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# EFFECT OF BITTER ORANGE OR SWEET ORANGE JUICES ON THE CHARACTERISTICS OF BEEF SAUSAGES

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**Keywords:** inlaid, meat product, proximate composition, texture, color

#### Abstract

This study evaluated the effects of replacing water with bitter orange and pear orange juices on the properties of beef sausages. Three formulations were prepared: control with water (F1) and two experimental formulations with bitter orange juice (F2) and pear orange juice (F3). Moisture, protein, lipid, ash, carbohydrate content, pH, water activity, shear force, and instrumental color were analyzed. Formulations complied with legislation for moisture (< 70 %), protein (> 12 %), and lipid (< 30 %) content. Moisture content ranged from 55.96 % (F3) to 60.32 % (F1), with the reduction in F2 and F3 attributed to the soluble solids in the juices. Protein content remained stable across formulations (22.24–23.39 %), reflecting the consistent meat proportion. Lipid content varied significantly, with F1 (15.25 %) showing the lowest value and F3 (19.29 %) the highest due to altered lipid retention influenced by acid-matrix interactions. F2 exhibited the highest acidity (0.40 g 100 mL $^{-1}$ ) and the lowest pH (4.68), leading to the lowest shear force (56.68 N). Due to particularities in the preparation, meat and fat portions were analyzed for colorimetry. Lighter sausages with lower redness ( $a^*$ ) values in F2 (5.20) and F3 (4.48) compared to F1 (8.50), and higher yellowness ( $b^*$ ) in F2 (7.26) were observed for the meat portion. The total color difference ( $\Delta E^*$ ) values indicated noticeable differences, particularly for F2 and F3 in the meat portion and F3 in the fat portion. These findings demonstrated that orange juices can modify the properties of beef sausages, enhancing their functional attributes, offering opportunities for the development of new food products.

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# Introduction

Meat is a highly perishable product, with its shelf life strongly influenced by storage conditions. Throughout history, humans have sought to preserve its quality, leading to the development of preservation techniques that have evolved from rudimentary methods to advanced technologies [1].

Sausages are spiced meat products encased in natural or artificial casings made from a variety of meats, including beef, pork, poultry, lamb, or fish. Available globally in butcher shops, they are prepared through cooking, curing, maturing, or as fresh products [2,3]. Sausages rank among the oldest processed foods, with hundreds of varieties shaped by local ingredients, climate, and cultural traditions. While their production initially relied on traditional practices, understanding the scientific principles of their physical, chemical, and biological processes is a more recent advancement. This knowledge has improved control over sensory qualities, nutritional value, and safety [4].

Maracaju sausage, originating from the Maracaju region in Mato Grosso do Sul, Brazil, began as a method of meat preservation and evolved into a cultural tradi-

tion. It is made from premium beef cuts such as striploin, rump, and brisket. It includes up to 30% fat and is seasoned with salt, pepper, garlic, and bitter orange juice (*Citrus aurantium*). Recognized with a Geographical Indication (GI) by the Brazilian Institute of Intellectual Property (INPI), it is protected against origin and production falsification [5].

Bitter orange is a member of the Rutaceae family commonly found in domestic orchards. Cultivated from seeds, the medium-sized plants produce thick-skinned, acidic fruits [6]. Native to Maracaju, bitter orange is more acidic than sweet orange, which dominates global citrus production, accounting for nearly 50% of total citrus output [7]. In Brazil, the pear orange (*Citrus sinensis*) is the most cultivated variety, used for both fresh consumption and juice production [8].

The use of citrus fruit juices, fibers, and extracts in sausages has varied effects on the products' physicochemical, sensory, and technological properties, depending on the type and concentration of the citrus used. Extracts from grapefruit, lemon, tangerine, and orange peel can increase the yellow intensity of sausages due to the presence of

natural pigments such as carotenoids and flavonoids, without negatively affecting the typical red color of cured sausages [9].

The use of citrus fibers or orange juice residue can add citrus sensory notes, in addition to increasing characteristic acidic flavors and aromas, especially at higher concentrations [10]. The addition of citrus fibers may also make the product firmer and less elastic, particularly at high levels (e. g., 2%) [11]. Citrus fibers and juice residue help reduce lipid oxidation, which extends shelf life and maintains the red color for a longer period [10,11].

Moreover, citrus extracts can serve as natural reducing agents, potentially replacing synthetic additives such as sodium ascorbate without compromising curing efficiency or oxidative stability [9]. The addition of citrus fibers does not significantly alter the yield, emulsion stability, or sensory characteristics at moderate levels [11,12]. Specifically, orange juice/residue reduces oxidation, adds flavonoids, and improves shelf life, while imparting a slightly citrus flavor to sausages [10,11].

While recent research often focuses on the valorization of citrus by-products like peel and bagasse extracts for their rich bioactive compounds and to address waste management [12–15], the direct use of orange juice in food formulations, such as in Maracaju sausages, is also a viable method to leverage its natural acidity and antioxidant properties.

Given this context, this study aimed to investigate the specific effects of bitter orange and sweet orange juices, which are integral to the traditional preparation of Maracaju sausage, and to evaluate how they influence the physical, chemical, and microbiological characteristics of this beef product.

## Objects and methods

Beef sausages

The beef sausages were prepared following the traditional Maracaju sausage manufacturing process, using three different formulations: F1 (control with water), F2 (traditional recipe with bitter orange juice), and F3 (with pear orange juice), as detailed in Table 1.

For that, the beef brisket was cleaned and stored together with the beef fat at  $4^{\circ}$ C for 30 min. Then, the meat was chopped into  $1\times2$  cm rectangles and the fat into  $1\times1$  cm cubes. Meat, fat and the other ingredients were weighted according to the sausage formulation and then mixed in a CAF M60 mixer (CAF Máquinas, Brazil) for 10 min at  $4^{\circ}$ C. Each mixture was embedded using an EP-8 manual filler (Picelli, Brazil), in natural bovine casings hydrated for 1 h in water before embedding and packaged in a spiral. Sausages were immediately utilized for the analysis.

Beef (brisket) and beef fat were purchased at the market (Maracaju, Mato Grosso do Sul, Brazil). Bitter oranges (*Citrus aurantium*) were harvested (Maracaju, Mato Grosso do Sul, Brazil), selected and washed, and the bitter orange juice obtained by manually squeezing. Commercial

pasteurized orange juice from pear orange (*Citrus sinensis*) was purchased from the local commerce (Dourados, Mato Grosso do Sul, Brazil). The condiments were supplied by Cavenaghi Eireli (Dourados, Mato Grosso do Sul, Brazil).

Table 1. Formulations of beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Ingredient, %	Formulation			
	F1	F2	F3	
Beef (brisket)	67.70	67.70	67.70	
Beef fat	20.32	20.32	20.32	
Water	8.81	_	_	
Bitter orange juice	_	8.81	_	
Pear orange juice	_	_	8.81	
Salt	1.76	1.76	1.76	
Pepper	0.85	0.85	0.85	
Garlic	0.35	0.35	0.35	
Scallion	on 0.18		0.18	

Microbiological analysis

To assess microbiological analysis, duplicate 25 g samples were aseptically transferred into a stomacher bag containing 100 mL of sterile distilled water containing 0.1% peptone (1% for *Salmonella spp.* determination). Samples were homogenized for 1 min. Ten-fold serial dilutions were prepared using sterile 0.1 peptone solution (9 mL) and spread plated (0.1 mL) in duplicate onto broths and/or agars for detection of typical colonies, biochemical confirmation and identification, and plate counting for thermo-tolerant total aerobic mesophilic microorganisms, *Escherichia coli*, and *Salmonella spp.*, in accordance with the methodology described elsewhere [16].

## Water activity and pH

Water activity of the samples was determined in triplicate using a hygrometer Aqualab, model CX-2, series 3 (Decagon Devices, Inc., United States) at 25 °C with 1 ml of sample. pH of the samples was measured in triplicate using a digital pH meter model HI99163 (Hanna Instruments, Brazil) [17].

# Titratable acidity

For the determination of the titratable acidity, the titration apparatus was a standard laboratory burette and stand setup. The equipment consisted of a 50 mL glass burette (Schott Duran, Germany), a magnetic stirrer model: C–MAG HS7 (IKA, Germany), and a stand. It was used for the chemical titration of 10 g of sample diluted in 90 ml of distilled water in a 250 mL beaker, using phenolphthalein as a final indicator of the reaction until the appearance of the pink color. The titrant used was 0.1 N sodium hydroxide and titratable acidity was expressed in g 100 mL<sup>-1</sup> [18]. The procedure was carried out in triplicate.

## Shear force

Texture analysis was carried out using a texture analyzer Model TAXTplus (Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Sausages were equilibrated at room temperature (28–30 °C) before

analysis. Cylindrical samples of 20 mm diameter and 30 mm length were cut, placed in the texture analyzer, and submitted to a cutting/shearing test (speed of 1.0 mm s<sup>-1</sup>, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the shear force (N). A minimum of 10 replicates of each treatment were analyzed [19].

## Proximate composition

Moisture, crude protein, and crude ash contents were determined in triplicate according to the methods described by AOAC (2012) [20]. Moisture was determined by the oven drying method at 105 °C until constant weight (method 950.46), protein by the Kjeldahl method (method 928.08) utilizing a conversion factor of 6.25, ash by using the muffle oven technique (method 920.153), and crude fiber by chemical digestion (method 978.10). The lipid content was obtained in triplicate by the extraction method with cold organic solvent [21]. The carbohydrate content was estimated by difference.

## Instrumental color

The color [CIE  $L^*$ (lightness),  $a^*$  (redness),  $b^*$  (yellowness)] was evaluated using a colorimeter Chroma Meter CR410 (Konica Minolta Inc., Japan), with measurements standardized with respect to the white calibration plate [22]. After the transversal cut of the sausages, five readings were made for the portion containing meat and another five readings for the portion containing fat, for each formulation.

The hue saturation index or chromaticity ( $C^*$ ) and the hue angle ( $h^\circ$ ) were obtained from Equations 1 and 2.

$$C^* = \sqrt{a^*2 + b^*2}. (1)$$

$$h^{\circ} = \frac{\tan - 1b^*}{a^*}.\tag{2}$$

The total color difference ( $\Delta E^*$ ) was obtained using Equation 3. For  $\Delta E^*$  < 1.5, the sample color was considered almost identical to the original. For  $1.5 \le \Delta E^* \le 5$ , the color difference was considered distinguishable. For  $\Delta E^* > 5$ , the color difference was considered evident.

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}.$$
 (3)

Statistical analysis

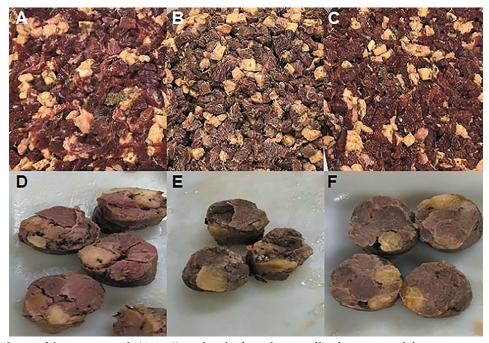
Statistical results were evaluated through analysis of variance (ANOVA) and Tukey's test for comparison of means, at a level of 5% of significance, using the statistical software Statistica 7.0. The results were presented as means ± standard deviations.

#### Results and discussion

The mixture of ingredients and the corresponding beef sausages can be observed for F1 (control with water) (Figure 1A and Figure 1D, respectively), F2 with bitter orange (*Citrus aurantium*) juice (Figure 1B and Figure 1E, respectively) and F3 with pear orange (*Citrus sinensis*) juice (Figure 1C and Figure 1F, respectively).

# Microbiological analysis

To evaluate the microbiological quality of the fresh beef sausages, microbiological evaluations were carried out in triplicate for aerobic mesophilic bacteria, *Escherichia coli* and *Salmonella spp*. According to Brazilian Legislation, all samples met the legal standards for sausage from meat from butcher's animals (Table 2), which limits *E. coli* to  $1 \times 10^2$  CFU/g and aerobic mesophilic bacteria to  $1 \times 10^6$  CFU/g, beyond the absence of *Salmonella* spp. in 25 g of the product [23]. The absence of *Salmonella* spp. is important due to the serious food poisoning caused by these microorganisms [24].



**Figure 1.** Photos of the raw materials (A, B, C) used in the formulations of beef sausages and the cross-sections (D, E, F) of the respective sausages after stuffing, for F1, F2 and F3, respectively

Table 2. Microbiological analyses of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Determination	Formulation			
	F1	F1	F1	
Aerobic mesophilic bacteria (CFU g <sup>-1</sup> )	$6.4\times10^{3a}$	$6.2\times10^{3a}$	$6.4 \times 10^{3a}$	
Escherichia coli (CFU g <sup>-1</sup> )	$3.1 \times 10^{1a}$	$2.5 \times 10^{1a}$	$2.9 \times 10^{1a}$	
Salmonella spp.	Absence	Absence	Absence	

CFU: colony-forming units. Means with the same letter in the same line do not differ statistically at 5 % (p > 0.05). p: probability.

Water activity, pH, titratable acidity and shear force

Table 3 presents the results of determinations of water activity, pH, titratable acidity and shear force of the beef sausages. The water activity varied from 0.965 (F3) to 0.983 (F1), showing significant differences between them (p < 0.05). This range of water activity is in line with research by Akbar et al. [25], which verified water activity in cooked beef sausages prepared with different fat sources ranging from 0.96 to 0.97 after one day of storage. Water activity is the aspect utilized to determine the viability of microbial growth in food. For fresh sausages, the recommendation is a water activity below 0.92 [26]. In this sense, the values obtained here are propitious to bacterial growth, which inspires attention during production and storage.

Table 3. Water activity, pH, titratable acidity and shear force of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

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Determination	Formulation			
	F1	F2	F3	
Water activity	$0.983 \pm 0.001^a$	$0.971 \pm 0.002^b$	$0.965 \pm 0.001^{c}$	
pH	$5.09 \pm 0.07^{\mathrm{a}}$	$4.68\pm0.20^b$	$4.73\pm0.10^{b}$	
Titratable acidity (g 100 mL <sup>-1</sup> )	$0.25\pm0.02^{\mathrm{b}}$	$0.40 \pm 0.03^{a}$	$0.26 \pm 0.02^{b}$	
Shear force (N)	$64.33 \pm 2.40^{a}$	$56.68 \pm 1.51^{b}$	$61.04 \pm 2.52^a$	

Means with the same letter in the same line do not differ statistically at  $5\,\%$  (p > 0.05). p: probability.

The pH value for F1 was 5.09, which differed significantly (p<0.05) from F2 and F3. These two formulations did not differ from each other (p>0.05), with pH values of 4.68 and 4.73, respectively (Table 3). Normal pH values for meat products typically range from 5.4 to 6.2 [27] and the pH indicated for fresh sausages is 5.6 [26]. All samples (F1, F2, and F3) had pH levels below this range. de Carvalho et al. [28], analyzing Cuiabana sausage, reported pH values between 5.61 and 5.83. Unlike Maracaju sausage, Cuiabana sausage contains milk, which contributes to a slight pH decrease. In contrast, the presence of orange juice in Maracaju-type sausages more significantly reduces pH, explaining the difference between the control (F1) and the formulations with orange juices (F2 and F3).

For total titratable acidity, F2 differed significantly (p < 0.05) from F1 and F3, which did not differ from each other (p > 0.05) (Table 3). Although the samples exhibited low acidity levels  $(0.25-0.40 \text{ g } 100 \text{ mL}^{-1})$ , these values were within acceptable limits [2]. The formulation containing bitter orange (F2) showed the highest acidity  $(0.40 \text{ g } 100 \text{ mL}^{-1})$ , while F1 and F3 (with sweet orange) presented the lowest value  $(0.26 \text{ g } 100 \text{ mL}^{-1})$ . The higher acidity in

F2 aligns with findings that bitter orange can have acidity levels up to 45 times higher than sweet orange [29].

When comparing pH and total titratable acidity, a clear relationship is observed: higher acid concentrations in the juice increased H<sup>+</sup> ion availability, lowering the pH and making the sausages more acidic (Table 1). However, since pH measures the strength of acid dissociation rather than the total acid content, total titratable acidity provided a more accurate assessment of bitter orange's effect on enhancing food acidity [30].

The shear force results showed that F1 and F3 required the application of a greater force to be cut. There was a significant difference (p > 0.05) between samples F1 and F3 in relation to F2, probably due to the lower pH and higher acidity which may led to the denaturation of proteins. So, it can be assumed that the amount of bitter orange juice added produced a higher reduction in shear force and a reduction in cohesion due to the tenderization of the meat, which involves both uptake of liquid and solubilization of connective tissue collagen [31]. One of the main factors that affect the texture of the sausage is the protein structure [32]. In finely comminuted products, the salt-soluble meat proteins myosin and actin form a three-dimensional cross-linked matrix that can entrap water and fat. When sausages are made from minced meat rather than finely chopped meat, the shear forces are lower because the meat is mixed, not chopped. This mixing process results in a weaker, less cohesive three-dimensional, cross-linked matrix. Moreover, raw briskets present higher shear forces than the thick flank, which is directly related to the collagen content [33] and in some cases to the presence of actomyosin bridges [34] in the cuts. As example, some other authors reported a shear force of 12.3 N for beef sausages prepared with minced meat [35].

# Proximate composition

The results of the proximate composition analysis for the sausages are presented in Table 4.

Table 4. Proximate composition of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Determination (0/)	Formulation			
Determination (%)	F1	F2	F3	
Moisture	$60.32 \pm 0.06^{a}$	$57.37 \pm 0.19^{b}$	$55.96 \pm 0.16^{c}$	
Protein	$23.39 \pm 0.39^{a}$	$22.99 \pm 0.58^{a}$	$22.24 \pm 0.73^{a}$	
Lipids	$15.25 \pm 0.35^{b}$	$17.74 \pm 0.01^{a, b}$	$19.29\pm1.25^a$	
Ash	$\boldsymbol{0.78 \pm 0.24^b}$	$1.50 \pm 0.23^{a, b}$	$2.16 \pm 0.32^{a}$	
Carbohydrates*	0.26	0.40	0.35	

Means with the same letter in the same line do not differ statistically at 5 % (p > 0.05). p: probability. \*Calculated by difference.

Moisture

Samples F1, F2 and F3 differed (p < 0.05) from each other for moisture, as observed (Table 4). In F1, the added water (8.81%) directly increases the moisture content. In F2 and F3, water is replaced by orange juices, which contain soluble solids (e. g., sugars, organic acids, and minerals) that reduce the amount of free water in the final product, which explains the lower moisture content in F2 (57.37 %) and F3 (55.96%) compared to F1 (60.32%). It possibly occurred due to water-solute interactions, where the compounds in the juices, particularly acids and salts, interact with proteins and other matrix components, reducing water retention and increasing water release during processing. Thus, it is possible that F2 and F3 presented lower moisture values due to the pH values below the isoelectric point of the myofibrillar proteins, causing the protein to denature, and consequently reducing moisture.

The obtained values were in accordance with the limit of 70% established by the Brazilian legislation [2]. For comparison, a 59.30% moisture content for fresh beef sausage made at very similar conditions was reported in [36], coinciding with the result obtained for sample F1 (control) (Table 4). Other authors report an average value of 64.6% for the moisture content of a low-calorie beef sausage, which was attributed to the fat replacement and reduction and to the addition of ice [37].

Protein

The protein content of F1, F2 and F3 did not differ significantly (p > 0.05) from each other (Table 4), ranging between 22.24% and 23.39%. It occurred because all formulations contained the same amount of beef brisket (67.74%) as the primary source of protein and the juices contributed with negligible amounts of protein. Thus, the protein content remained stable across F1, F2, and F3. While the acids in the juices may cause slight protein denaturation, this did not significantly impact the total protein content measured.

The protein contents were well above the minimum limit established for sausages, which is 12 % [2]. The brisket beef has 17.6 to 22.20 % protein [38], which agrees with the protein content of the sausage (Table 4). Literature reports 20.65 % of protein for fresh beef sausage prepared with 65 % beef [36]. For Cuiabana sausages prepared with the rear cut of cattle, the average value was 16.53 % of protein content [28].

Lipids

The lipid content of F1, F2 and F3 differed significantly (p<0.05) from each other (Table 4). Despite the identical fat proportion (20.32%) in all formulations, the presence of juices, especially pear orange juice in F3, may affect lipid extraction, distribution, and retention during emulsification and/or cooking. Acidic compounds and their contents in the juices could have altered the protein structure, decreasing the binding of lipids to the matrix and resulting in higher apparent lipid content in F3 (19.29%) compared

to F2 (17.74%) and F1 (15.25%). Moreover, the lower pH in the formulations with juices influences emulsion stability, potentially impacting lipid distribution in the final product.

All formulations were within the limit of 30 % lipids established by the Brazilian legislation [2]. For comparisons, an average of 15.5 % of lipids was reported for fresh beef sausage with 15.0 % sheep fat tail [36]. Only 5.75 % of lipids was recorded in Cuiabana sausage because milk and mozzarella cheese were the main sources of fat [28].

Ash and carbohydrates

The ash content differed significantly from each other (p < 0.05), as can be seen in Table 4. The significant increase in ash content in F2 (1.50%) and F3 (2.16%) can be attributed to the mineral contribution of citrus juices, which have a higher concentration of salts compared to water. The carbohydrate content obtained by difference was 0.26, 0.40 and 0.35% for F1, F2 and F3, respectively (Table 4).

The legislation does not establish limits for the analysis of mineral matter and the carbohydrates content cannot surpass 7%, including 2% starch [2]. Shahin [36] reported ash content value of 1.92% and carbohydrates content of 2.63% in fresh beef sausages. These values are higher than those found here (except for ash in F3) and are due to the differences in the added ingredients, which have mineral content, including sodium nitrite and nitrate, sodium polyphosphate and various spices, and the addition of 5% starch as reported by these authors. Another example is the carbohydrate content of 3.70% found for Cuiabana beef sausages formulations made with rump and 2.28% for formulations made from the fillet cover. These higher values were explained due to the addition of milk and mozzarella cheese, which are rich in the carbohydrate lactose [28].

Instrumental color

The colorimetric characterization of beef sausages (Table 5) revealed significant differences between formulations containing water (F1), bitter orange juice (F2), and pear orange juice (F3), particularly in the meat and fat portions.

In the meat portion, lightness  $(L^*)$  values were significantly higher for F2 (52.49), indicating that bitter orange juice enhanced the lightness of the meat compared to F1 (43.21) and F3 (45.64). The redness  $(a^*)$  values were highest for F1 (8.50), with F2 (5.20) and F3 (4.48) showing significantly lower redness, suggesting that the inclusion of orange juices reduced the red tones in the meat. Yellowness  $(b^*)$  was greater in F2 (7.26) compared to F3 (4.41), with F1 (5.18) showing an intermediate value. These differences indicate that bitter orange juice contributed to a more yellowish hue. Chroma  $(C^*)$  values followed a similar trend, with F3 (6.38) showing the lowest saturation, significantly differing from F1 (10.04). Hue angle (h°) was highest in F2 (53.79), indicating a shift towards a more yellowish tone. The color difference ( $\Delta E^*$ ) for F2 (10.06) and F3 (10.69) was above 5, indicating noticeable differences from the control (F1) (Table 5).

Table 5. Instrumental color analysis of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Parameter	Formulation (portion)					
	F1 (meat)	F2 (meat)	F3 (meat)	F1 (fat)	F2 (fat)	F3 (fat)
$L^*$	$43.21 \pm 3.52^{b}$	$52.49 \pm 2.89^{a}$	$45.64 \pm 2.72^{b}$	$65.49 \pm 3.3^{a}$	$68.26 \pm 3.63^{a}$	$66.65 \pm 3.72^{a}$
a*	$8.50^{a} \pm 1.62^{a}$	$5.20 \pm 1.94^{b}$	$\textbf{4.48} \pm \textbf{0.50}^{\text{b}}$	$7.15 \pm 1.07^{a}$	$4.78 \pm 1.18^{b}$	$2.89 \pm 0.98^{\mathrm{b}}$
$b^*$	$5.18 \pm 1.47^{a, b}$	$7.26 \pm 3.33^{a}$	$4.41 \pm 2.03^{b}$	$20.29 \pm 1.07_{a}$	$18.56 \pm 2.36^{a}$	$18.98 \pm 3.36^{a}$
<b>C</b> *	$10.04 \pm 1.38^a$	$8.98 \pm 3.69^{a}$	$6.38 \pm 1.75^{b}$	$21.70 \pm 0.96^{a}$	$19.21 \pm 2.18^{a}$	$19.21 \pm 3.40^{a}$
<b>h</b> °	$31.04 \pm 9.68^{c}$	$53.79 \pm 7.66^{a}$	$42.45 \pm 9.55^{b}$	$70.72 \pm 7.78^{b}$	$75.26 \pm 4.39^{b}$	$81.50 \pm 2.60^{a}$
$\Delta E^*$	_	10.06	10.69	_	4.03	39.52

Means with the same letter in the same line do not differ statistically at 5 % (p > 0.05). p: probability;  $L^*$ : lightness;  $a^*$ : chroma  $a^*$  (intensity of red);  $b^*$ : chroma  $b^*$  (intensity of yellow); chroma  $C^*$ : hue saturation index;  $h^\circ$ : hue angle;  $\Delta E^*$ : color variation.

For the fat portion, lightness  $(L^*)$  values were similar across formulations, ranging from 65.49 (F1) to 68.26 (F2), indicating that all formulations were bright. Redness  $(a^*)$ was highest in F1 (7.15), with F2 (4.78) and F3 (2.89) showing significantly lower values, particularly F3, which exhibited the least redness. Yellowness  $(b^*)$  values were consistent across formulations, with F1 (20.29) and F2 (18.56) showing no significant differences, while the values were lower in F3 (18.98), indicating a similar yellow hue across all formulations. Chroma  $(C^*)$  values were also similar between formulations, ranging from 19.21 (F2 and F3) to 21.70 (F1). The hue angle (h°) was significantly higher in F3 (81.50), indicating a greater shift towards yellow. The color difference ( $\Delta E^*$ ) was notably higher in F3 (39.52), indicating a significant color variation compared to the other formulations (Table 5).

The color of the meat portion showed a shift from red to yellow due to the orange juices, with a slight grayish tone due to the salt. In contrast, the fat portion exhibited greater differences, especially between F3 and the other formulations, with a shift from red to yellow. The inclusion of orange juices, particularly pear orange juice, caused notable changes in both the meat and fat portions, with pear orange juice leading to the most significant color alterations. The color differences may also be attributed to a limited degree of fat dispersion, commonly observed in other sausages. This is particularly due to the spread of oily lipids, which contributes to an increase in lightness [25]. These

changes may influence consumer perception, as color plays a key role in sensory evaluation [39].

#### Conclusion

The replacement of water with orange juices in beef sausage formulations significantly influenced the product's physicochemical properties. All formulations complied with Brazilian legislation regarding moisture, protein, and lipid content. The addition of orange juices resulted in lower water activity and reduced moisture content due to the presence of soluble solids. This effect, combined with lower pH values caused by the juices, contributed to protein denaturation and altered matrix interactions. While protein content remained consistent across all formulations due to the fixed meat content, lipid content showed significant variation, with higher lipid retention observed in formulations containing pear orange juice. These differences were attributed to acid-matrix interactions during emulsification and cooking. Bitter orange juice, with its higher acidity, resulted in the lowest shear force, which is likely due to enhanced protein denaturation, leading to softer product consistency. In terms of color, orange juices caused noticeable changes, with lighter tones and shifts from red to yellow observed in both the meat and fat portions. Bitter orange juice led to a more pronounced yellowish hue in the meat, while pear orange juice caused the most significant color differences overall. These color alterations, alongside the structural modifications, could influence consumer acceptance and sensory perception.

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