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Wheat dietary fiber-added to low-fat semi-dry fermented buffalo sausage: proximate composition, physical-chemical, microbiological and sensory characteristics

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Abstract

The main objective of this study was to evaluate the incorporation of buffalo meat and wheat fiber into a semi-dry fermented sausage with reduced fat content. Nine different formulations were prepared with varying the buffalo meat content and the wheat fiber proportions in the mass then evaluated. Buffalo meat incorporation did not affect the pH and moisture content during the ripening period, nor the proximate composition of the final product, only increased the protein content of the fermented sausages. Fiber addition influenced the moisture content where decreased the drying yield. Buffalo meat and wheat fiber did not affect: the microbiological quality, the instrumental texture and color, nor the sensory acceptance of the final product. Semi-dry fermented buffalo sausage added with 1.5% wheat fiber presented itself as a promising product to the new consumer market, having microbiological stability (low pH), desirable nutritional value (low-fat, dietary fibers),technological (color, texture) and sensory characteristics. The present study proposed the elaboration of a new product with high added value that combines the functional properties of vegetable compounds with the high nutritional value of an under explored protein source, i.e. the buffalo meat.

Introduction

Although several studies have demonstrated the association of high intake of saturated animal fat with increased risk of cardiovascular disease, obesity, hypertension, atherosclerosis, and others (WHO, 2003), excessive consumption of such food is still a frequent habit of the population. According to a survey conducted in 2014 by the Brazilian Ministry of Health through the VIGITEL ("Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico" – Surveillance of Risk Factors and Protection for Chronic Diseases by Telephone Survey), about 30% of the population has the habit to eat meat with excess fat (without removing the visible fat) (Brazil, 2014).

Fat in meat products is directly responsible for quality attributes such as juiciness, firmness, taste, flavor and appearance. Thus, one of the major challenges of the meat industry is the fat reduction in products such as fermented sausages, as these have a high lipid content (35%) (Brazil, 2000; Morin *et al.*, 2002). In this sense, functional foods are presented as a potential tool (Brasil, 2008).

Dietary fibers are some of the main ingredients used in the development of new functional foods (Campagnol *et al.*, 2012). Fiber incorporation in dry or semidry fermented sausages enables to fix a high amount of moisture within the capillary connection system through multiple levels. It has advantages such as water activity decrease, improving texture, reducing time of drying due to the fiber structure, reducing weight loss (drying yield) and increasing shelf-life (Bollinger and Sieg, 2006).

In addition to incorporating functional properties to the product, dietary fiber helps in maintaining the physical-chemical and technology characteristics of low-fat fermented sausages. In this sense, several dietary fibers such as those from cereals, fruits and vegetables have been studied as fat substitutes in meat products (García *et al.*, 2002; Fernández-Ginés *et al.,* 2003; Eim *et al.,* 2008; Fernández-López *et al.*, 2008; Sánchez-Zapata *et al.*, 2013; Ham *et al.*, 2016). The advantages associated with their low cost of obtaining make their use feasible as a food ingredient, increasing the nutritional value also providing technological functionality, such as inhibition of lipid oxidation in meat products (Mendes *et al.,* 2014). Moreover, fibers have important physiological and physical actions of forming volume and maintain bowel regularity. Depending on their composition, they may have actions in the control of blood glucose

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and blood cholesterol levels (Francisco *et al*., 2013).

Another alternative for increasing the nutritional value of this meat product is the replacement of pork and/or bovine meat by buffalo meat, which considered healthier nutritionally due to its high protein and low fat content. Lira *et al.* (2005) evaluated different cuts of buffalo meat *(Bubalis bubalis)* confirming the low levels of lipids and cholesterol compared to beef. In addition, essential fatty acids were found, highlighting the hypocholesterolemic. According to Rodrigues and Andrade (2004), buffalo meat has less fat content and its softness is related to the lower growth rate of the diameter of muscle fiber, associated with less consistency of connective tissue. However, this protein source is still poorly explored and studied in the literature.

Thus, this study aimed to develop a low-fat semi-dry fermented wheat fiber-added buffalo sausage. The pH and moisture were monitored during the ripening process. The effects of fat replacement by wheat fiber and the buffalo meat incorporation were evaluated in the proximal composition, microbiological, physicochemical and sensory characteristics of the final product. In addition, the study also aimed to contribute to the appreciation of buffalo meat.

Material and Methods

Fermented sausages preparation

The fermented sausage was processed in the Laboratory of Meat Technology at the Federal University of Santa Catarina (UFSC).The meat cuts used in the fermented sausages were the knuckle *(Biceps femoris)* for cattle (beef) or buffalo meat, and the center loin *(Longissimus dorsi)* for pork meat. All raw materials were obtained from local commerce (Florianópolis, SC, Brazil). The wheat fiber was purchased from Vitacel® (WF 200, J. Rettenmaier Latioamericana Ltda., SP, Brazil).

Vitacel® wheat fiber (WF 200) is a bright dietary fiber, insoluble, neutral, inert, white in color, defibrated, of thin consistency, free of gluten, phytic acid, fatty acids and GMO (Genetically Modified Organisms). It has a total dietary fiber content of up to 97% and is produced from wheat by a special method. It presents different types of fiber lengths, between 30 and 500 µm, and can be used in basically all areas of the food industry (JRS, 2015).

For fermented sausages processing, the beef and buffalo meat were passed twice in a grinder with a disk diameter of 5 mm. In the cutter, the meats (beef, buffalo and pork) were added first; followed by the bacon (15%) and the mass was homogenized until the desired particle size. Sodium chloride (NaCl, 2.5%), milk powder (1%), red wine (1%), sucrose (0.3%), dehydrated garlic (0.15%), ground white pepper (0.1%) and nutmeg (0.04%) were then added. Next, the starter culture consisting of *Staphylococcus carnosus* and *Lactobacillus pentosus* (Flora Carn SL 200, Christian Hansen) was added by spreading (2.5 g into 100 mL of water - 0.04%). And lastly, the antioxidant (0.25% of sodium ascorbate - AI - Exato Laboratory, Brazil) and the curing agents (0.25% of sodium nitrate and nitrite - Griffith do Brasil S.A, Brazil) were added. The mass was transferred to the mixer, where it was homogenized for about 2 min. Each formulation was divided into 3 parts and wheat fiber was added. In total, nine formulations were performed with different percentages of beef and buffalo meat (0%:15%, 7.5%:7.5% and 15%:0%) and wheat fiber Vitacel® (0%, 1.5% and 3%). The pork was fixed at 75% in all formulations.

Each batch was stuffed into collagen casing (diameter of 45 mm and length of 20 cm) using a manual stuffer, with a final weight of 400 g. Later, the samples were smoked at a temperature of 24°C during 19 h. The fermentation and drying processes were performed at 12-18°Cwith ambient relative humidity for28 days. For storage of the final product, the fermented sausages were washed with chlorinated water (5 ppm) to remove the characteristic fungi, the collagen casing was removed and each fermented sausage was vacuum-package in polyethylene bags and stored at 4°C.

The ripening step (fermentation and drying) was monitored in terms of pH and moisture content. Proximate composition, color, texture (hardness and cohesiveness), weight loss, microbiological and sensory analysis were carried out on the final product. To perform the analysis, the fermented sausages were cut, removing portions of the center and the tips to ensure that they were representative of the whole. The proximate composition of the raw material was also performed.

Physico-chemical analyses and proximate composition

Fat, protein (Kjeldahl N x 6.25), ash and moisture contents of the fermented sausage samples and raw materials were determined according to the official methods: 991.36, 940.25, 938.08 and 950.46, respectively (AOAC, 1995).

The pH was measured with a pH meter (Hanna Instruments pH 21, USA) in a slurry made by mixing 5 g of sample with 5 ml of distilled water (AOAC, 1995).

Microbiological analysis

The fermented sausage samples were analyzed for total plate count, lactic acid bacteria, coliforms at 45°C, *Salmonella* sp., and *Staphylococcus* coagulase positive according to standard procedures (APHA, 1976; ICMSF, 1978). For all analysis, samples of 25 g were diluted with 225 mL of peptone water 0.1% and homogenized. Total viable counts were made on Plate Count Agar (PCA, 37°C/48h), lactic acid bacteria were numerated on de Man, Rogosa and Sharpe Agar (MRS, 30°C/48 h) by spreading method, coliforms at 45°Cwere counted using themultiple-tube fermentation technique and the *Staphylococcus* coagulase positive was determined using the Baird-Parker Agar. Pre-enrichment in Lactose broth (37°C/24h), selective enrichment in Selenite Cystine broth and Tetrathionate broth were carried out for the detection of *Salmonella* sp., before being finally followed by biochemical identification.

Texture analysis and drying yield

Texture profile analysis was performed with a texturometer (mod. TA.XT2i, Stable Micro Systems-SMS), using as parameters: probe p/5S; load cell of 5 kg, distance covered by probe of 11 mm; pretest speed of 1 mm/s; test speed of 0.83 mm/s; and post-test speed of 10 mm/s. Three cylinders in the direction of the fibers were taken for each fermented sausage sample (diameter of 4.5 cm and length of 7 cm) with a manual punch. The samples were compressed twice and the average of the three evaluations was obtained with the use of Texture Expert software version 1.15 (SMS). The following parameters were determined: hardness (N), maximum force required to compress the sample, and cohesiveness, the extent to which the sample could be deformed prior to rupture (A2/A1, A1 was the total energy required for the first compression and A2 the total energy required for the second compression).

The semi-dry fermented sausages were weighed before and after the ripening process (28 days). Drying yield was calculated according to Equation (1) below.

$$\text{Drying yield}(\%) = \frac{\text{Initial sample weight} - \text{Final sample weight}}{\text{Initial sample weight}} \times 100 \quad (1)$$

Color measurement

The instrumental colorof each treatment was measured using a colorimeter, Minolta CR 200 (Chroma Meter, Konica Minolta, Osaka, Japan), using CIELab system with L^*, a^* and b^* coordinates as indicators of lightness (L), redness (a) and yellowness (b). The measurements were performed in 3 distinct regions on the surface of sausages.

Sensory analysis

The preference ordering test (preference ranking) was applied with a panel composed of 38 untrained persons. The fermented sausages were individually presented to each panelist in a separate area where distractions, noises and odors were minimized. The panelists were not informed about the experimental approach and the samples were blind-coded with 3-digit random numbers. They were instructed to order the samples in the ascending order of preference. The results were evaluated by the Friedman method, using the Newell and MacFarlane table to see if there was a significant difference between the samples, at a significance level of 5%.

A nine-point hedonic scale (9 = like extremely to 1 = dislike extremely) was used for evaluation of the overall acceptability. Jointly, the buying intention was evaluated through a five-point hedonic scale (5 = certainly buy to 1 = certainly not buy). This test was performed with a panel composed of 50 untrained persons. Samples were provided in the same manner as described in the preference ordering test. The panelists were instructed to evaluate the samples globally.

Statistical analysis

All assays were performed in triplicate and the results were treated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test at a confidence level of 95% ($p<0.05$).

Results and Discussion

Proximate composition of raw materials

In order to characterize the pork, beef and buffalo meats and evaluate the replacements possibilities of the same in the development of a fermented sausage (salami), proximal composition was carried out (Table 1).

The buffalo meat presented the lowest values of moisture and lipids and the highest protein content compared to beef and pork. According to data in the literature, the lower fat and higher protein content (lean meat with high biological value protein) of the buffalo meat is well-known, in addition to tenderness and stronger red coloration than bovine meat (Ferraraand Infascell; 1994; Lira *et al*., 2005; Oliveira, 2005). Despite this, buffalo meat is still poorly explored. Thus it is important to develop products that add value to this meat.

The lower moisture content directly influences the ripening time (fermentation and drying) of the fermented sausage and the microbiological quality of the final product, reducing bacterial multiplication and

Table 1. Proximate composition (wet basis, mean ± standard deviation) of beef, pork and buffalo meat

	Beef	Buffalo	Pork
Moisture (%)	75.1 ± 0.2^{a}	72.7 ± 0.8^{b}	74.0 ± 1.0^{a}
Protein (%)	19.6 ± 0.2^{b}	23.2 ± 0.9^{a}	20.3 ± 0.6^{b}
Fat (%)	3.3 ± 0.2^{a}	2.0 ± 0.8^{b}	4.2 ± 0.01^{a}
Ash (%)	1.0 ± 0.1^{a}	1.1 ± 0.02^{a}	1.0 ± 0.02^{a}

Equal letters (same row) indicate that there is no significant difference among the values (Tukey, $p > 0.05$; $n = 3$).

enzymatic reaction, as well increasing the shelf-life of the product. Regarding the fat content, this directly influences the sensory characteristics of fermented sausages and it is essential for the development of aroma and flavor characteristics of these products. Thus, a decrease in the lipid content is advantageous from a nutritional point of view, but harmful from a sensory viewpoint. Therefore, it is important to study the replacement of beef and the decrease of fat in meat products, searching for compounds which assist this reduction such as dietary fiber.

Fermented sausages ripening

The moisture and pH results were monitored during the ripening process, as illustrated in Figures 1 and 2. At time zero, it was observed that the fermented sausage with buffalo meat showed higher initial moisture content (63.8%) compared to the other formulations. During ripening, the incorporation of wheat fiber had greater influence on the pH and moisture profiles than buffalo meat incorporation.

During the 28 days of fermentation, there was a considerable loss of water and this was almost linear with time, due to the drying process. As expected, samples without addition of fiber showed lower moisture content at the end of fermentation, with values between 41.3 and 42.6%. The addition of 1.5% and 3% of wheat fiber resulted in higher final moisture values, between 45.7-46.3% and 43.6-47.9%, respectively.

According to Campagnol *et al.* (2006), dietary fibers are used to improve the yield due to their water and fat retention capacity, improving the texture of products as well. Similar results were obtained by Ham *et al.* (2016), who also observed that the addition of collagen fiber resulted in a higher retention of water by the sausages, increasing the yields and obtaining high moisture in the final product. They attributed this to the gelling and water-binding properties of collagen and dietary fiber.

Regarding pH, all formulations showed similar behavior. It was possible to see a more pronounced decrease in the first 48 h after the stuffing step, even in formulations with added fiber. This decrease is

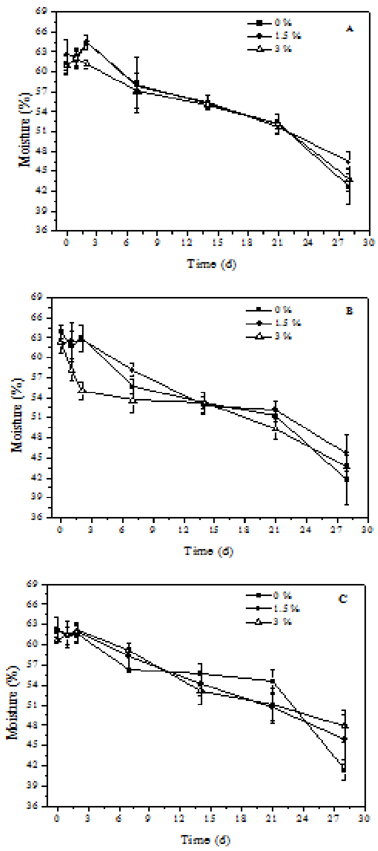

Figure 1. Water loss during ripening period of fermented sausages added with beef-BO (a), buffalo meat-BU (b) and beef/buffalo meats-BB (c) with different wheat fiber contents (0, 1.5 and 3%)

important for inhibition of undesirable spoilage bacteria, for color conversion and for the formation of the characteristic flavor of the dry fermented sausage (Lücke, 1994). The final pH values were between 4.7 and 5.0.

Fermented sausages generally present this pronounced decrease in pH values during fermentation (Eim *et al.*, 2008; Marco *et al.*, 2008; Salem and Ibrahim, 2010). The characteristic decrease in pH of fermented sausages is a consequence of lactic fermentation by lactic acid bacteria (Eim *et al.*, 2008). The reduction of pH of the fermented sausages to levels close to 5.0 (isoelectric point of the meat myofibrillar proteins) causes a water loss in the product, reducing its water activity, and favoring the preservation of the product (Ammor and Mayo, 2007). Similar values of final pH (4.91 to 5.12) in fermented sausage with added fiber were also achieved in other studies in the literature (Campagnol *et al.*, 2012; Mora-Gallego *et al.*, 2013).

Eim *et al.* (2008) observed that the pH was critically affected by the amount of dietary fiber incorporated in the fermented sausage during ripening. Contrary to what occurred in the present work, the addition of wheat fiber did not influence the decrease of pH during fermentation, thus achieving

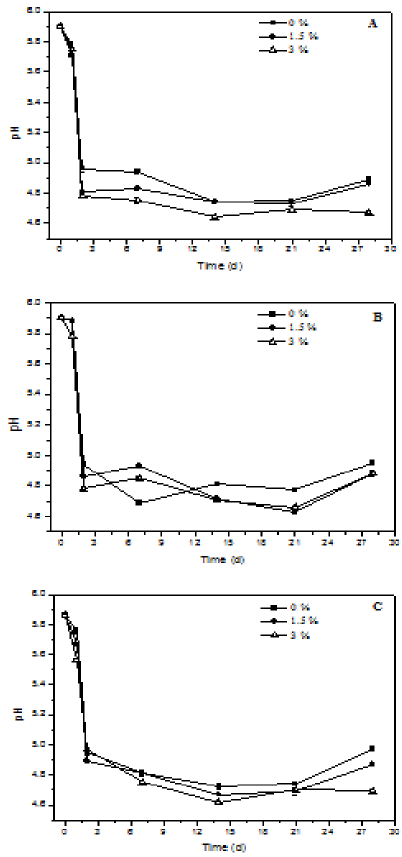

Figure 2. pH evolution during the ripening period of fermented sausages added with beef-BO (a), buffalo meat-BU (b) and beef/buffalo meats-BB (c) with different wheat fiber contents (0, 1.5 and 3%).

values of pH characteristic for this product.

At the end of fermentation, there was an increase in the pH value in all formulations. Salem and Ibrahim (2010) also observed an increase in the pH value from 4.69 to 5.53 in the fermented sausage formulated with buffalo meat. This fact can be attributed to the production of ammonia and other compounds such as peptides, amino acids, aldehydes, amines and fatty acids resulting from the proteolytic activity (Mauriello *et al*., 2004) and due to the decomposition of the organic acids (Salem and Ibrahim, 2010).

This increase could be due to the destruction of organic acid formed. These findings are in agreement with previous results of Kayaardi and Gok, (2003) and Vural (1998), who indicated that the production of organic acids by bacteria might be the cause of the noted decrease in pH, also, the observed increase in pH values might be due to the decomposition of acids and the formation of basic nitrogenous compounds.

Proximate composition

At the end of the ripening period, the proximate composition of each formulation was determined (Table 2). The moisture content of the nine formulations showed no significant differences (p>0.05), differing only from the commercial fermented sausage. In all

Table 2. Proximate composition (mean ± standard deviation) of fermented buffalo sausages with wheat fiber

Formulation	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
BO$_0$	42.6 ± 0.6^{b}	26.6 ± 0.9^{a,b}	18.6 ± 1.4^{b}	5.2 ± 0.3^{a}
BU$_0$	41.7 ± 1.7^{b}	28.5 ± 0.4^{a}	19.9 ± 0.9^{b}	4.5 ± 0.1^{a,b}
BB$_0$	41.3 ± 0.8^{b}	27.5 ± 1.6^{a,b}	19.3 ± 1.6^{b}	4.6 ± 0.2^{a,b}
BO$_{1,5}$	46.3 ± 0.5^{b}	26.2 ± 0.9^{a,b}	18.3 ± 1.4^{b}	3.9 ± 0.4^{b}
BU$_{1,5}$	45.7 ± 0.6^{b}	28.1 ± 0.4^{a,b}	19.6 ± 0.9^{b}	4.8 ± 0.2^{a,b}
BB$_{1,5}$	45.9 ± 0.3^{b}	27.1 ± 1.6^{a,b}	19.0 ± 1.6^{b}	4.9 ± 0.01^{a,b}
BO$_3$	43.7 ± 0.8^{b}	25.4 ± 0.9^{b}	17.8 ± 1.3^{b}	4.7 ± 0.7^{a,b}
BU$_3$	43.6 ± 0.3^{b}	27.3 ± 0.4^{a,b}	18.9 ± 0.9^{b}	4.9 ± 0.2^{a,b}
BB$_3$	47.8 ± 0.5^{b}	26.3 ± 1.5^{a,b}	18.5 ± 1.5^{b}	4.5 ± 0.5^{a,b}
CO	31.9 ± 0.6^{a}	25.3 ± 0.4^{b}	28.8 ± 0.3^{a}	5.0 ± 0.4^{a}

Equal letters (same column) indicate that there is no significant difference among the values (Tukey, p>0.05; n=3); CO = commercial sample;
BO$_0$, BO$_{1.5}$ and BO$_3$ = beef with 0, 1.5 and 3% of fiber;
BB$_0$, BB$_{1.5}$ and BB$_3$ = beef and buffalo meat (1:1) with 0, 1.5 and 3% of fiber;
BU$_0$, BU$_{1.5}$ and BU$_3$ = buffalo meat, with 0, 1.5 and 3% of fiber.

samples, the moisture content (41.3 and 47.8%) was higher than 40%, the maximum limit of moisture content for dry fermented sausages (Brazil, 2000). According to Vignolo *et al.* (2010), fermented sausages with moisture content higher than 35% can be labeled as semi-dry fermented sausage, a meat product popularly consumed in Northern European countries and in North America. The final pH of semi-dry sausages should be explicitly acidic (4.7 to 5.2 – 5.4) with a lactic acid content of 0.5% to 1.3% (Vignolo *et al.*, 2010), as achieved in the present study (pH values of 4.7-5.0). A lower pH is needed for satisfactory protection against undesired microorganisms. However, due to their high moisture and protein content, semi-dry sausages require refrigeration.

As it can be seen in Table 2, the fermented sausages with 1.5 and 3% wheat fiber achieved higher moisture contents, due to the gelling and water-binding properties of dietary fiber, as mentioned before. The hydroxyl groups on the fiber interact with water molecules forming a gel network (Terra, Fries and Terra, 2004).Insoluble fibers have strong hygroscopic properties; they swell and can absorb up to 20 times their weight in water. In a simplified way, water bound to insoluble polysaccharides can be presented in two forms: water bound by surface tension in the pores of the matrix; and water bound by hydrogen bonds, ionic bonds and/or hydrophobic interactions (Thebaudin *et al*., 1997).

Regarding the protein content, there was a variation from 25.3% (fermented sausage with bovine meat – BO$_0$) to 28.5% (fermented sausage with bubaline meat – BU$_0$) and only the BU$_0$ formulation was statistically different from the commercial sample. All formulations presented protein values above the minimum established by current Brazilian legislation (20%) (Brazil, 2000). The higher protein content of the formulations added with buffalo meat is noteworthy remarkable, this makes the product developed in this study attractive from the nutritional point of view, being recognized as a good source of high biological value proteins.

In the study of Petridis *et al.* (2015), higher protein values were also obtained in traditional Greek sausages with buffalo meat. A sample with 70% of bubaline meat resulted in a protein content of 42%, while sample with 70% of pork meat resulted in a protein content of 36.6%. These results show once more the high protein content of buffalo meat. In the study by Nassu *et al.* (2001), protein contents between 19.80 and 22.96% were obtained for samples of fermented sausages made with different proportions of goat and pork meat, both lower than those products made with buffalo meat.

The addition of wheat fiber did not interfere in protein content of the formulations. In the work of Eim *et al*. (2008), the increase of fiber concentration also did not influence the protein content of dry fermented sausages, having an influence on moisture, ash and fat content. However, depending on the fiber type and/or meat product the protein content may decrease with an increase in fiber concentration (Mehta *et al*., 2015; Ham *et al.*, 2016).

The nine formulations presented fat contents (17.8 to 19.9%) lower than commercial sample (28.8%) and the maximum established by the current Brazilian legislation (Brazil, 2000); that is 35%. Thus, the fermented sausage developed in this study can be considered low-fat, being healthier compared to traditional product (commercial).

According Lira *et al.* (2005) there is a tendency of products developed with bubaline meat to present lower fat content than products made with bovine meat, contributing to health and minimizing cardiovascular disorders. Thus, the use of buffalo meat becomes attractive for the new focus that has been given to healthy nutrition with low fat intake.

Despite the lower fat content of buffalo meat compared to beef, there was no significant difference between the nine formulations. This low lipid content is due to the cut of the meats used in this study (knuckle and center loin), which are already characterized by having a lower fat content. Although there was no statistically significant difference, it is observed that wheat fiber incorporation caused a reduction in lipid content by 4.3%, 5% and 4.2% in the semi-dry fermented sausages of beef (BO), buffalo meat (BU) and bovine/bubaline meat (BB), respectively. This reduction of fat content with the dietary fiber incorporation was also observed in other studies in the literature (Eim *et al*., 2008; Ham *et al*., 2016).

The addition of dietary fiber in meat products usually resulted in an increase in ash percentage (Ham *et al*., 2016). However, in the present study the increase in fiber concentration up to 3% was not enough to change the ash content of the final product. Statistical analysis showed that the $BO_{1.5}$ sample (bovine meat + 1.5% of fiber) was significantly different only from the commercial and BO_0 (bovine meat without fiber) samples, being statically equal to the other samples. The other formulations showed statistically equal values with each other. Campagnol *et al.* (2012) and Choi *et al.* (2014) obtained an increase in the ash content of the products with higher fiber concentration.

Microbiological analysis

The results of microbiological analysis were statistically equal for all formulations of fermented sausages and the averages obtained in each analysis were: <3 MPN/g for Coliforms at 45°C (tolerance: 103MPN/g); $<10^2$ CFU/g for *Staphylococcus* coagulase positive (tolerance: 5×10^3 CFU/g); absence in 25 g for *Salmonella* sp. (tolerance: absence in 25 g); 4.81 $\log_{10}$CFU/g for total plate count (TPC); and 3.78 $\log_{10}$ CFU/g for lactic acid bacteria (LAB). Where "MPN" is the "most probable number" and "CFU" is the "colony forming units". The results of coliforms at 45ºC, *Staphylococcus* coagulase positive and *Salmonella* sp. are within the acceptable limits for consumption, established by the Board Resolution n° 12 of ANVISA/MS (Brazil, 2001).

Regarding viable aerobic microorganisms, in the Brazilian federal legislation, there are no references for the evaluation of meat and meat products, however, the total plate count may be used as an indicator of hygienic-sanitary quality of food products (Siqueira, 1995; Salem and Ibrahim, 2010; Ham *et al.,* 2016). Ahmad and Amer (2013) described that the spoilage condition normally corresponds to a total plate count of about 10^7 CFU/g in meat products. In this work,a low total plate count was obtained for the fermented sausages, which indicates a good hygienic-sanitary condition. This is due to the microbiological quality of raw materials and adequate sanitation of the equipment used during processing. Moreover, the lactic acid bacteria inhibit the growth of spoilage and pathogenic microorganisms (Ahmad and Srivastava, 2007).

The TPC showed similar result with LAB count, indicating that most of the microorganisms were lactic acid bacteria, once again proving the adequate

Table 3. Drying yield, texture, color and sensory analysis of the semi-dry fermented buffalo sausages (mean ± standard deviation).

Formulation	Drying yield (%)	Hardness (N)	Cohesiveness	L^*	a^*	b^*	Overall acceptability	Buying intention
BO_0	$27.6 \pm 3.1^{a,b}$	$144.8 \pm 14.8^{b,c}$	$0.35 \pm 0.03^{a,b}$	47.9 ± 1.0^{a}	13.1 ± 0.8^{a}	3.1 ± 0.4^{c}	7.4	3.8
BU_0	29.3 ± 0.1^{a}	$164.3 \pm 23.7^{a,b,c}$	$0.36 \pm 0.06^{a,b}$	51.0 ± 1.6^{a}	12.1 ± 1.1^{a}	$3.5 \pm 0.6^{b,c}$	-	-
BB_0	24.7 ± 2.2^{b}	108.3 ± 20.4^{c}	$0.31 \pm 0.04^{a,b}$	48.8 ± 0.6^{a}	14.2 ± 0.3^{a}	$3.2 \pm 0.4^{b,c}$	-	-
$BO_{1.5}$	$27.0 \pm 1.0^{a,b}$	$131.9 \pm 11.1^{b,c}$	$0.41 \pm 0.17^{a,b}$	48.6 ± 2.2^{a}	13.3 ± 0.6^{a}	$3.6 \pm 0.5^{b,c}$	-	-
$BU_{1.5}$	$27.4 \pm 1.2^{a,b}$	$185.7 \pm 33.3^{a,b}$	0.26 ± 0.12^{b}	53.8 ± 8.6^{a}	12.4 ± 3.9^{a}	$4.1 \pm 1.0^{b,c}$	7.2	4.1
$BB_{1.5}$	$24.3 \pm 2.2^{a,b}$	229.1 ± 91.6^{a}	0.30 ± 0.03^{b}	53.2 ± 2.0^{a}	12.8 ± 1.3^{a}	$4.1 \pm 1.4^{b,c}$	-	-
BO_3	$25.7 \pm 0.5^{a,b}$	$118.7 \pm 14.1^{b,c}$	0.57 ± 0.03^{a}	50.0 ± 1.8^{a}	14.2 ± 0.3^{a}	$4.5 \pm 0.5^{a,b,c}$	-	-
BU_3	$26.3 \pm 0.6^{a,b}$	$122.6 \pm 3.2^{b,c}$	$0.40 \pm 0.11^{a,b}$	49.6 ± 0.8^{a}	14.2 ± 0.6^{a}	$4.2 \pm 0.5^{b,c}$	6.9	3.8
BB_3	$23.5 \pm 0.8^{a,b}$	$138.8 \pm 32.1^{b,c}$	$0.49 \pm 0.07^{a,b}$	52.9 ± 4.9^{a}	13.0 ± 1.5^{a}	5.2 ± 0.4^{a}	6.7	3.6
CO	-	$181.8 \pm 21.4^{a,b}$	0.29 ± 0.01^{b}	47.4 ± 2.1^{a}	9.8 ± 1.4^{b}	6.4 ± 0.7^{a}	-	-

L^*: lightness; a^*: redness; b^*: yellowness; Equal letters (same column) indicate that there is no significant difference among the values (Tukey, $p>0.05$; n=3);
CO = commercial sample;
BO_0, $BO_{1.5}$ and BO_3 = beef with 0, 1.5 and 3% of fiber;
BB_0, $BB_{1.5}$ and BB_3 = beef and buffalo meat (1:1) with 0, 1.5 and 3% of fiber;
BU_0, $BU_{1.5}$ and BU_3 = buffalo meat, with 0, 1.5 and 3% of fiber.

hygienic-sanitary condition of raw materials, equipment and processing. Bedia *et al.* (2011), Conte *et al*. (2012) and Ham *et al.* (2016) also obtained similar values for the TPC and LAB counts in their fermented sausages.

The major role of lactic acid bacteria is to produce lactic acid rapidly and reliably from sugars added to the sausage mix, although they may also show proteolytic and lipolytic activity. This acidification promotes the inhibition of pathogenic and microorganism contaminants, faster drying and improve the texture, among other factors (Bedia *et al.*, 2011).

At the end of ripening (28 days),a lactic acid bacteria count of about 3.78 log_{10}CFU/g was obtained for the formulations of fermented sausage. Depletion of fermentable substrate probably occurred, indicated by the slight increase in pH during the 21st and the 28th day. Sawitzki (2000) obtained a LAB count of 7.00 log_{10} CFU/g in a fermented sausage using a starter culture of *Lactococcus lactis* ssp *lactis*.

Salem and Ibrahim (2010) found values of TPC between 3.75 and 3.61 log_{10}CFU/g and a LAB count between 4.99 and 5.01 log_{10} CFU/g at the end of the curing period (15 days) of fermented sausages produced with buffalo meat and sage oil extract. Conte *et al*. (2012) achieved values in the order of 8.82-9.33 log_{10} CFU/g for TPC and 8.37-8.97 log_{10} CFU/g for LAB count in a fermented sausage elaborated with buffalo meat. Bedia *et al.* (2011) obtained a TPC of 7.93-8.90 log_{10} CFU/g and a LAB count of 7.82-8.83 log_{10} CFU/g in a semi-ripened salami stuffed in swine gut.

In the small caliber fermented sausages added with collagen and dietary fiber prepared by Ham *et al.*(2016), the LAB count increased from 6.11-6.39 to 7.55-7.92 log_{10} CFU/g during the ripening period. The TPC increased from 6.29-6.49 to 7.60-7.89 log_{10} CFU/g.

Texture analysis and drying yield

The weight loss (drying yield) of the meat product is influenced by the drying process, and is directly related to the yield of the final product. As explained by Rust (1994), during the maturation period of fermented sausage, there is a decrease in pH value, reaching the protein isoelectric point. Therefore, there is a decrease in the ability of the proteins to remain bound to the water, occurring water loss and consequent weight loss.

Addition of fiber to the fermented sausages was crucial to reduce the drying yield in this study, resulting in higher water retention and reducing dehydration. That is, a higher yield was obtained in the semi-dry fermented sausages added with wheat fiber compared to the same formulation without the fiber (Table 3).

This behavior was also observed by other authors. Ham *et al*. (2016) developed small caliber fermented sausages added with a mixture of collagen and dietary fiber. The authors verified that the increase of collagen and fiber concentration caused a decrease in the drying yield by up to 22%. Terra, Terra and Terra (2004) verified that the addition of 1.5% of fiber in a

40 mm caliber fermented sausage caused a reduction of the drying yield from 50% to 46%.

The compression parameters obtained with TPA have been employed by many authors in their evaluations of meat products, such as dry fermented sausages, as an index to determine the quality of the finished product or to select the best functional ingredients (García *et al*., 2002; Hoz *et al*., 2004; Herrero *et al*., 2007; Eim *et al*., 2008; Rey *et al*., 2011; Conte *et al*., 2012; Petridis *et al*., 2015).

Regarding texture analysis of semi-dry fermented sausages developed in this study (Table 3), values between 108.3 and 229 N were obtained for hardness and between 0.26 and 0.57 for cohesiveness. The addition of 1.5 and 3% of wheat fiber in the semi-dry fermented sausages as well as the substitution of beef by bubaline meat at 7.5 and 15% caused no significant changes in the texture of the final product.

Hoz *et al*. (2004) developed an n-3 fatty acid and α-tocopherol enriched dry fermented sausage. The final product presented a hardness of 145.2 N and cohesiveness of 0.52. Herrero *et al.* (2007) performed the texture profile analysis of five different dry fermented sausages (salami) and obtained values of hardness between 98.2 and 276.1 N and values of cohesiveness between 0.32 and 0.45; therefore, the values obtained in this study are in accordance with literature. Using a principal components analysis, Herrero *et al.* (2007) observed that the salami texture is induced by water activity (a_w) and fat content. High values of aw and fat resulted in higher adhesiveness and intermediate breaking strength; being with hardness, springiness and cohesiveness not being much affected significantly.

García *et al.* (2002) evaluated the utilization of cereal and fruit fibers in low fat dry fermented sausages. Using wheat fiber, the authors obtained values of hardness and cohesiveness of 207-315 N and 0.49-0.69, respectively. The incorporation of wheat fiber results in higher values of hardness and cohesiveness. Similar behavior was obtained by Eim *et al*. (2008). The addition of carrot dietary fiber on dry fermented sausage (sobressada) increased the values of hardness from 35.6 N to 52.8. The lower hardness value is probably due to the higher fat used during manufacture of the fermented sausages (64.8%) compared to the percentage used in this study (15%), besides the lower meat content (27.8%).

Petridis *et al.* (2015) evaluated the effect of buffalo meat on the texture of traditional Greek sausages. The hardness increased proportionally to the increase in buffalo meat content in the samples due to the higher protein and lower lipid content. Rey *et al*. (2011) also observed a greater hardness in the sausages with buffalo meat.

Color analysis

The addition of wheat fiber and buffalo meat in the semi-dry fermented sausage causes no statistical change in the lightness *(L^*)* and redness *(a^*)* values. Regarding the a^* coordinate, the nine formulations were only different from the commercial product (CO). The samples showed intermediate coloration for L^*, ranging from 47.9 and 53.8.

The L^* coordinate was significantly influenced by the fat in meat products, so higher fat levels resulted in higher values of L^* (Pietrasik, 1999; Ham *et al*., 2016). Cavenaghi and Oliveira (1999) studied the instrumental color of six commercial dry fermented sausages. They obtained values between 47.6 and 49.6 for lightness *(L^*)* and between 11.6 and 15.5 for redness *(a^*)*.

The redness *(a^*)* is indicative of quality in meat products, with the red color being recognized by the consumer as a quality factor. Values between 12.1 and 14.2 were obtained for the semi-dry fermented buffalo sausages, indicating the predominance of red color in all samples, which was expected for meat product.

Coordinate b^* indicates the presence of yellow coloration, so, b^* values higher than a^* correspond to a product with medium brown and whitish colorations, and this is unpleasant from the consumer viewpoint, whereas did not occur with the fermented sausages developed in this work. The b^* values (yellowness) ranged from 3.1 to 5.2, and the highest values were obtained in the formulations with wheat fiber.

Soyer and Ertas (2007) reported that lightness and yellowness of reduced-fat fermented sausages were significantly lower than high-fat ones, whereas redness was significantly higher in reduced-fat treatments. The same behavior was obtained by Ham *et al.* (2016) in small caliber fermented sausages added with a mixture of collagen and dietary fiber.

Eim *et al*. (2008) evaluated the effect of the addition of carrot fiber on the instrumental color of dry fermented sausages (sobrassada). The coordinate that had more influence was the lightness *(L^*)*, with a decrease being observed in the treatments with higher fiber content. A slight decrease in redness *(a^*)* and yellowness *(b^*)* were verified with the increase of fiber content.

Bedia *et al.* (2011) evaluated different starter cultures in semi-ripened salami stuffed in swine gut. They obtained values between of 50.9-53.2 for coordinate L^*, 13.2-16.7 for coordinate a^* and 1.81-6.49 for coordinate b^*. The authors verified that the parameters a^* and b^* decreased after ripening due to the nitrosylmyoglobin formation and the transition from red-yellowish to red-bluish, typical for ripening in dry-cured meat. The authors also report that the levels of fat, haematic pigments, nitrate/nitrite and moisture among other factors affect the reflectance values of fermented sausages (salami). In the study of Hoz et al. (2004),a^*, b^* and L^* values of around 12.0, 7.0 and 45.6, respectively, were obtained for a dry fermented sausage enriched with n-3 fatty acid and α-tocopherol.

Sensory analysis

Firstly, the preference test with the nine formulations was performed. Friedman's test showed no significant difference between the samples, i.e., the incorporation of fiber and buffalo meat did not affect the sensory characteristics that are expected of a semi-dry fermented sausage. However, the formulation with buffalo meat only and without wheat fiber (BU0) had a stronger flavor, precisely because the buffalo meat presented a more pronounced flavor than bovine meat. But, the formulation with the addition of 1.5 to 3% of fiber showed a milder taste, that is, wheat fiber softened the strong buffalo meat flavor and increasing the preference of buffalo semi-dry fermented sausage. Samples with only bovine meat and 1.5 or 3% of fiber showed a pale coloration and the wheat fiber negatively interfered with the flavor of fermented sausages. Further, the formulation with the mixture of the two meats (bubaline and bovine) was not well accepted by the panelists.

The four most preferred semi-dry fermented sausages by the judges (BO_0, BB_3, $BU_{1.5}$ and BU_3) were subjected to acceptance testing (overall acceptability) and buying intention (Table 3).Semi-dry fermented sausages BO_0 and $BU_{1.5}$ presented the higher overall acceptability, 7.4 and 7.2, respectively, i.e., with an acceptance between "liked extremely" and "liked moderately". 96% of panelists gave a score between 9 (liked extremely) and 6 (like slightly) for BU1.5 sample. Sample BO_0 also presented a high acceptance, 88% of the panelists liked the product (score between 9 and 6). Samples BB3 and BU3 presented overall acceptability of 6.7 and 6.9, respectively, i.e., with an acceptance between "like slightly" and "liked moderately". The formulation with lower acceptance was BB3; 76% of the panelists gave a score between 9 and 6, and 14% disliked (score below 4 – disliked slightly).

The BB_3 and BU_3 samples showed lower overall acceptances because they presented a more pronounced acid taste, according to comments exposed by the panelists. It is worth mentioning that most of the panelists pointed out that the flavor of the samples was very close to commercial sample.

Buying intention presented similar results of global acceptance (Table 3). The $BU_{1.5}$ sample had the highest average, which was 4.1, meaning a buying intention between 4 (probably buy) and 5 (certainly buy), where 72% of the panelists gave a score between 4 and 5. BO_0, BB_3 and BU_3 samples showed scores of 3.8, 3.6 and 3.8, respectively, meaning a buying intention between 3 (maybe buy/maybe not buy) and 4 (probably buy), while 68% of the panelists gave a score between 4 and 5 for the BO_0 formulation. The lowest percentage was obtained for BB_3 sample, which reached 56% of buying intention.

Sensory analysis results demonstrated that the incorporation of buffalo meat and wheat fiber in semi-dry fermented sausages can be made without interfering in the acceptance of the product, thus conserving its sensory characteristics. In the literature, other fermented sausages produced with buffalo meat presented high overall acceptability. The dry fermented sausage with sage oil developed by Salem and Ibrahim (2010) presented an overall sensory quality score of between 6.5 and 7.4 at the end of ripening period. The panelists gave scores from 1 (worst) to 10 (best). The dry fermented buffalo sausage of Conte *et al.* (2012) presented a taste score between 6 and 7 in a nine-point hedonic scale. The authors reported that the sausages were well accepted. Petridis *et al.* (2015) evaluated different sensory attributes of a traditional Greek sausage elaborated with buffalo meat using a 15 cm long unstructured scale. Redness and hardness increased significantly and the juiciness declined with buffalo meat enrichment, presumably due to gradual pork content increase in the samples, which was expected due to the characteristic protein and fat content of the buffalo meat.

Eim *et al.* (2008) evaluated the influence of the addition of carrot fiber on the sensory attributes of a dry fermented sausage (sobrassada). They verified that the treatments until 3% of fiber presented a high overall acceptability, over 75% for all attributes, achieving higher scores than the control sample (without fiber). The beef sausages formulated with powdered cellulose, concentrated barley beta-glucan and potato fibers produced by Ktari *et al*. (2014) had higher acceptability scores than the control. The addition of the three fibers induced no negative effects on the flavor, texture and overall acceptability of the sausages. The product obtained scores between 4.29 and 4.79 in a five-point hedonic scale.

Ham *et al*. (2016) studied the effect of fat replacement (from 25 to 75%) with a mixture of collagen and dietary fiber (microcrystalline cellulose and carboxymethyl cellulose) on small caliber fermented sausages. The sensory attributes were evaluated with a 10-point hedonic scale. Three formulations (25, 50 and 75% of pork fat replaced with mixture of collagen and fiber) achieved high overall acceptability (score of 8.2). Most sensory properties were not significantly affected by the incorporation of collagen and fibers and fat reduction. Texture was the attribute that got lower scores than control; ranging from 7.9 to 8.0 (the control got 8.3). The results obtained by authors demonstrated that the substitution level of 50% had the highest score of overall acceptability and the incorporation of collagen and dietary fibers promoted no significant changes in sensory properties of the dry fermented sausages.

García *et al.* (2002) verified that the reduced-fat dry fermented sausage with 1.5% of wheat fiber presented an overall acceptability statistically equal to the control; the scores were 6.1 and 6.5, respectively, in a 10-point hedonic scale. Although, 3% wheat fiber sample obtained a 4.04 score (overall acceptability). Once again, the texture was the sensory property most affected by the addition of fiber.

Conclusion

At the end of ripening, the fermented sausages achieved a moisture content higher than 40% and acid pH value (4.8), fitting in the standard of a semi-dry fermented sausage. The lower dehydration improved the texture and decreased the drying yield. The semi-dry fermented sausage was elaborated with low fat content and high protein content, adding higher nutritional value to the product. Microbiological, physicochemical characteristics (texture and color) and sensory properties were not affected by fat reduction and incorporation of wheat fiber and buffalo meat. Formulation with 15% of bubaline meat and 1.5% of wheat fiber gained an acceptability of 96% by panelists and 72% of buying intention. This study showed a new product with reduced fat content and high biological value and functional properties of dietary fibers. Additionally, the present work contributes to the appreciation of buffalo meat, a protein source that is stillundervalued, butthat has high nutritional value.

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