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Research Article
Evaluation of the Physicochemical Properties of Fermented Sausages with *Staphylococcus xylosus* and *Lactobacillus Sakei*

Leidys Sarmiento Ortíz, Leidy Laura Sinning and Piedad M. Montero Castillo

Faculty of Engineering, Food Engineering program, Research Group IDAA, University of Cartagena Av. El Consulado, St. 30 No. 48152. Cartagena de Indias, Colombia

Abstract
Objective: The objective of this research was to evaluate the effect of the replacement of fat by yam starch (*Dioscorea rotundata*) and addition of pea fiber (*Pisum sativum* L.) in fermented sausages by means of a starter culture composed of *Staphylococcus xylosus* and *Lactobacillus sakei*. **Materials and Methods:** A factorial design 2^2 was carried out, which shows two independent variables with two levels each, for a total of four treatments and a control sample. The independent variables were starch and pea fiber, the levels of addition were 25, 50 1.5 and 3.0% respectively. The physicochemical and bromatological properties were evaluated, each treatment was analyzed in triplicate. **Results:** Treatment T4 presented a higher percentage of moisture at nine days of fermentation with respect to the other samples ($p<0.05$) with the exception of treatment T3, with which no significant differences were found. The pH values decreased considerably during the first six days of fermentation in all the sausages as expected, this is attributed to the presence of bacterial strains from the initial culture and carbohydrates that were metabolized by lactic acid bacteria to produce acids. **Conclusion:** The results of this study demonstrate that the partial substitution of fat by yam starch in a proportion of 10% does not negatively affect the main sensory, bromatological and physicochemical attributes of fermented sausages.

Key words: Acid lactic bacteria, fat substitute, fermented sausages, yam starch, pea fiber, physicochemical

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Corresponding Author: Piedad M. Montero Castillo, Universidad de Cartagena, Colombia, Av. El Consulado, St. 30 No. 48152 BS02

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fermented sausages have a high fat content; however, in recent years, there has been an effort to reduce their lipid content using fat substitutes in proportions that retain their main characteristics. Consumer interest in foods with a high added nutritional value has led to increased interest in producing meat products with a positive impact on health; fermented products with low fat levels are in high demand but consumers are also concerned about sensory quality and safety, which is why an important balance between diet and health, as well as the requirements of healthy foods[1], must be maintained.

Low fat meat derivatives have been replaced by carbohydrates such as starches, which are added to meat products to increase cooking yield, increase water holding capacity and modify the texture of the products. Starches have gained approval for use in many standardized and non standardized meat products to increase stability and yield during processing. In general, native starches are used to regulate and stabilize the texture of foods and their thickening and gelifying properties[2]. with cereals, fruits and tubers being excellent alternative sources for these starches[3]. The yam belongs to the Dioscorea ceae family and is a recommended energy food because of its high starch content (70-80% of its weight in dry matter) and 12% protein concentration, which is higher than in other tropical roots and tubers[2].

On the other hand, various organizations, such as the WHO, promote the reduction of saturated fats and cholesterol in daily diets, recommending the consumption of fiber to prevent cardiovascular disease, obesity and diabetes[4]. The addition of dietary fiber to meat products improves the nutritional value and quality characteristics such as texture and cooking performance. Ayo *et al.*[5] reported that carrot fiber increases the quality characteristics of fermented sausages. Similarly, peas, like all legumes, are an important source of fiber, containing soluble and insoluble fiber, where soluble fiber helps to reduce high levels of cholesterol and blood sugar and insoluble fiber helps regulate the proper functioning of the intestine, avoiding constipation[6].

When lactic acid bacteria are used as the starter culture in the fermentation of meat products, the use of a microbial starter culture increases shelf life, improves characteristics and guarantees consistency in the metabolic transformations of this type of product[7]. This study aimed to evaluate the effects of fat replacement with yam starch (Dioscorea rotundata) and pea fiber (*Pisum sativum* L.) in sausages fermented with the use of a starter culture composed of *Staphylococcus xylosus* and *Lactobacillus sakei*.

MATERIALS AND METHODS

Raw materials: Yam espino (*Dioscorea rotundata*) from Cartagena, Bolívar, Colombia was used to obtain the yam starch. Pea fiber (*Pisum sativum* L.) was obtained from the company Ingredientes y Productos Funcionales S.A.S., located in Medellín. The bacterial strains *Staphylococcus xylosus* ATCC® 2997™ and *Lactobacillus sakei* ATCC® 15521™ were obtained from C.M.L.A.B., located in Bogotá. The raw meat materials and other supplies were purchased from distributors of chemical inputs and supermarkets in Cartagena, Colombia.

Experiment design: The 2^2 factorial design in Table 1 was used, which had two independent variables with two levels each for a total of four experimental treatments. The independent variables were starch and pea fiber and the added level were 25 and 50 and 1.5 and 3.0%, respectively. The physicochemical, bromatological and acceptability properties were evaluated; each treatment was analyzed in triplicate.

Preparation of fermented sausages: The pork, beef and ground fat were mixed. This mixture was subjected to a curing process (common salt and nitrate salt) for 30 min at a refrigeration temperature of 4°C. The resulting meat paste was added to a cutter with the rest of the ingredients and the bacterial strains, which were prepared following the methodology proposed by Acevedo, *et al.*[8] until the cultures had a concentration of 10^8 CFU mL^{-1} on the Mc Farlan scale. The fine paste was embedded in resistant synthetic covers. The treatments were subjected to a temperature of 25°C for 24 h and then placed in an incubator at 25°C, with 75, 80% relative humidity to achieve fermentation; the pH, acidity, water activity and moisture were analyzed at 0, 3, 6 and 9 days. The fat, protein, carbohydrates, ash, soluble and insoluble fiber and starch were determined at 0 and 9 days[7].

Formulations: To carry out this study, four treatments were formulated with partial replacement of fat using yam starch, pea fiber and starter cultures (*L. sakei*+*S. xylosus*) with a 1:1 ratio and one treatment (5) as a control sample (Table 2).

Characterization of the physicochemical and bromatological properties of fermented sausages: The physicochemical and bromatological analyses of the different treatments were carried out after 9 days of fermentation, with the exception of pH, titratable acidity and moisture, which were analyzed at 0, 3, 6 and 9 days of fermentation. The moisture, fat, protein, titratable acidity and ash were

Table 1: Experimental design in the preparation of fermented sausages

Treatments	Yam starch as a substitute for animal fat (%)	Pea fiber (%)
1	25	1,5
2	25	3,0
3	50	1,5
4	50	3,0

Table 2: Formulation of sausages

	Treatments				
Raw materials	T1	T2	T3	T4	T5
Lean beef (%)	40	40	40	40	40
Lean pork (%)	40	40	40	40	40
Fat Pork (%)	15	15	10	10	20
Yam starch (%)	5	5	10	10	0
Meat paste (%)	100	100	100	100	100
Pea fiber (%)	1.5	3	1.5	3	0
Starter culture (mL kg^{-1})	10	10	10	10	10
Neutral salt (%)	0.2	0.2	0.2	0.2	0.2
Seasoning for sausage (%)	0.6	0.6	0.6	0.6	0.6
Polyphosphate	0.02	0.02	0.02	0.02	0.02
Glucose (%)	1.5	1.5	1.5	1.5	1.5
Sausage spreader	3	3	3	3	3
Antioxidants (%)	0.02	0.02	0.02	0.02	0.02

determined according to A.O.A.C[9]. To determine the pH, 10 g of the meat product sample were taken, dissolved in 100 mL of distilled water and mixed for 1 min. Then, the sample was filtered and measured with a pH meter.

Evaluation of the acceptability of fermented sausages: The sensory evaluation of the different treatments was carried out once the sausages met the stipulated time of fermentation (9 days) with the help of 50 untrained tasters, aged between 17 and 47, female and male, using acceptance tests. The acceptance test took into account the scores that each treatment reached for each parameter evaluated (taste, smell, texture, appearance and general appearance) on a scale of five points, where was (1) Very displeased, (2) I do not like it, (3) I do not like or dislike it, (4) I like it and five and (5) I like it a lot[10].

Statistical analysis: The data were presented as the mean±standard deviation. The various regressions were calculated with the statistical program STATGRAPHICS Centurion XVI.I® in Windows 10. An analysis of variance (ANOVA) with a level of significance of 95% ($p<0.05$) was applied. The treatment differences were compared using the least significant difference (LSD) test and significant differences were reported at 5% probability.

RESULTS AND DISCUSSION

Physicochemical analysis of fermented sausages: There were no significant differences in the different samples with respect to pH and acidity; however, the control sample had the lowest pH values and the highest acidity values. The pH values decreased considerably during the nine days of fermentation in all the sausages; the pH values decreased to 4.98, 4.97, 4.90, 4.90 and 4.80 for treatments T1, T2, T3, T4 and T5, respectively (Fig. 1). These results were consistent with Sun *et al.*[7], who reported that the pH values decreased from 6.22-5.33, 4.80, 5.08 and 4.73 in inoculated sausages. This can be attributed to the presence of bacterial strains in the initial culture and carbohydrates that were metabolized by lactic acid bacteria (LAB) and coagulase negative staphylococci to produce acids such as lactic acid.

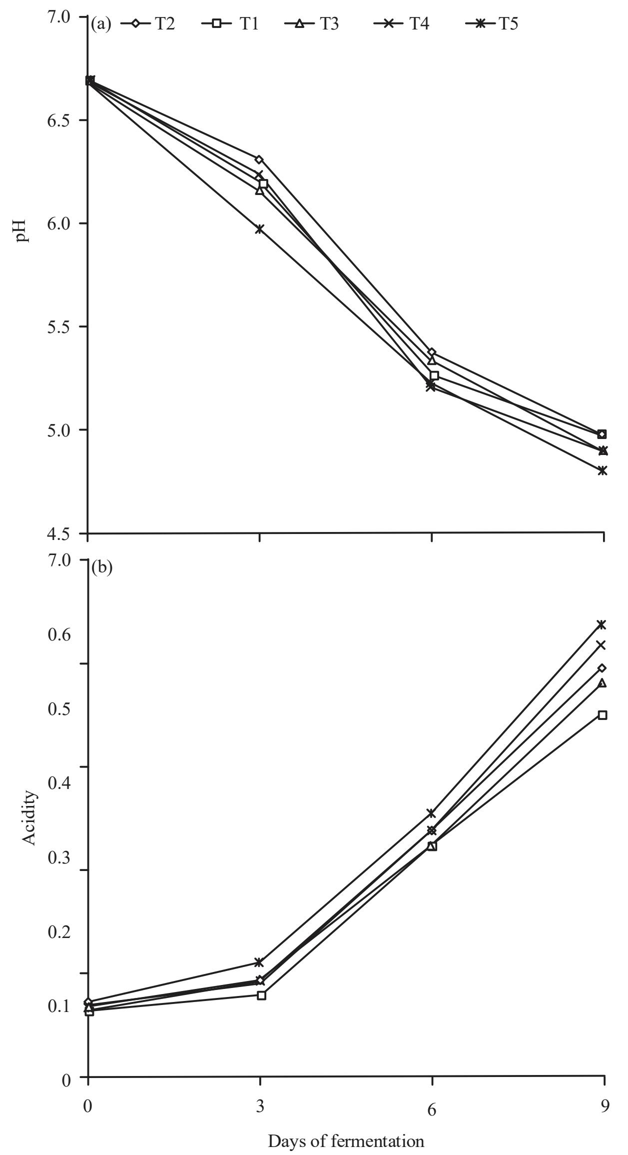

Fig. 1(a-b): Changes in pH and acidity of fermented sausages during the fermentation period

This result was similar to that reported by Nie *et al.*[11], who showed the same tendency in the pH values of carp sausage (*Cyprinus carpio*) inoculated with *Lactobacillus plantarum* and *Staphylococcus cerevisiae*. However, a lower pH decrease was observed at the end of the fermentation; this could have been due to the accumulation of alkaline compounds such as ammonia and trimethylamine, among others, mainly derived from microbial actions[12]. Wang *et al.*[13] stated that "the decrease in pH in fermented meat foods is due to the degradation of proteins by endogenous or microbial enzymes". A decrease in pH is vital to inhibiting the growth of unwanted bacteria, increasing the conversion rates of the desired color and reducing the formation of unwanted flavors in fermented meat products[14].

Changes in the moisture content of the sausages are shown in Fig. 2. The initial moisture content of the sausages was 61.02% on average; no statistically significant differences were reported; however, after the fermentation period, these values decreased to 33.72, 33.67, 34.28, 34.31 and 32.86% on the ninth day of fermentation in the treatments T1, T2, T3, T4 and T5, respectively. T4 showed a higher percentage of moisture after nine days of fermentation with respect to the other samples ($p<0.05$), except for T3, with which no significant differences were found. As can be seen in Fig. 2, the moisture content of the sausages decreased faster during the first six days of fermentation than in the last three days; these results are similar to those reported by Sun *et al.*[7] in the preparation of Harbin sausages fermented using *Staphylococcus xylosus*, *Lactobacillus plantarum* and a mixture of bacterial strains, who showed that the moisture content decreased from 64.23-37.53, 32.16, 35.04 and 31.85 for the ninth day of fermentation and that the moisture decreased more during the first six days than the last three days. Changes in water content are mainly associated with changes in pH, leading to acid denaturation in some muscle proteins and the promotion of gelation, which leads to a decrease in the water retention capacity of the protein[15].

The initial water activity (a_w) of the sample ranged from 0.82-0.9 and decreased to 0.79, 0.79, 0.78, 0.76 and 0.72 for samples T1, T2, T3, T4 and T5, respectively, after nine days of fermentation. Sun *et al.*[14] reported similar a_w values; Yongin *et al.*[16] reported a_w values that were initially in the range of 0.92-0.93 and decreased from 0.82-0.89 during 48 h of fermentation, with slightly higher a_w values for the inoculated sausages than for the control in fermented sausages using a mixed starter culture consisting of *Lactobacillus plantarum*, *Staphylococcus xylosus* and *Pediococcus pentosaceus*. This could have been due to the

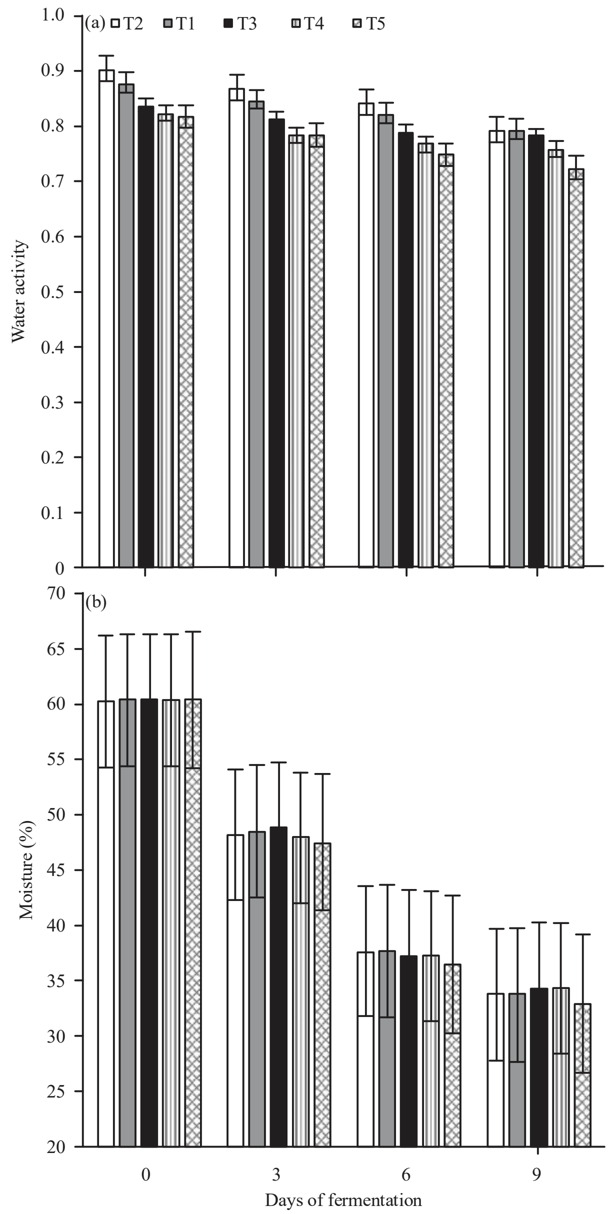

Fig. 2(a-b): Moisture and water activity of sausages during the fermentation period

addition of fiber in the other treatments since the study carried out by Ramirez *et al.*[17] reported that the development of a mixture of fiber and starch as a fat substitute in the elaboration of meat products increased the water retention capacity, especially in the formulations with the highest concentration of pea and orange fiber. Fiber has been extensively researched in food science and technology because of its technological properties, which depend on its chemical composition, the presence of pores and its microstructure, which affect its ability to absorb and retain water, gel formation and viscosity[18]. It should be noted that, in this study, the sausages that had 3% fiber added (T2 and T4) had less moisture loss during the fermentation period than the control sample ($p<0.05$). Moisture content and water activity are associated with pH changes because a decrease in pH during fermentation leads to acid denaturation in some muscle proteins, which leads to a decreased water retention capacity in the protein[19].

The bromatological parameters of the different sausages after nine days of fermentation can be seen in Table 3, which show that the control sample presented significant differences ($p<0.05$) with respect to the other samples for the total fat content because no substitution was done in this treatment; therefore, treatments 3 and 4, which had the highest percentage of fat substitution using yam starch (50%), had the lowest percentage of fat, with an average of 16.73 and 15.36% (Day 9), respectively, which differed significantly from the other treatments ($p<0.05$). The sausages that had 10% yam starch added presented lower moisture loss during the fermentation period, lower than the control sample ($p<0.05$).

It can also be observed that there was a considerable increase in the proportion of fat in all samples on the ninth day of fermentation, resulting from the reduction in humidity, which caused the other values to increase; the control sample (T5), which initially presented a fat content of 21.54%, increased to 33.89% at the end of the fermentation period, a value that was higher than that of the T3 and T4 ($p<0.05$).

On the other hand, the total protein content of the different treatments exceeded 20%; T5 obtained the lowest percentage of protein content, meaning that the substitution of fat using yam starch and the addition of fiber in fermented sausages resulted in meat products with a high percentage of protein.

Sucu and Turk[20] also demonstrated protein values above 20% in their study on sausages fermented using *Staphylococcus carnosus*, *Pediococcus acidilactici* and *Lactobacillus sakei*. Results different from those of the present study were reported by Choi *et al.*[21], who investigated the effects of partially substituted pork fat (30-20, 15 and 10%) using makgeolli fiber in a sausage type meat product, which had lower protein values, in the range of 12- 13%. It should be noted that, in this case, the sausages were not fermented, meaning the use of starter cultures to ferment this type of food results in meat products with a high protein content.

The addition of yam starch increased the carbohydrate values in treatments 1, 2, 3 and 4; it can also be observed that there were significant differences ($p<0.05$), which indicated that there was a significant increase in the CHO in the sausages, depending on the percentage of fat substitution using yam starch. As expected in this study, the control treatment had the lowest percentage of carbohydrates (2.03%), with significant differences ($p<0.05$) from the other formulations. On the other hand, there were significant differences in the ash content of the samples with the control treatment, which showed the lowest ash values at the beginning and end of the fermentation.

Table 4 shows the analysis of the soluble and insoluble fiber and sausage starch at 0 and 9 days of fermentation. It is clear that the values of these parameters varied according to the concentration that was added to each treatment. Therefore, there were significant differences between the treatments; the control sample had the lowest values of soluble and insoluble fiber and starch ($p<0.05$) and treatments 2 and 4 presented the highest values of soluble and insoluble fiber.

Table 5 shows the average of the results of the acceptance test for the five treatments, where it can be clearly observed that there were no significant differences for texture in different treatments, meaning that texture was favored by the fermented sausages. On the other hand, treatment 5 had the highest results for taste, texture, appearance and general acceptance, while treatment four, which had the highest percentage of pea fiber and yam starch substitution, had the best result for the color attribute. *Staphylococcus xylosus* is a grampositive bacterium that is often found in fermented

Table 3: Bromatological analysis of fermented sausages

Treatments	Fat (%)	Protein (%)	CHO (%)	Ash (%)
Day 0				
1	13.70 ± 0.58^b	17.41 ± 0.34^c	6.37 ± 0.71^b	1.48 ± 0.13^b
2	12.28 ± 0.95^b	17.78 ± 0.45^c	7.38 ± 0.85^b	1.53 ± 0.14^b
3	10.28 ± 0.31^a	16.20 ± 0.54^b	10.90 ± 0.53^c	1.40 ± 0.12^b
4	10.04 ± 0.40^a	15.47 ± 0.80^b	12.02 ± 0.31^d	1.43 ± 0.15^b
5	21.54 ± 0.49^c	15.18 ± 0.15^a	1.20 ± 0.12^a	1.05 ± 0.06^a
Day 9				
1	19.88 ± 0.18^b	30.28 ± 0.03^c	11.83 ± 0.22^b	4.27 ± 0.11^b
2	19.91 ± 0.23^b	30.20 ± 0.11^c	12.02 ± 0.20^b	4.18 ± 0.04^b
3	16.73 ± 0.87^a	28.65 ± 0.26^b	16.14 ± 0.09^c	4.19 ± 0.14^b
4	15.36 ± 0.64^a	28.45 ± 0.22^b	17.78 ± 0.13^d	4.09 ± 0.16^b
5	33.89 ± 0.10^c	26.57 ± 0.04^a	2.03 ± 0.03^a	4.64 ± 0.08^a

Table 4: Analysis of soluble and insoluble fiber and sausages starch at 0 and 9 days of fermentation

	Soluble fiber (%)		Insoluble fiber (%)		Starch (%)	
Treatments	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
T1	0.35±0.03[b]	0.57±0.03[b]	1.29±0.03[b]	3.14±0.06[b]	4.42±0.09[b]	7.24±0.11[b]
T2	0.61±0.05[c]	0.71±0.02[c]	1.98±0.06[c]	4.18±0.06[c]	4.31±0.12[b]	7.08±0.14[b]
T3	0.32±0.02[b]	0.55±0.04[b]	1.31±0.03[b]	3.19±0.04[b]	8.59±0.04[c]	11.96±0.08[c]
T4	0.60±0.04[c]	0.73±0.02[c]	1.95±0.03[c]	4.21±0.08[c]	8.55±0.08[c]	12.01±0.06[c]
T5	0.06±0.02[a]	0.13±0.01[a]	0.12±0.02[a]	0.19±0.03[a]	0.08±0.02[a]	0.14±0.02[a]

Table 5: Sensory analysis of fermented sausages

	Average				
Treatments	Flavor	Color	Texture	Appearance	General aspect
T1	3.38±0.12[a]	3.36±0.07[a]	3.19±0.19[a]	3.13±0.02[a]	3.29±0.15[a]
T2	3.43±0.10[a]	3.30±0.08[a]	3.06±0.34[a]	3.29±0.09[a]	3.45±0.05[a]
T3	3.57±0.22[ab]	3.41±0.09[a]	3.04±0.28[a]	3.34±0.08[a]	3.49±0.06[a]
T4	3.89±0.29[bc]	3.83±0.07[c]	3.40±0.27[a]	3.72±0.02[b]	3.67±0.05[b]
T5	4.08±0.08[c]	3.57±0.02[b]	3.42±0.35[a]	3.81±0.04[c]	3.93±0.04[c]

sausages produced traditionally without a starter culture[22]. It is generally believed that *S. xylosus* and other members of the *Micrococcaceae* family are important for the development of aromas in such sausages.

Studies carried out by Sayas Barberá *et al*.[23] reported that the addition of fiber does not affect the color intensity of sausages fermented using *L. casei*. In this study, the addition of fiber favored the color attribute, resulting in a more attractive product for the untrained tasters. Treatment 1 obtained the lowest values for flavor, despite having the lowest substitution of starch and a lower concentration of fiber. Finally, the general appearance results did not have significant differences in the different formulations; the control sample and treatment 4 had the highest results in all of the sensory attributes ($p < 0.05$); therefore, it can be inferred that the sausages fermented using *Staphylococcus xylosus* and *Lactobacillus sakei*, with fat replacement using yam starch up to 50% and added fiber up to 3%, resulted in functional fermented sausages with optimal sensory characteristics for consumption. Sun *et al*.[7] found that sausage inoculated with *Lactobacillus plantarum* and *Staphylococcus Xylosus* had higher color, flavor and acidity scores than the control sausages ($p<0.05$). The lactic acid produced from the bacterial metabolism of carbohydrates directly contributes to acidity and indirectly contributes to flavor and texture. For color, the sausages inoculated with *S. xylosus* had a higher score than the control sausages and those inoculated with *L. plantarum* ($p<0.05$) because *S. xylosus* contains nitric oxide, forming nitrosomyoglobin, making the product redder. The sugar and fermentation temperature determine, to a large extent, the rate of lactic acid formation and, therefore, the growth of microorganisms of potential importance flavor[24].

The present study suggests that the partial substitution of fat by yam starch in a proportion of 10% does not negatively affect the main sensory, bromatological and physicochemical attributes of fermented sausages. Future studies on meat products should increase fiber incorporation and include higher percentages of yam starch as a potential fat substitute and evaluate the effect on other parameters such as texture and microstructure.

CONCLUSION

The pH values decreased considerably during the first six days of fermentation in all formulations. The initial moisture content of the different sausages was in the average range of 61.02%. Sausages to which 10% yam starch was added had less moisture loss during the fermentation period. The total protein content of the different treatments exceeded 20%, with T5 obtaining the lowest percentage of protein content. Finally, the general appearance results showed significant differences in the different formulations, with the control sample and treatment 4 being the ones that obtained the highest results in all the sensory attributes evaluated ($p<0.05$).

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