present study (190 °C/7 min), which may have led to their partial denaturation.

Currently, natural antioxidant sources have been considered as good alternatives both for health and to reduce or replace synthetic antioxidants used in the meat industry, since the increased of phenolics and

#### Table 3

Proximate composition, phenolic compounds (d.b.\*) and antioxidant activity (d.b.\*) (mean  $\pm$  standard error) of raw and cooked beef burgers with isolate soy protein (CN) or whole sorghum flours of BR 501 and BRS 305 genotypes.

	CN		BR 501 <sup>§</sup>		BRS 305 <sup>§§</sup>	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Moisture (%) Ash (%) Protein (%) Fat (%) Carbohydrate Total anthocyanins Condensed tannins Total phenols Antiovidant canacity	$\begin{array}{l} 59.01 \pm 0.37^{aA} \\ 2.85 \pm 0.06^{aA} \\ 19.13 \pm 0.68^{aA} \\ 17.98 \pm 0.34^{aA} \\ 1.03 \pm 0.72^{bA} \\ 0.04 \pm 0.00^{cA} \\ 0.29 \pm 0.02^{bA} \\ 3.90 \pm 0.04^{bA} \\ 6.68 \pm 0.44^{bA} \end{array}$	$\begin{array}{r} 49.66 \pm 0.32^{bB} \\ 3.20 \pm 0.01^{aB} \\ 28.19 \pm 1.28^{aB} \\ 19.39 \pm 0.18^{aA} \\ 0.47 \pm 0.24^{bA} \\ 0.03 \pm 0.00^{bA} \\ 0.48 \pm 0.05^{aA} \\ 3.45 \pm 0.03^{abB} \\ 2.94 + 0.39^{bB} \end{array}$	$\begin{array}{rl} 64.11 \pm 0.31^{\mathrm{bA}} \\ 2.45 \pm 0.03^{\mathrm{bA}} \\ 15.80 \pm 0.28^{\mathrm{bA}} \\ 14.40 \pm 0.16^{\mathrm{bA}} \\ 3.24 \pm 0.39^{\mathrm{aA}} \\ 0.05 \pm 0.00^{\mathrm{bA}} \\ 0.37 \pm 0.10^{\mathrm{bA}} \\ 3.85 \pm 0.04^{\mathrm{bA}} \\ 8.05 \pm 0.62^{\mathrm{bA}} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{r} 61.90 \pm 0.23^{cA} \\ 2.93 \pm 0.03^{aA} \\ 16.20 \pm 0.16^{bA} \\ 16.13 \pm 0.18^{cA} \\ 3.07 \pm 0.19^{abA} \\ 0.06 \pm 0.00^{aA} \\ 0.73 \pm 0.02^{aA} \\ 4.73 \pm 0.06^{aA} \\ 16.86 \pm 0.52^{aA} \end{array}$	$\begin{array}{r} 53.26 \pm 0.34^{aB} \\ 2.92 \pm 0.03^{bB} \\ 20.55 \pm 0.35^{bB} \\ 19.63 \pm 0.23^{aB} \\ 3.64 \pm 0.31^{aB} \\ 0.05 \pm 0.00^{aB} \\ 0.57 \pm 0.05^{aA} \\ 3.56 \pm 0.04^{aB} \\ 12.52 \pm 0.78^{aB} \end{array}$

Means with different lowercase letters, in the same line, are significantly different by the Tukey test (P < .05) among the raw or cooked samples. Means with different capital letters, in the same line, are significantly different by the paired t-test (P < .05) comparing the raw and cooked samples. Total anthocyanins (mg equivalent luteolinidine /g); Antioxidant capacity (mmol Trolox equivalent/g); Total phenols (mg gallic acid equivalents/g); Condensed tannins (mg equivalent of catechin/g sample). \*d.b.: on dry basis.

<sup>§</sup> Whole sorghum flour of BR 501: moisture 10.40%; ash 3.79%; protein 12.00%; fat 4.10%; carbohydrate 58.33%; 0.08 mg equivalent luteolinidine/g; 0.09 mg equivalent of catechin/g sample; 3.92 (mg gallic acid equivalents/g); 3.9 (mmol Trolox equivalent/g).

<sup>§§</sup> Whole sorghum flour of BRS 305: moisture 11.00%; ash 2.55%; protein 11.57%; fat 3.20%; carbohydrate 53.67%; 0.26 mg equivalent luteolinidine/g; 57.10 mg equivalent of catechin/g sample; 19.70 (mg gallic acid equivalents/g); 186.7 (mmol Trolox equivalent/g).



Fig. 1. Scores (1 and 3) and Loadings (2 and 4) plots of PCA for the physicochemical characteristics of raw and cooked beef burgers. CN: added isolate soy protein; BR 501: added whole sorghum flour of BR 501 genotype; and BRS 305: added whole sorghum flour of BRS 305 genotype.

other bioactive compounds in foods can be effective in preventing the initiation or propagation reaction during the lipid and protein oxidation process (Kumar, Yadav, Ahmad, & Narsaiah, 2015). Thus, sorghum flours with higher antioxidant compound contents have potential for use in the food industry to reduce lipid oxidation and to improve the nutritional quality of products.

# 3.6. Principal component analysis (PCA)

Using principal component analysis (Fig. 1) it was possible to determine patterns or structure in the data as well as identify the relative importance of individual variables in this study, thus facilitating the understanding of the dataset. Two principal components (PC1 and PC2) were used and together they explained 83.53% of the total variance observed among raw samples (Fig. 1.1 and 1.2), and PC1 explained the majority of the variations (60.18%) (Fig. 1.1). According to the loadings plot (Fig. 1.2), the variables fat, protein and color parameters (L \*, a \* and b \*) were positively correlated with PC1, whereas carbohydrates, moisture and TA correlated negatively with PC1. Ash, TP and AC correlated negatively with PC2 (Fig. 1.2).

The scores plot (Fig. 1.1) indicated different physicochemical characteristics among the raw formulations. According to the loadings

plot (Fig. 1.2) the raw BRS 305 was characterized by higher TA, CT, TP contents and higher AC. On the other hand, the raw BRS 305 presented lower fat, protein and ash contents, as well as lower values of L \*, a \* and b \*. BR 501 showed higher moisture, carbohydrate content and higher pH compared to other formulations. The CN showed lower moisture, carbohydrate, TA, CT, contents and AC, but intermediate values mainly for fat, protein, ash and L \*, a \* and b \* parameters.

Regarding the cooked samples, the sum of the principal components 1 and 2 (PC1 and PC2) accounted for 69.90% of the data variation (Fig. 1.3 and 1.4), and PC1 explained 49.55% of the variation. The variables cooking loss, diameter reduction and protein and ash correlated positively and significantly with PC1. The variables yield, moisture, carbohydrate and TA and AC, were negatively correlated with PC1. On the other hand, the variables fat and TP correlated positively with PC2 (Fig. 1.4).

The scores plot (Fig. 1.3) indicated different physicochemical characteristics among the cooked formulations. According to the loadings plot (Fig. 1.4) the BRS 305 was characterized by higher yield, moisture retention, fat content, CT, TA, TP and higher AC. On the other hand, this formulation showed lower shear force and diameter reduction, lower a \* and b \* values, as well as lower protein and ash contents than the other formulations. CN was positioned on the opposite side of



**Fig. 2.** Scores (1) and Loadings (2) plots of PCA for the sensorial attributes of cooked beef burgers. CN: added isolate soy protein; BR 501: added whole sorghum flour of BR 501 genotype; and BRS 305: added whole sorghum flour of BRS 305 genotype.

the BRS 305, indicating a characterization contrary to BRS 305. The BR 501 presented intermediate results, mainly for diameter reduction, cooking loss, pH, and a \* and b \* color parameters.

In addition, PCA (Fig. 2) indicated that two components (PC1 and PC2) were used and together explained 93.34% of the variations observed among sensory attributes of the samples. All sensory attributes correlated positively and significantly with PC1 (73.32%), indicating greater acceptance of the formulations with WSF added (BRS 305 and BR 501). This result is probably associated with the higher moisture retention observed in these samples, especially the BRS 305, which resulted in higher product softness. On the other hand, all the sensorial attributes were negatively correlated with CN.

#### 3.7. Consumer study

There was no significant difference between BR 501 and BRS 305 for all sensory attributes evaluated, as well as for purchase intention (P > .05; Table 4). CN showed lower taste, texture, overall acceptability and purchase intention scores than BRS 305 and BR 501 (P < .05). The CN color was less accepted than BR 501 (P < .05), but did not differ from BRS 305. These results were confirmed by PCA (Fig. 2).

The consumers "would probably buy" the beef burgers with added WSF (BRS 305 and BR 501), while "don't know if would buy" predominated for that with added ISP (CN). It is reported that products with added soy can influence the flavor, texture and overall quality of processed meat, depending on the type of product and the amount used

#### Table 4

Sensorial attributes and purchase intention (mean  $\pm$  standard error) of beef burgers with isolated soy protein (CN) or whole sorghum flours of BR 501 and BRS 305 genotypes.

	CN	BR 501	BRS 305
Aroma Color Flavor Texture Overall acceptability Purchase intention	$\begin{array}{rrrr} 7.1 \ \pm \ 0.14^{a} \\ 7.4 \ \pm \ 0.14^{a} \\ 7.2 \ \pm \ 0.15^{a} \\ 6.8 \ \pm \ 0.17^{a} \\ 7.1 \ \pm \ 0.13^{a} \\ 3.8 \ \pm \ 0.11^{a} \end{array}$	$\begin{array}{rrrr} 7.4 \ \pm \ 0.11^{a} \\ 7.8 \ \pm \ 0.09^{b} \\ 7.8 \ \pm \ 0.11^{b} \\ 7.5 \ \pm \ 0.12^{b} \\ 7.7 \ \pm \ 0.11^{b} \\ 4.2 \ \pm \ 0.08^{b} \end{array}$	$\begin{array}{rrrr} 7.4 \ \pm \ 0.12^{a} \\ 7.7 \ \pm \ 0.10^{ab} \\ 7.8 \ \pm \ 0.12^{b} \\ 7.4 \ \pm \ 0.13^{b} \\ 7.7 \ \pm \ 0.11^{b} \\ 4.2 \ \pm \ 0.09^{b} \end{array}$

Means with different letters, in the same line, are significantly different by the Tukey test (P < .05). Sensorial attributes: 9 points-hedonic scale: 9 = extremely liked; and 1 = extremely disliked. 5 point-purchase intention: 5 = surely buy the product and 1 = certainly would not buy the product).

(Danowska-Oziewicz, 2014). For example, its flavor is describe as bitter or astringent, unlike the sorghum which has a neutral flavor.

A study with gluten-free chicken nuggets showed that the addition of 5% sorghum flour did not differ from the control (5% wheat flour), regarding all the sensorial attributes (P > .05), but differed from the formulation with the addition of 10% sorghum flour (P < .05) (Devatkal et al., 2011). This result might be explained by the increase of firmness and alteration of flavor after this added percentage of sorghum flour. Another study that evaluated chicken burgers with added cereals, like wheat, sorghum and corn, showed no significant difference in color, taste, odor, appearance and overall acceptability (Ramadan & Sorour, 2016).

# 4. Conclusion

The use of WSF to replace ISP in beef burgers might be considered a technologically feasible option, since it did not alter most of the physical characteristics (except color). The formulations with added WSF, especially the BRS 305 genotype (with tannin), presented higher moisture retention and yield, and lower cooking loss resulting in a softer product. Nutritionally, both raw and cooked CN (with ISP) had higher protein and ash contents, and lower carbohydrate content, whereas BRS 305 showed higher lipid, TP, TA and CT contents, which resulted in a higher AC compared to the other formulations. Thus, is suggested that WSF with the presence of tannin (BRS 305) has the potential to replace ISP in meat products, bringing additional benefits to consumer health, as well as being able to reduce or replace synthetic antioxidants. The formulations with added WSF, independent of genotype, showed higher sensory acceptance and purchase intent than CN. The presence of CT did not interfere in the acceptance of the BRS 305. This research provides scientific support for the development of meat products with added sorghum of different genotypes, increasing the possibility of using this cereal in human nutrition. However, more studies should be conducted to identify which sorghum flour concentration is optimal to promote health benefits for the population and economic benefits for the industry, as well as which food matrix components are responsible for such benefits, ensuring quality and commercial viability.

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