

# Production and Microbiological and Sensory Quality of Food Based on Sheep (*Ovis aries*) Meat in an Agro-Ecological Family Farm

António Elísio José<sup>1,\*</sup>, Heloisa Helena Chaves Carvalho<sup>2</sup>,  
Rafael Francisco Nanelo<sup>3</sup>, Philippa Nomagugu Ncube<sup>4</sup>, José Maria Wiest<sup>5</sup>

<sup>1</sup>Higher Polytechnic Institute of Gaza, College of Agriculture, Mozambique

<sup>2</sup>Institute of Food Science and Technology, Universidade Federal do Rio Grande do Sul

<sup>3</sup>Center for Dryland Agriculture, Bayero University Kano, Nigeria.

<sup>4</sup>Higher Polytechnic Institute of Gaza, College of Agriculture, Mozambique

<sup>5</sup>Institute of Food Science and Technology, Universidade Federal do Rio Grande do Sul

\*Corresponding author: [aelisiojose@gmail.com](mailto:aelisiojose@gmail.com)

Received July 16, 2023; Revised August 17, 2023; Accepted August 24, 2023

**Abstract** A study was conducted to assess the hygienic and sanitary practices as well as the microbiological and sensory quality of sausages produced on an agroecological family farm located in the Quilombola Remnant Community of Limoeiro in the district of Bacopari, in the municipality of Palmares do Sul, on the Northern coast of Rio Grande do Sul in Brazil. Six different formulations of produced from fresh and smoked sheep meat using different spices from locally available resources were evaluated. Physicochemical and nutritional analyses, and microbiological tests and sensory analysis of the Hedonic Scale type were carried out to determine the nutritional value, the hygienic-sanitary quality and the level of acceptance of the final products. The production process was assessed using a list of 30 elements, subdivided into 3 groups based on the RDC 275/2002 – ANVISA for good manufacturing practices. About the physicochemical and nutritional characteristics, it was observed that the smoked products had higher levels of protein and lipids compared to the fresh ones, and the linguça had greater acceptance than the salsichões. All the final products met the hygienic and sanitary standards required by law and the percentage of non-compliance was 10.1% for handling of the products. The property was classified under the regular group with a compliance of 54.3%. The handlers showed to have basic knowledge about good manufacturing practices for meat products.

**Keywords:** Good manufacturing practices, hygienic-sanitary quality, nutritional quality, sensory analysis, smoked products, sheep meat

**Cite This Article:** António Elísio José, Heloisa Helena Chaves Carvalho, Rafael Francisco Nanelo, Philippa Nomagugu Ncube, Philippa Nomagugu Ncube and José Maria Wiest, “Production and Microbiological and Sensory Quality of Food Based on Sheep (*Ovis aries*) Meat in an Agro-Ecological Family Farm.” American Journal of Food Science and Technology, vol. 11, no. 3 (2023): 96-103. doi: 10.12691/ajfst-11-3-3.

## 1. Introduction

Food production based on sustainable technologies and the need to consume agroecologically-based foods has become an important alternative for populations with low income, thereby counteracting the need for expensive industrially processed foods. The promotion of small-scale food producers [1] leads to equity and social inclusion and simultaneously to a greater and more diversified supply of sustainably produced food. There is a virtually unexplored market opportunity in sheep farming. This is highlighted by [2], who highlight the tradition among the *gauchos* who developed this vocation and appropriate production technologies; the presence of available natural resources and a favorable environment for breeding and, above all,

the growing demand for quality sheep meat. Currently, the return of sheep production to the meat sector is because of factors such as the increased purchasing power of the population and the adaptation of new consumption habits that lead to an appreciation for sheep meat. The trends for this market are promising. However, there are some obstacles to its growth, which include the lack of product standardization and the sector disorganization [3].

After production, it is important to analyze the properties of food products to ensure that they meet the legal requirements for safety and nutritional quality. It is also important to ensure that food products retain their desirable properties until the time they are consumed.

The good condition of the raw material, the handling hygiene, the manufacturing and conservation conditions and the cleaning of equipment are important factors that are directly linked to the quality of fresh sausages [4]. For

microbiological assessment, fecal coliforms have been used to determine unsatisfactory food sanitary conditions and the potential presence of pathogens [5]. High counts of thermotolerant coliforms indicate unsatisfactory hygienic conditions during processing and the possibility of the presence of pathogenic microorganisms.

The present study was carried out on a family agroecological farm in the Limoeiro, Bacupari region, Municipality of Palmares do Sul, RS, in Brazil. In order to valorize the knowledge and skills related to the characteristic food and alimentation habits of rural *quilombola* families, the study aimed to evaluate the hygienic-sanitary, physicochemical and sensory quality of sausage products, *linguiças* and *salsichões*, made from sheep meat processed and seasoned with locally available resources. Furthermore, it classified the production operations according to the level of compliance conforming to the current legislation.

## 2. Materials and Methods

### 2.1. Production Process

For the processed products, free range castrated male sheep, with an average age of 30 months, were skinned, eviscerated and the external fat cleaned. The locally bred sheep are a cross with an Australian merino. The processed products were as follows: (A) fresh *salsichão*, (B) *linguiça* without fresh saffron, (C) *linguiça* with fresh saffron, (D) smoked *salsichão*, (E) smoked *linguiça* without saffron, (F) smoked *linguiça* with saffron, (G) frozen *salsichão*, (H) frozen *linguiça* without saffron and (I) frozen *linguiça* with saffron.

To prepare fresh and smoked *salsichões* types, the meat was ground using an “8 mm” disc. For each kg of ground meat, 10% skinned pork bacon (*Sus scrofa*), 2,5% cooking salt, 2% crystal sugar, 0,25% pounded *Piper nigrum* (white pepper), 15% flaked ice from pasteurized water, 5% *Allium cepa* (onion), 0,2% *Myristica fragrans* (ground nutmeg), 2% red wine vinegar, 0,2% *Allium sativum* cloves (garlic). A good quantity of chopped green herb spices such as *Apium graveolens* (celery), *Petroselinum sativum* (parsley), *Allium schoenoprasum* and *Allium fistulosum* (all-year green onions or spring onions), *Origanum vulgare* (oregano or black marjoram), *Origanum x applii* (white marjoram) and *Allium tuberosum* (garlic chives) were also used. All ingredients were manually homogenized for 20 minutes. They were then left to marinate in a refrigerator at a temperature of 4°C for 3 hours, after which they were homogenized again for 20 minutes. This was followed by stuffing into casings and tying up the sausages to a maximum length of 10 cm. The stuffing was done using a sausage stuffer and artificial casings purchased from a local supplier. The casings were prepared in advance by rehydrating them in pasteurized water, vinegar and lemon. With the aid of a thorn of the *Citrus sinensis* (orange tree), micro holes were made in the casing. This is a traditional method used to remove small air pockets formed during filling.

Two types of *linguiças* were manufactured, one with saffron and the other without saffron. Boneless meat, with excess connective tissue, clots and surface fat removed,

was ground on disk n° 5 together with 3% of cooking salt, 0,3% of pounded black and white pepper, 1% chopped garlic clove, 2% *Cominum cyminum* (cumin) powder and 10% previously pasteurized ice water. In addition to the spices already mentioned, 3% *Curcuma longa* (turmeric) powder was added to the fresh and smoked sausages with saffron. This mixture was homogenized for 20 minutes, left to marinate in a refrigerator for 3 hours and then homogenized again. This was followed by stuffing into artificial casings and the sausages were tied at a minimum length of 30 cm.

Part of the fresh *salsichões* and *linguiças* were frozen in a 200-liter domestic horizontal freezer (Consul®) at -18°C for a period of 3 months. The rest of the fresh sausages were placed in smokers immediately after being stuffed. The smokers were handcrafted with a fiber-asbestos coating. In these smokers, intense natural smoke was generated at a temperature of 50 ± 2°C through carefully controlled burning of orange tree wood added with dry leaves of *Bambusa taquara* (taquara plant). The products were placed at an initial height of 1.0 m for two hours; to 1.50 m for three hours and 2.0 m for the remaining time until the fire went out, in alternating daily sessions for an average period of 8 hours. The sausages were kept in the smoker for 3 days of treatment. The water used in all stages of the process was obtained from a well available on site and was pasteurized (75 to 80°C) for a period of 45 minutes. Sanitation operations consisted of manual cleaning of all equipment, utensils and work surfaces with pasteurized water and of disinfection carried out using a product locally called “arnique” placed in plastic spray bottles. Also called tincture, the “arnique” was prepared by mixing 750 ml of pasteurized water, 250 ml of 96° GL ethyl alcohol and medicinal plants: *Allium tuberosum* (garlic chives), *Apium graveolens* (celery), *Casearia sylvestris* tea (*bugre*), *Allium porrum* (leek) and *Citrus bergamia* bark (bergamot orange) coarsely chopped and left to macerate for ten days in bottles. All handlers wore personal protective equipment and frequently sanitized their hands throughout processing.

### 2.2. Microbiological Analysis

The samples, in triplicate, were taken from the formulations fresh from the grinder and after the stuffing process (fresh) and from the frozen and smoked sausages. These were examined for fecal coliforms, *Salmonella sp*, *Staphylococcus aureus* and *Clostridium* sulfite reducer. In addition to these microorganisms, deteriorating total aerobic mesophiles from the well water were counted, before and after pasteurization. All analyses were carried out at the food physicochemical and microbiological laboratory at the Food Science and Technology Institute of the Federal University of Rio Grande do Sul, RS, Brazil. The procedures followed are detailed in the Manual of methods for the microbiological examination of foods [6] and based on the Normative Instruction N°. 62 of August 26, 2003 of the Ministry of Agriculture, Livestock and Water Supply (*Ministério da Agricultura, Pecuária e Abastecimento* – MAPA), which approves the analytical methods for microbiological analysis for the control of animal products and water [7]. The analyses were also based on meeting the required standards established by

RDC 12 of January 2, 2001, since they are variable for each commercialized food [8].

## Most Probable Number (MPN) of Fecal Coliforms

The multiple tube fermentation technique was used to determine the MPN of fecal coliforms. Sodium lauryl sulfate broth (MERCK, Germany), in simple concentration, was used for the presumptive examination with dilutions of 1, 0.1 and 0.01 ml. The tubes were incubated at  $36 \pm 1^\circ\text{C}$  for 24 and 48 hours. In the tubes with a positive result (lactose fermentation and Durhan gas production), an aliquot of sodium lauryl sulfate broth was added into tubes containing EC broth (MERCK, Germany) and incubated at  $45 \pm 2^\circ\text{C}$  for 24 - 48 hours. The Most Probable Number by sample weight (MPN/g, [9]) was determined from the combination of tubes that showed positive results in each of the exams (the presumptive and the confirmatory for fecal coliforms).

## *Salmonella* sp

For the analysis of *Salmonella* sp.,  $25 \pm 0.2$  g of the sample was weighed and 225 ml of 1% buffered saline peptone solution (DIFCO, France) was added. This was homogenized for 60 seconds in a stomacher and incubated at  $36 \pm 1^\circ\text{C}$  for 20 hours. After this, 1 ml of the sample was simultaneously inoculated into a tube containing tetrathionate broth (MERCK, Germany) and selenite cystine broth (OXOID, England), which were incubated at  $41 \pm 0.5^\circ\text{C}$  for 30 hours. These samples were then separately strained in Brilliant Green Phenol Red Lactose Sucrose Agar (OXOID, England) and Xylose Lysine Deoxycholate Agar (MERCK, Germany) and incubated at  $36 \pm 1^\circ\text{C}$  for 18-24 hours. Characteristic colonies were confirmed by biochemical and serological tests [7] and the results expressed as the presence or absence of *Salmonella* sp. in 25 g of the sample.

## *Staphylococcus Aureus*

$25 \pm 0.2$  g of the sample were weighed and 225 ml of 0.1% peptone saline solution was added and homogenized for 60 seconds in a stomacher. From this solution, the other dilutions were made for the analyses. The coagulase positive *Staphylococcus* count was triplicated, where 100  $\mu\text{L}$  of the sample was inoculated in Baird-Parker Agar (DIFCO, France) and the plates were incubated at  $36 \pm 1^\circ\text{C}$  for 48 hours. Plates containing between 15 and 150 colonies were selected for the count of typical (shiny black surrounded by a light halo/ black and shiny with a thin white border, surrounded by a light area) and atypical (grayish or black without a halo) colonies. From each plate, 3 to 5 colonies were subcultured onto Brain Heart Infusion Broth (OXOID, England) and Brain Heart Infusion Agar (OXOID, England) and incubated at  $36 \pm 1^\circ\text{C}$  for 48 hours. The presence of *Staphylococcus aureus* was determined through DNase and catalase tests [10].

## Sulfite Reducing *Clostridium* Count

The analysis of sulfite reducing *Clostridium* followed the same procedure used with *Staphylococcus aureus*. The count was performed in Sulfite Polymyxin Sulfadiazine Agar (DIFCO, France), using the overlay pour-plate technique. The plates were incubated in anaerobic conditions at  $36 \pm 1^\circ\text{C}$  for 24 hours, followed by counting of characteristic colonies (black colonies) which were confirmed by Gram stain and specific biochemical tests.

## Aerobic Mesophilic Count

The deep plating technique (Pour Plate) in Plate Count Agar® (PCA) medium was used. The plates were incubated at  $35^\circ\text{C}$  for 48 hours and the counts were carried out in plates containing from 30 to 300 colony-forming units using a colony counter. The number of aerobic mesophilic microorganisms per gram of the sample was obtained by the average of the number of colonies counted, multiplied by the dilution factor of the corresponding samples, in accordance with the American Public Health Association [11].

## 2.3. Assessment of Good Manufacturing Practices

The analysis of the operations was based on an evaluation list, conforming to the Resolution RDC n° 275, of October 21, 2002/ANVISA [12]. The list was composed of elements related to the physical structure, the working environment, the handlers, the food preparation, the sinks in the production area, the cleanliness and hygiene of the work areas and quality control. These were categorized into three main areas: (i) equipment, furnishings and fixtures; (ii) handlers and (iii) water supply totaling 30 items analyzed. A quantitative and descriptive analysis of the data was done to verify the percentage of adequacy of the elements that made up the evaluation list, according to their lesser or greater conformity in compliance with the Brazilian legislation. The classification was performed through a score [13], with group 1 equivalent to 76 to 100%; group 2 from 51 to 75% and group 3 from 0 to 50%. For each item, only one answer from the three possible answers was marked: adequate, inadequate and not applicable. According to [14], group 1 is considered good; group 2 is regular and group 3 is bad.

## 2.4. Physicochemical Analysis

Samples of the finished products were collected, in triplicate, for the analysis of physicochemical properties according to the standards described by Horwitz and Latimer [15]. The proteins were obtained by using the Kjeldahl method, which determines the total nitrogen. A conversion factor of 6.25 was used to convert the result into proteins. The percentage of moisture was obtained by the weight loss of the sample subjected to heating in an oven at  $105^\circ\text{C}$ . The lipids were obtained by direct extraction with ethyl ether in a Soxhlet extractor. For

mineral salts, the gravimetric method was used where the samples were subjected to incineration in a muffle furnace at 550°C until a constant weight was obtained. The total energy value was calculated based on the calorific coefficients (kcal/g) for proteins (4) and for lipids (9).

## 2.5. Sensory Analysis

The sensory analysis was carried out using the level of consumer acceptance test. The test was carried out by 13 volunteer tasters, of both sexes, untrained, randomly selected from habitual consumers of sheep meat and its products, and aged between 35 and 80 years. This was done in compliance with the Resolution n° 466 [16] and the recommendations of the Ethics Committee for Research with Human Beings. The meat formulations mentioned in 2.1 were divided into small cubes of 2 cm in length and served to each taster to assess the sensory characteristics (color, flavor, appearance, texture, aroma, and general evaluation). Considering the methodology of [17], a 5-point hybrid hedonic scale was used, with indications 1 (disliked very much), 2 (disliked), 3 (neither liked nor disliked), 4 (liked) and 5 (liked very much). Between tastings, water and cookies were served to the tasters so that the taste of the previous sample would not interfere with the evaluation of the next sample.

## 2.6. Statistical Analysis

The sensory evaluation was carried out using a completely randomized design, with 6 treatments and 13 repetitions, and the acceptability of the products was evaluated by the frequency test. The analysis of variance was done according to the procedures of the statistical program Statistical Analysis System (SAS), version 9.0 [18] through the general linear model (GLM), considering the significance level of 5%, and the averages of the results were compared by Tukey's test.

## 3. Results and Discussion

### 3.1. Microbiological Analysis

In the assessment of the hygienic and sanitary conditions, the microbiological analyzes showed, for the water used in the well, a very high count of total aerobic deterioration for the  $10^{-3}$  dilution before the thermal treatment was carried out. After pasteurization, its load decreased considerably, at the same dilution. The bacteria of interest to health showed satisfactory results both before and after the treatment (Table 1). [19] also observed that the fecal coliforms were within the acceptable levels by law in their research on the quality of water used, before its treatment, in family agro-industries.

Undesirable and potentially pathogenic microorganisms (*Salmonella sp.*, *Staphylococcus aureus* and sulfite reducing *Clostridium*) were absent in the different samples collected, characterizing good practices in all stages of the process. It is interesting to note that RDC legislation n°. 12, of January 2, 2001 [8], stipulates that for these types of products, the maximum microbial load limits are  $3 \times 10^3$

CFU/g for *Staphylococcus aureus*, of  $3 \times 10^2$  CFU/g for sulfite reducing *Clostridium* and  $10^3$  NMP/g CFU/g for fecal coliforms, and absent in 25g of the collected sample for *Salmonella sp.* Although authors such as [20], state that in ground meat, grinding is an additional factor that can favor the contamination and multiplication of pathogenic microorganisms. This fact was not observed in this study.

In relation to fecal coliforms (MPN/g), it was observed that the samples of the formulations that had just left the stuffing and freezing processes had concentrations slightly higher than those that had just left the grinder (M1 and M2). After the smoking process, these microorganisms were below 0.3 NMP/g, suggesting that their presence in non-smoked products was probably related to the contamination of the artificial casing used for stuffing. The innocuousness of the smoked products can be explained by the good operating practices on the one hand, and on the other hand the use of natural additives or spices with antimicrobial properties (parsley and/or celery, year-round green onions, oregano, white marjoram, garlic chives) as well the smoking process itself. This means that the microbial control in the formulations was guaranteed by the applied technological processes, therefore highlighting the importance of hygiene during processing. In their studies on the antibacterial activity of plants with ethnographic indications for medicinal and flavoring, [21], [22] found an intense activity of these plants against *Salmonella sp.*, *Escherichia coli* and *Staphylococcus aureus*.

As with the results of this study, authors such as [23], on evaluating the proximate, microbiological and sensory composition of silver catfish (*Rhamdia quelen*) subjected to the smoking process, observed that their smoked products had no presence of *Salmonella sp.* in 25g and sulfite reducing *Clostridium*. They found the positive *Staphylococcus* coagulase count lower than 10 CFU/g, Total coliforms  $1.1 \times 10^3$  MPN/g, and Coliforms at 45°C less than 0.3 MPN/g. [24], verified the absence of *Salmonella sp.* and *Escherichia coli*, and values below  $10^2$  CFU/g for *Staphylococcus sp.*, Total coliforms and Enterobacteria in smoked fillets. In their researches, [25], [26], did not consider the compromising loads of harmful microorganisms such as *Salmonella sp.*, *Staphylococcus aureus* and Coliforms in smoked meat products. In this regard, [27], report that many of the microorganisms that occur naturally in environments where food is produced can be controlled by proper handling and storage practices, good hygiene practices, the manufacturing process and the control of time and temperature of the processes. The same authors also found that there are food processing procedures that result in the formation of substances with antimicrobial properties in food and these include smoking of meat products. This is one of the stages of the production process from this study. [28] cite numerous smoking compounds (phenols, carboxylic acids, and formaldehyde) at concentrations similar to those in heavily smoked products as being effective antimicrobial agents and also as producing a desiccation that contributes to inhibiting bacterial growth.

**Table 1. Sanitary-hygienic quality samples of the well water available on the property, the masses of the formulations fresh from the grinder and mixed with other ingredients and the sheep meat formulations after the freezing and smoking processes**

| Sample | Undesirable Microorganisms |                          |   |                                      |  |
|--------|----------------------------|--------------------------|---|--------------------------------------|--|
|        | Salmonella (in 25g)        | Faecal Coliforms (MPN/g) | Positive Staphylococcus coagulase (CFU/g) | Sulfite reducing Clostridium (CFU/g) | Total aerobics deterioration (CFU/g or mL) |
| WATER  | Absent                     | < 0.3                    | Absent                                    | Absent                               | Countless                                  |
| WATER* | Absent                     | < 0.3                    | Absent                                    | Absent                               | 103  |
| (A)    | Absent                     | 24.0                     | Absent                                    | Absent                               | NCO  |
| (B)    | Absent                     | 4.3                      | Absent                                    | Absent                               | NCO  |
| (C)    | Absent                     | 24.0                     | Absent                                    | Absent                               | NCO  |
| (D)    | Absent                     | < 0,3                    | Absent                                    | Absent                               | NCO  |
| (E)    | Absent                     | <0.3                     | Absent                                    | Absent                               | NCO  |
| (F)    | Absent                     | <0.3                     | Absent                                    | Absent                               | NCO  |
| (G)    | Absent                     | 24.0                     | Absent                                    | Absent                               | NCO  |
| (H)    | Absent                     | 4.3                      | Absent                                    | Absent                               | NCO  |
| (I)    | Absent                     | 24.0                     | Absent                                    | Absent                               | NCO  |
| (M1)   | Absent                     | <0.3                     | Absent                                    | Absent                               | NCO  |
| (M2)   | Absent                     | <0.3                     | Absent                                    | Absent                               | NCO  |

(WATER) locally available well water, \* after pasteurization, (A) fresh *salsichão*, (B) *linguiça* without fresh saffron, (C) *linguiça* with fresh saffron, (D) smoked *salsichão*, (E) smoked *linguiça* without saffron, (F) smoked *linguiça* with saffron, (G) frozen *salsichão*, (H) frozen *linguiça* without saffron and (I) frozen *linguiça* with saffron, (M1) *salsichão* paste fresh from grinder, (M2) *linguiça* paste fresh from grinder, (MPN) most probable number, (CFU) colony formation units and (NCO) not carried out because it is not in the scope of the RDC 12/2001/ANVISA.

### 3.2. Physicochemical analysis

Table 2 indicates the chemical-nutritional composition of the studied formulations. It can be observed that the smoked products had lower moisture content and higher content levels of protein, lipids, carbohydrates, mineral salts and energy in relation to the corresponding fresh products. No significant differences ( $p < 0.05$ ) were observed in the moisture content of the smoked formulations, and their energetic value showed significant differences. Similar values of moisture (62.5%), protein (18.1%) and lipids (19.4%) were found by [29] in fresh sheep meat. [30] found, in their study on the chemical composition and yield of fresh sheep meat, 74.05% of moisture, 18.85% of protein, 1.15% of ash, results almost similar to the ones obtained in this study. The mean values found in this study also tally with those of [31], who recorded in their research that the values of the physicochemical characteristics of fresh and processed meat ranged from 45.8 to 68.5% for moisture, 12.10 to 16.40% for protein, 11.10 to 38.10% for fat and 0.3 to 3.7% for salts in *linguiças*. In their study on the effect of smoking on the proximate composition, [32] also found that the percentage of moisture decreased in smoked meat products, while the content of nutritional components such as protein, lipids and fixed mineral residue increased. In this regard, other studies such as the one by [23], refer to the increase in the nutritional value of smoked products compared to the corresponding fresh products. This is similar to [33], [34], who, in their studies, observed that

the average content levels of protein, lipids, ash and salts increased in smoked products. [35] verified the loss of 37% of moisture content in their smoked formulations and values that increased from 17 to 44%, from 0.3 to 1.25% and from 0.89 to 3.1% of crude protein, lipids and ash from the fresh to the smoked product, respectively.

**Table 2. Evaluation of the physicochemical and nutritional qualities of the different formulations based on sheep meat studied**

| FM  | Moisture (%)             | Protein (%)               | Lipids (%)               | Carbohydrates (%)       | Mineral salts (%)       | Energy (Kcal)             |
|-----|--------------------------|---------------------------|--------------------------|-------------------------|-------------------------|---------------------------|
| (A) | 66.7 ± 0.67 <sup>a</sup> | 10.5 ± 0.37 <sup>d</sup>  | 18.6 ± 0.43 <sup>c</sup> | 2.1 ± 0.12 <sup>e</sup> | 2.2 ± 0.01 <sup>e</sup> | 217.1 ± 0.92 <sup>f</sup> |
| (B) | 58.0 ± 2.37 <sup>b</sup> | 14.2 ± 0.68 <sup>c</sup>  | 19.5 ± 0.42 <sup>c</sup> | 4.8 ± 0.19 <sup>c</sup> | 3.4 ± 0.03 <sup>d</sup> | 251.9 ± 0.70 <sup>d</sup> |
| (C) | 59.1 ± 0.65 <sup>b</sup> | 13.0 ± 0.88 <sup>c</sup>  | 19.5 ± 0.47 <sup>c</sup> | 4.7 ± 0.17 <sup>c</sup> | 3.8 ± 0.06 <sup>c</sup> | 246.3 ± 0.06 <sup>e</sup> |
| (D) | 35.5 ± 0.52 <sup>e</sup> | 24.0 ± 0.12 <sup>a</sup>  | 31.5 ± 0.45 <sup>b</sup> | 4.0 ± 0.02 <sup>d</sup> | 5.1 ± 0.10 <sup>b</sup> | 394.9 ± 0.69 <sup>c</sup> |
| (E) | 32.2 ± 1.11 <sup>c</sup> | 21.4 ± 1.20 <sup>b</sup>  | 33.0 ± 0.12 <sup>a</sup> | 7.7 ± 0.06 <sup>a</sup> | 5.6 ± 0.03 <sup>a</sup> | 413.7 ± 0.16 <sup>a</sup> |
| (F) | 33.8 ± 1.05 <sup>c</sup> | 22.4 ± 1.19 <sup>ab</sup> | 31.8 ± 0.17 <sup>b</sup> | 6.6 ± 0.04 <sup>b</sup> | 5.5 ± 0.02 <sup>a</sup> | 401.8 ± 0.51 <sup>b</sup> |

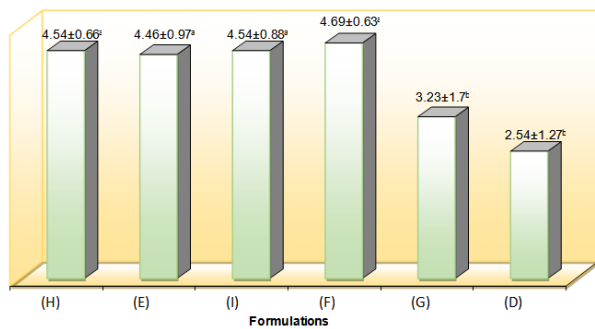
Same letter in column indicates non-significant differences at 95% probability. (A) fresh *salsichão*, (B) *linguiça* without fresh saffron, (C) *linguiça* with fresh saffron, (D) smoked *salsichão*, (E) smoked *linguiça* without saffron, (F) smoked *linguiça* with saffron, FM formulation

### 3.3. Sensory analysis

The percentage values of acceptance of the formulations according to the chosen categories (liked very much, liked, neither liked nor disliked, disliked and disliked very much) are described in Figure 1, where the *linguiças* with or without saffron, smoked or not, had higher percentages in relation to the *salsichões*. Among the *linguiças*, the highest acceptance was attributed to the smoked ones, a fact that would probably be related to the specific aroma and flavor that these products acquired during smoking. The low score presented by the *salsichões* can be attributed to the fact that these products had an abundant mass of spice plants that, in turn, was not contained in the *linguiças* formulations. Confirming these results, [33], [36], [37] also found in their studies on

sensory evaluation between smoked and non-smoked meat products, higher average values of general acceptance to the smoked ones. [38], state that smoking improves the flavor, color and commercial value of products. This is in addition to the fact that smoking compounds induce distinct color and flavor by themselves and by interaction with meat-based components, which results in creation of other active sensory substances. [28] associate the desirable taste of smoked products with the presence of a mixture of syringol (1.3-dimethoxy-2-hydroxybenzene) and 4-methylsyringol (2.6-dimethoxy-4-methylphenol), although 4-allylsyringol (1.3-dimethoxy-2-prop-2-enoxybenzene), guaiacol (2-methoxyphenol), 4-methylguaiacol (2-Methoxy-4-methylphenol), and trans-isoeugenol (2-Methoxy-4-[(1E)-1-propen-1-yl]phenol) also contribute to the typical sensory sensation.

There were no statistical differences between the tasters, demonstrating the homogeneity of their perception in relation to the products presented. The *salsichões* and the *linguiças* differed significantly in terms of acceptance, and the addition or not of saffron to *linguiças* did not significantly change ( $p < 0.05$ ) the means.

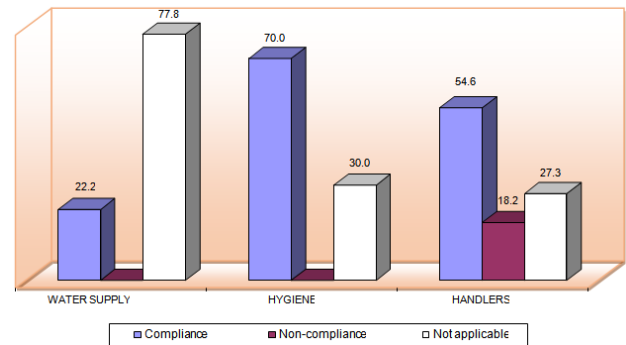


**Figure 1.** Evaluation of the acceptance of the formulations according to the tasters' perception on a scale from 1 (disliked very much) to 5 (liked very much). (D) smoked *salsichão*, (E) smoked *linguiça* without saffron, (F) smoked *linguiça* with saffron, (G) frozen *salsichão*, (H) frozen *linguiça* without saffron and (I) frozen *linguiça* with saffron. Equal lowercase letters indicate non-significant statistical differences at 5% significance.

### 3.4. Good handling practices

The use of the checklist of good practices produced the results shown in Figure 2, where the percentages of compliance are higher than those of non-compliance in all items evaluated. In the water supply aspect, many of the items on the list could not be evaluated, since the production unit did not have industrial dimensions but rather characteristics of a family agro-industry. The rest of the aspects were checked accordingly. Taking the results of the three blocks (water supply, handlers and hygiene) and considering that the items that were not applied did not interfere in the production stages and in the preparation of the products, the unit was classified in group 2 (regular). Similar results were found by [39], using the same method in their study to assess good manufacturing practices in a food production unit. In line with the results obtained in this study, [40], evaluating hygiene conditions and adequacy to good practices in food production units, found that the units studied had at least 50% of compliance with the entire checklist. Specifically, for hygiene and food preparation, these authors reported

higher percentages of non-compliance, differing considerably from this study especially with the hygiene block where there was no non-compliance.



**Figure 2.** Level of compliance in the execution of operations in the production chain of sheep meat products

## 4. Conclusion

The procedures for the preparation of meat products, from meat preparation, preservation, addition of spices and smoking, including good handling practices, ensured that the meat products were healthy and safe. The products, therefore, meet the microbiological standards required by legislation. The smoking process increased the content levels of nutritional requirements analyzed in relation to fresh ones. Of the stuffed sausages, the *linguiças* with or without saffron, smoked or not, had greater acceptance while the *salsichões* were less accepted by the tasters. The handlers showed to have basic knowledge of the good handling practices of the processed products, which ensured healthy food, and the property was classified in the regular group.

## Acknowledgements

To the Quilombola Community of Limoeiro, Bacupari, Municipality of Palmares do Sul, RS, which greatly contributed to the collection of data for this research and to the research group "Foods of Animal Origin" of the CNPq Directory of Research Groups for their support.

## Statement of Competing Interests

There are no conflicts of interest in this research

## List of Abbreviations

RDC (Collegiate Directive Resolution)  
 ANVISA (National Health Surveillance Agency)  
 MAPA (Ministry of Agriculture, Livestock and Supply)  
 SAS (Statistical Analysis System)  
 GLM (General Linear Model)  
 MPN (Most Probable Number)  
 CFU (Colony Formation Units)  
 NCO (Not Carried Out)

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