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Effect of Inulin Addition on Physicochemical, Microbiological, Textural, and Sensorial Characteristics of Fermented Butifarra with *Lactobacillus sakei*

Piedad Margarita Montero Castillo [1,*], Verónica Morelos Martelo [1], Katiuska Gómez Acevedo [1], Yesid Alejandro Marrugo Ligardo [2] and Diofanor Acevedo-Correa [3]

1 Food Engineering Program, Research Group in Agricultural and Agro-Industrial Innovation and Development, Faculty of Engineering, University of Cartagena, Cartagena 130015, Colombia; morelosmarteloveronica@hotmail.com (V.M.M.); kgomeza@unicartagena.edu.co (K.G.A.)
2 Environment, Food and Health Research Group (MAAS), Faculty of Nursing, Zaragocilla Campus, University of Cartagena, Cartagena 130015, Colombia; ymarrugol@unicartagena.edu.co
3 Tourism Administration Program, Research Group in Agricultural and Agro-Industrial Innovation and Development, Faculty of Economic Sciences, University of Cartagena, Cartagena 130015, Colombia; dacevedoc1@unicartagena.edu.co
* Correspondence: pmonteroc@unicartagena.edu.co

Abstract: Butifarra, with the addition of inulin, was produced for the first time. The objective of this study was to investigate the effect of inulin in butifarra fermented with *Lactobacillus sakei* ATCC® 15521™ on physicochemical properties, instrumental texture, microbiology, and sensory evaluation. Initially, fermented butifarra was prepared with the addition of 5% and 7.5% inulin and a control butifarra was prepared without inulin addition. The butifarra was analyzed by physicochemical, microbiological, instrumental texture, and sensory evaluation. The results indicated that the analysis of physicochemical properties, fat, protein, and ash content showed no significant differences between the experimental and control butifarra ($p > 0.05$); in other words, this type of fiber did not cause alterations in the butifarra. The mesophilic microorganism count, total and fecal coliforms, and positive staphylococcus were assessed according to Colombian Technical Standard 1325 of 2008. The hardness of the butifarra with higher inulin content on day 9 was, on average, ~55% greater than the control. The adhesiveness increased in each formulation as the days of fermentation increased, showing significant differences relative to the control. In general, the best sensory properties evaluated were related to the butifarra samples with higher inulin content in the formulation, so it is established that inulin did not alter the sensory properties of the butifarra, but rather potentiated the sensory attributes, making it suitable for use in fermented meat formulations. It can be stated that the addition of inulin to fermented butifarra is possible.

Keywords: butifarra; fermententation; inulin; *Lactobacillus sakei*

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1. Introduction

Inulin is a prebiotic present in more than 3000 vegetables and fruit and has been part of our daily diet for centuries. The plant most commonly used to produce inulin is chicory root. Inulin is one of the most popular prebiotics used in the food industry, along with pectin and β-glucan. It provides unique nutritional properties and stands out for its important technological benefits. In addition, it is used in food matrices as a fat or sugar substitute, as a low-calorie sweetener, or to generate desirable characteristics because, when mixed with water, it forms gels that improve the texture of various foods, increase water-retention capacity, and improve viscosity, engendering a sensation similar to that produced by fat [1–3]. In addition, the addition of inulin specifically to fermented products can improve their textural and sensory properties. It also provides health benefits, including cholesterol reduction and reduced risk of cancer development, e.g., colon cancer, acting as a bulking agent, promoting healthy gut flora, and stabilizing blood sugar [4,5]. Fermentation is an important method of food preservation, providing unique sensory properties to a variety of meat products. Fermented sausages further exhibit longer shelf life relative to their unfermented counterparts, and enjoy a higher quality as a result of fermentation [6,7].

In the last two decades, world meat consumption has increased by 58%. Meat is considered the world's largest source of protein, as it contains a variety of essential vitamins and minerals, such as iron and zinc, as well as being particularly rich in protein and fat. More than 50% of the protein consumed in the world comes from meat. According to forecasts, world meat consumption will reach 72% by 2030 [8,9]. Although consumers are increasingly influenced to consume meat, the sensory properties of food remain one of the most important reasons why people choose one food over another [9].

Now, meat sausages purchased on the market contain an average of 30% fat, which improve the quality characteristics of these products and sensory properties such as color, flavor, texture, juiciness, and general acceptance of this type of food. However, it is well known that the high content of animal fat in meat products related to higher consumption can influence different types of diseases that are in turn related to general food consumption, among which are coronary heart disease, hypertension, and obesity, among others [10–12].

Low-fat diets are increasingly attracting worldwide attention; however, fat substitution can affect the sensory properties of foods. Polysaccharides, including dietary fibers such as inulin and microcrystalline cellulose [13], konjac gum, sodium alginate, and xanthan gum [14], κ-carrageenan and methyl cellulose [15], guar-xanthan gum [16], and amorphous cellulose fiber (Z-trim®) [17], are important fat replacements widely used in a vast array of meat products.

Chemically, inulin is a straight-chain linear polysaccharide made from d-fructose linked by a $\beta(1 \rightarrow 2)$ glycosidic bond, usually with a glucose residue at its end [13]. It is a source of dietary fiber that acts as a prebiotic and has a beneficial effect on intestinal habits, due to the ability to change the composition of the colon microbiota which has beneficial effects on the human host [18–20].

In general, the addition of healthy fibers to meat products causes a positive effect during cooking, improving water-retention capacity and exerting an impact on texture depending on the type and structure of the dietary fiber. In addition, dietary fibers derived from plant polysaccharides are widely accepted and used as prominent nutrients in human nutrition due to its ability to improve intestinal functions, decrease the incidence rates of cardiovascular and gastrointestinal diseases, and have a protective effect against weight gain and obesity without decreasing the feeling of satiety [21,22].

The Colombian Technical Standard NTC 1325 [23] defines butifarra as a "homogenized processed meat product, cooked, stuffed in edible casing, made from meat, with added salt, fat, and spices. It has a spicy flavor and may or may not contain curing salts, maintaining its characteristic pale color, and does not contain colorants." Fermentation is not a phase in the production of butifarra.

Some research has analyzed the effect of inulin and microcrystalline cellulose on the storage characteristics of pork sausages [24]—characterized as the butifarra marketed in Cartagena (Colombia) [25] and Soledad Atlántico [26]—and has studied the quality and acceptability of sausages formulated with bovine blood plasma and sesame paste [27]; however, most of the research has been carried out on fresh meat products, such as hamburgers, and less so on sausages such as chorizo. Nonetheless, the addition of inulin to fermented chorizo has not been studied. Traditionally, many researchers have focused on studying the effect of starter cultures on fermented meat products. However, no studies have investigated the effect of the addition of inulin and microbial culture composed of *Lactobacillus sakei* ATCC® 15521™ on the physicochemical, textural, microbiological, and sensory quality of fermented butifarra. Currently, no fermented sausages have been produced, nor has inulin been added to the formulation. Traditional Colombian butifarra is not fermented and polysaccharides such as inulin are not added. Therefore, this study can provide insights into this area of fermented products.

2. Materials and Methods

2.1. Materials and Supplies

The beef and pork meats, along with other ingredients to prepare the product, were purchased at a local supermarket in Cartagena (Bazurto Market, Cartagena, Colombia), Colombia, considering the organoleptic characteristics of smell, color, and freshness suitable for the production of fermented meat products [28]. The starter culture strain for fermented butifarra was *Lactobacillus sakei* ATCC® 15521™, purchased from CMLAB SAS., Bogotá, Colombia. Inulin was purchased in Itagüí-Medellín, Colombia (IPF Ingredientes y Productos Funcionales S.A.S., Fibruline®, Itagüí-Medellín, Colombia I, ≥90% purity; 4.32% moisture).

2.2. Preparation of Starter Cultures

The microbial strain consisting of *Lactobacillus sakei* ATCC® 15521™ was inoculated in de Man Rogosa and Sharpe (MRS) broth and cultured anaerobically at 37 °C/24 h. The cultures were then centrifuged at 6000× *g* for 10 min. Microbial cells were suspended in 0.9% saline and a 0.5 McFarland scale tube was used as a turbidity standard corresponding to 1×10^8 CFU/mL *L. Sakei* [29,30].

2.3. Experimental Design

A completely randomized design with a unifactorial structure was used. Three treatments were evaluated; a control sample (without inulin), sample A with 5% inulin, and sample B with 7.5% inulin [31]. The response variables included the physicochemical characteristics (moisture, oil, protein, ash, total fiber, and carbohydrates), microbiological characteristics (aerobic mesophiles, total and fecal coliforms, and coagulase-positive Staphylococcus), instrumental texture (hardness, adhesiveness, cohesiveness, springiness, and chewiness), and sensory properties (color, odor, flavor, and hardness).

2.4. Preparation of Fermented Butifarra

Beef and pork meats were stored under refrigeration at 4 °C in a domestic refrigerator (Daewoo® Electronics Inc., Ridgefield Park, NJ, USA), then cut into pieces and ground in a meat grinder M-12 (Javar Ltd.a., Bogotá, Colombia). The other ingredients, such as spices, inulin (IN), and curing agents, were added to the meat paste obtained according to the formulations shown in Table 1, taking into account the percentages calculated according to the Colombian Technical Standards (NTC 1325, 2008) [23]. Afterward, the bacterial strains were added, having been prepared according to the methodology proposed by Zuber and Horvat [29] whereby all cultures had to have reached a concentration of 1×10^8 CFU/mL. The emulsion obtained was stuffed into natural collagen-based casings of 40 mm (Alico® S.A., Medellín, Colombia). A rapid sausage filler SF-260 (Davison's Butcher Supply, CA., USA) was used, with portioning consisting of 15 g weight and 3 cm diameter; the tying was done manually [27]. All formulations were kept refrigerated at 12–15 °C and at a relative humidity of 70–80% for nine days to achieve fermentation. The samples were analyzed on days 0, 6, and 9 [32].

Table 1. Fermented butifarras formulations in this study.

Ingredients	Control (%)	A: 5% Inulin	B: 7.5% Inulin
Beef meat	60	60	60
Pork meat	22	17	14.5
Ice	14	14	14
Inulin	0	5	7.5
Starter culture (mL)	10	10	10
Red onion	0.2	0.2	0.2
Long onion	0.2	0.2	0.2
Ground black pepper	0.3	0.3	0.3
Monosodium glutamate	0.2	0.2	0.2
Garlic powder	0.2	0.2	0.2
Paprika	0.2	0.2	0.2
Salt	1.7	1.7	1.7
Total	100	100	100

2.5. Physicochemical Analysis

The methods of the Official Association of Analytical Chemistry [33] (AOAC, 2005) were followed. Moisture was determined by drying in a forced convection oven until a constant weight was attained [33] (AOAC-950.46), ashes were obtained by total incineration at 550 °C (method 942.05), proteins by the Kjeldahl method with a factor N = 6.25 (method 992.15), fat by Soxhlet extraction with petroleum ether at 60 °C (method 991.36). The total fiber was measured by gravimetric method 993.21, and the total carbohydrate content of the sample was calculated by the difference of 100% minus the sum of moisture, ash, protein, fat, and total fiber contents.

2.6. Microbiological Analysis

The tests were carried out for all samples at the end of the fermentation (day 9). Total mesophilic aerobes were performed using Plate Count Agar incubated at 35 °C for 48 h (NTC 4519, 2009), total and fecal coliforms were estimated by tube dilution with the technique of most probable number, Brilliant Green Bile Broth 2%, incubated at 37 °C for 48 h (NTC 4516, 1998). Coagulase-positive Staphylococcus was measured using Baird-Parker Agar incubated at 35 °C for 48 h (NTC 4779, 2007). The colonies were visually counted and recorded as the decadic logarithm of colony-forming units per gram product ($\log_{10}$ CFU/g) [34].

2.7. Texture Analysis

Texture profile analysis (TPA) of the different formulations (control, A, and B) was performed on days 6 and 9 of the fermentation process using a TA-XT plus texture analyzer with software texture expert exceeding version 2.64 for Windows (Stable Micro Systems Ltd., Godalming, Surrey, UK), available at the National University of Colombia, Medellin (Colombia) [35]. The device was equipped with a load cell capacity of 5 kg, a heavy-duty platform of aluminum (HDP/90), and a compression platen (P/100) made of stainless steel (100 mm diameter). Cylinder-shaped pieces weighing ~10 g (10 mm width and 15 mm height) were obtained from the central part of the fermented samples using a metal cutter. These sample pieces were covered with a plastic film from the moment they were cut until they were analyzed to avoid dehydration. The butifarra was analyzed 10 min after cutting, the same time for all the butifarras. The tests were performed at 25 °C with a speed of 10 mm/s to compress the samples by up to 50% of their original height, using two-cycle uniaxial compression, simulating the human bite with a delay time of 10 s.

The measurements were repeated three times for each formulation. The parameters used as indicators of textural changes were hardness (N), adhesiveness (N.· m), cohesiveness (dimensionless), springiness (dimensionless), and chewiness (hardness × cohesiveness × elasticity, N). All variables were calculated from the TPA curve.

2.8. Sensory Analysis

A sensory evaluation was performed with 50 untrained panelists, who were provided with the fermented samples (control, A, and B) in private booths inside the sensory room at 25 °C. A five-point hedonic scale was used, where the panelists indicated their degree of acceptability in the attributes of color, odor, flavor, and texture (hardness). The categories on the scale ranged from "I like it (5)" to "neutral (3)" and "I do not like it (1)". The data were collected in a spreadsheet and later transformed into numerical scores for analysis.

2.9. Statistical Analysis

The physicochemical analysis, along with the microbiological, sensorial, and textural assays were performed in triplicate. All results correspond to the arithmetic mean (±) standard deviation. One-way analysis of variance (ANOVA) was used to analyze significant differences, along with the HSD Tukey method at 95% confidence. The data were processed in the Statgraphics Centurion program version 16.2.04 (Stat-Point Technologies Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1. Physicochemical Properties

Table 2 shows the physicochemical properties of the fermented sausages. The moisture content of the butifarra differed, with statistical significance, between the control butifarra and the butifarra containing the highest percentage of inulin (butifarra B). The control butifarra attained 4.8% more moisture than the other treatments. This parameter tended to decrease with the addition of higher inulin content in the formulation, i.e., the presence of this type of soluble fiber had a significant effect on this property, similarly to the results published by Choi et al. [36] and Gadekar et al. [37] in sausages formulated with chicory (*Cichorium intybus* L.) fiber and inulin, respectively; both studies reported a trend similar to that observed in the present study. It was also evident that, when 7.5% inulin was added, significant differences were observed with the control. This was possible because inulin solubilizes water molecules at higher temperatures. Therefore, this fiber did not influence the deceleration of water loss during the fermentation period of meat products. Huang et al. [38] demonstrated that, with an increased level of inulin, the moisture content of sausages decreased, which was consistent with the results from the present study. In general, the physicochemical and textural properties of fermented sausages may depend on the type of dietary fiber, as well as the molecular weight. Depending on the interaction between this polysaccharide and water molecules, it can influence the ability to bind. Upon inspection, the water molecule shares a hydrogen bond donor region and a hydrogen bond acceptor region that is quite characteristic of this molecule. On the other hand, the sugar units observed in the structure of the inulin molecule share a region more likely to donate hydrogen molecules. The ability to donate or receive hydrogen bonds of inulin molecules may indicate their strong interaction with water, being even greater at higher temperatures, which in turn could help to understand the differences in these analyzed properties.

Table 2. Physicochemical composition of the fermented butifarras.

Samples		Components (% d.b)					
		Moisture	Fat	Protein	Ash	Fibre	Carbohydrates
Control		54.02 ± 1.11 [a]	15.98 ± 0.72 [a]	28.15 ± 2.35 [a]	1.26 ± 0.76 [a]	0.12 ± 0.04 [a]	0.47 ± 0.15 [c]
A		53.21 ± 1.24 [a]	15.07 ± 0.48 [a]	28.04 ± 4.17 [a]	1.31 ± 0.57 [a]	0.15 ± 0.03 [a]	2.22 ± 0.91 [b]
B		49.63 ± 1.33 [b]	14.88 ± 0.62 [a]	27.57 ± 1.33 [a]	1.19 ± 0.41 [a]	0.18 ± 0.02 [a]	6.54 ± 1.07 [a]
ANOVA	F-value	52.87	8.67	10.34	3.35	6.58	106.44
	p-value	0.042 < 0.05	0.765 > 0.05	0.962 > 0.05	0.973 > 0.05	0.592 > 0.05	0.016 < 0.05

Data are means ± standard deviation (n = 3). Different superscripts in the same column denote significant differences ($p < 0.05$).

In general, the main function of products enriched with dietary fibers is to increase water retention; however, in this study, as well as in Choi et al. [39], the presence of fiber in a meat product is important, especially because of the functional contributions that this polysaccharide can make. This type of fiber, specifically, can help digestive health by reducing the risk of diseases such as constipation, irritable bowel syndrome, and colon cancer [40].

However, in this study, fiber values were not altered in a statistically significant way with the presence of water-soluble dietary fiber, as reported by Huang et al. [38] in Chinese-style sausages. It can also be evidenced that fat, protein, ash, and fiber content exhibited no statistically significant reduction from control to test butifarra ($p > 0.05$), i.e., this type of fiber did not cause alterations to the sausages. In general, no reduction in fat content was observed in the butifarra. That is, the amount of inulin added was not sufficient to cause variations in the content of fat, protein, ash, and fiber. More precisely, no differences were found in terms of physicochemical properties between the three samples, due to the low percentage of inulin added to the butifarras.

3.2. Microbiological Characteristic

The microbiological results are shown in Table 3. The count of mesophilic microorganisms, total and fecal coliforms, and positive staphylococcus were by Colombian Technical Standard 1325 of 2008 [23]. The heat treatment and microbiological quality of the ingredients and the adequate disinfection of the raw materials have a direct influence on this type of test. Sodium chloride is a fundamental ingredient in meat products because it reduces water activity and stabilizes the microbiological characteristics of sausages. The low levels of microorganisms evaluated are associated with the use of additives, seasonings in good microbiological condition, and good manufacturing practices of the product, thus ensuring that the chorizos are safe for consumers [41]. It has been shown that the addition of inulin to different food matrices can promote the growth of probiotic microorganisms. This is associated with the prebiotic effect of fiber, favoring lactic acid bacteria to ferment fructooligosaccharides and degrade long-chain inulin-type fructans [42–44].

Table 3. Microbiological analysis of the fermented butifarras.

Microorganisms Analyzed	NTC 1325:2008 (CFU/g)	Day 9 (Maximum Fermentation)			ANOVA	
		Control	A	B	F-Value	*p*-Value
Total aerobic mesophiles (Log_{10} CFU/g)	100,000	<3.65 ± 0.09 [a]	<3.60 ± 0.05 [a]	<3.60 ± 0.08 [a]	9.26	0.516 > 0.05
Total Coliform MPN (Log_{10} CFU/mL)	<10	<3.20 ± 0.07 [a]	<3.15 ± 0.08 [a]	<3.15 ± 0.05 [a]	5.33	0.887 > 0.05
Fecal Coliform MPN (Log_{10} CFU/mL)	<10	0.00	0.00	0.00	NA	NA
Coagulase-positive staphylococcus (Log_{10} CFU/g)	<100	<3.40 ± 0.02 [b]	<3.25 ± 0.05 [a]	<3.25 ± 0.01 [a]	79.58	0.035 < 0.05

Data are means ± standard deviation (n = 3). Different superscripts in the same row denote significant differences ($p < 0.05$).

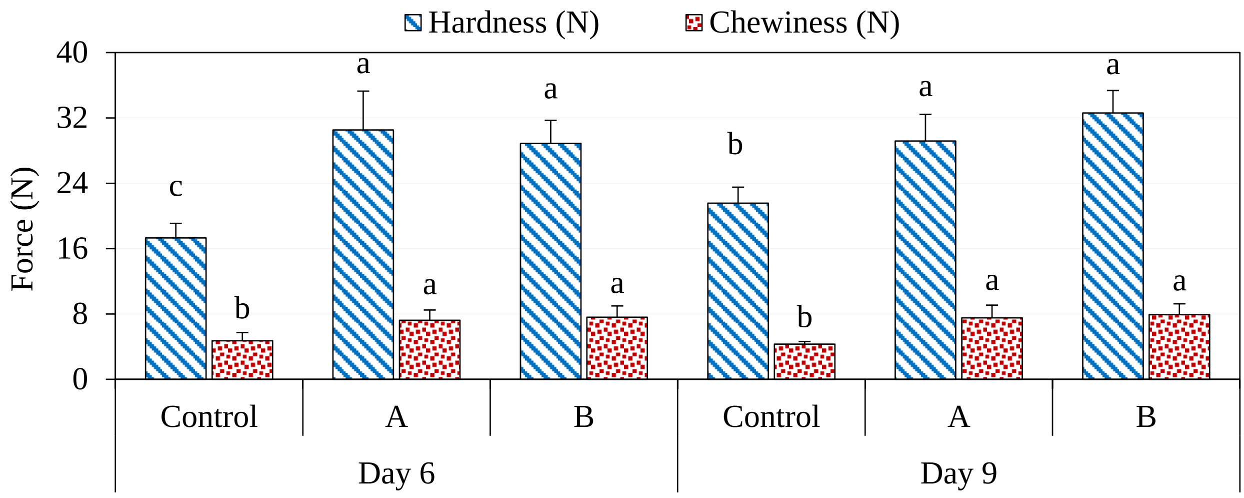
3.3. Textural Changes

Upon analysis of the textural results, inulin generally exerted a positive impact on the textural characteristics of sausages, such as firmness, elasticity, and chewiness, making this fiber a prominent ingredient in sausage matrices. The textural properties of the fermented sausages are shown in Figure 1 The hardness varied from 17.31 to 21.57 N in the control sausage during fermentation, while the hardness of the sausage with 7.5% inulin on day 9 increased, on average, by ~55% relative to the control.

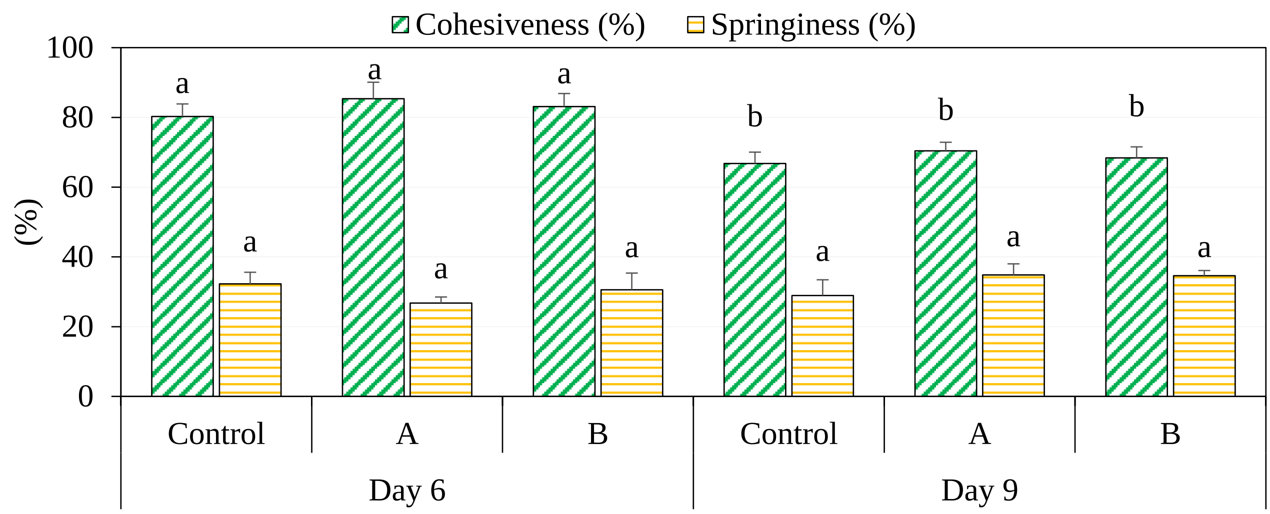
Regarding hardness, the values found were in a range of 17.31 to 32.60 N, i.e., the inulin formed a more compact structure because the OH groups favored greater interaction between the other groups of proteins, carbohydrates, and water; in addition, the reduction of fat in the emulsified meat product made the texture firmer [45], being different from those reported by Fernández-Diez et al. [46], who observed hardness values of 41.3 N in dry pork sausages with 50% fat substitution by cooked quinoa. It has been observed that one of the most important characteristics influencing texture is the higher moisture content and moisture/protein ratios. Therefore, this relation can explain the differences in these fermented meat products. Also, it is possible to highlight that, mainly, the hardness and chewiness of chorizos can be related to the amount of inulin added either in cream or powder form [47,48]. If the study carried out by Han and Bertram [49] is analyzed in meat products, these researchers did not observe significant differences between test and control butifarra when 2% inulin was added. However, when they added Carboxymethylcellulose and pectin, the hardness increased.

Alaei et al. [50] showed that increasing the proportion of inulin decreased textural properties such as hardness, cohesiveness, gumminess, and stringiness, but increased elasticity and chewiness when up to 25% fat replacement by inulin was added. Liaros et al. [51] reported that toughness increased with increasing maturation time, while other researchers, such as Salazar et al. [52], showed that both toughness and chewiness in sausages increased during fermentation.

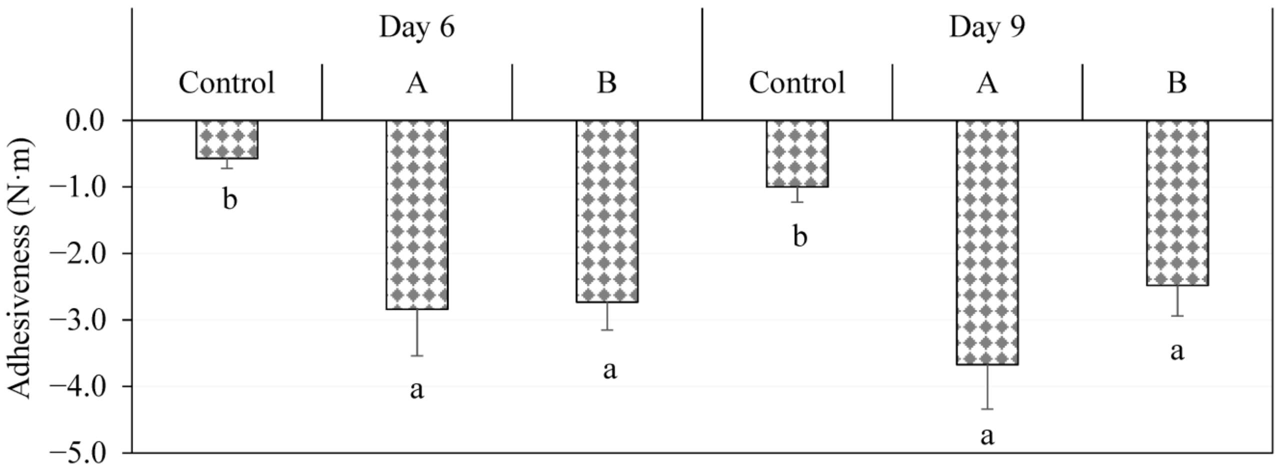
Beriain et al. [53] observed that inulin incorporated at 6% showed an increase in the hardness value of fermented sausages, going from 5.59 N to 14.21 N after 10 days of fermentation, and being even higher (37.24 N) with 3% inulin addition and 31 days of fermentation. The cohesiveness values did not show significant differences from control.



(**a**) Hardness and Chewiness



(**b**) Cohesiveness and Springiness



(**c**) Adhesiveness

Figure 1. Texture profile analysis of the different fermented butifarras developed in this study. (**a**) Hardness and chewiness; (**b**) cohesiveness and Springiness; (**c**) adhesiveness. Data are presented as means ± standard deviation ($n = 3$). Different superscripts in the same pattern denote significant differences ($p < 0.05$).

The chewiness values showed significant differences between the control and the sausages formulated with inulin on the sixth and ninth days of analysis. No differences were observed between the sausages formulated with inulin. The results were similar to those presented by Noguerol et al. [54] in sausages formulated with Psyllium Husk soluble fiber (0.65 to 6.3 N). Özer [44] reported that the replacement of beef fat with *Jerusalem artichoke powder* (40% inulin) in sausages fermented by a starter culture mixture (Lactobacillus curvatus, Staphylococcus xylosus, Staphylococcus carnosus and Pediococcus pentosaceus) showed a significant effect on hardness and adhesiveness. However, there were no significant changes in resilience, cohesiveness, or chewiness index.

De Carvalho et al. [55] reported that chewiness values were very variable (between 10.37 and 98.70 N·mm) in fresh lamb sausages due to the different percentages of pecans in the formulations, with the optimum formula, containing 20% of pecans, having a value of 12.12 N·mm. Salva Ruiz et al. [56] also reported similar chewiness values (14.19 N·mm) in llama sausages with pecans (*Carya illinoinensis*) and kañiwa (Chenopodium pallidicaule Aellen) as fat substitutes. The results of this study were different from those reported by Alves et al. [57], who observed a decrease in chewiness when green banana flour was used as a fat substitute in bologna-type sausages.

The elasticity values found ranged from 0.268 to 0.348, and no statistically significant differences were observed. Bis-Souza et al. [58] reported that decreasing fat content as a result of inulin in salami increased the elasticity values (from 0.302 to 0.445), which is different from the results presented in this study.

3.4. Sensory Acceptability

Sensory properties are one of the main factors used by consumers to accept many products and obtain satisfaction as a result of their consumption. Therefore, it is important to study the factors that affect them. Figure 2 shows the behavior of the sensory evaluation of fermented butifarra formulated with inulin. Regarding the attributes of flavor, color, and sensory odor, the butifarra formulated with inulin presented significant differences relative to the control; as for sensory hardness, no significant differences were observed. Souza et al. [59] demonstrated that sausages produced with inulin and oat fiber obtained similar ratings in color, flavor, and general acceptability; however, texture values were low when oat fiber was included.

In general, the best sensory properties evaluated were related to the sausage samples with higher inulin content in the formulation, thus establishing that inulin does not alter the sensory properties of sausages, but rather enhances the sensory attributes of sausages and is suitable for use in fermented meat formulations.

Chaharaein et al. [9] showed that the addition of inulin in higher concentrations improved the overall sensory properties, although the odor scores were low in chicken sausages. Furthermore, the authors reported that the sensory properties of inulin were close to those of the control, and all sensory properties improved on day 45; therefore, the evaluators reported better color and fragrance.

Guedes-Oliveira et al. [60] investigated the substitution of fats by carboxymethyl cellulose and inulin for the manufacture of low-fat lamb patties. The combination of carboxymethyl cellulose and inulin was found to be a suitable fat substitute, with the final product being accepted by consumers. The changes in sensory properties may be associated with the positive impact caused by the replacement of fat with inulin. The results showed that the perception of odor and flavor compounds improved, with greater release in this type of fermented product.

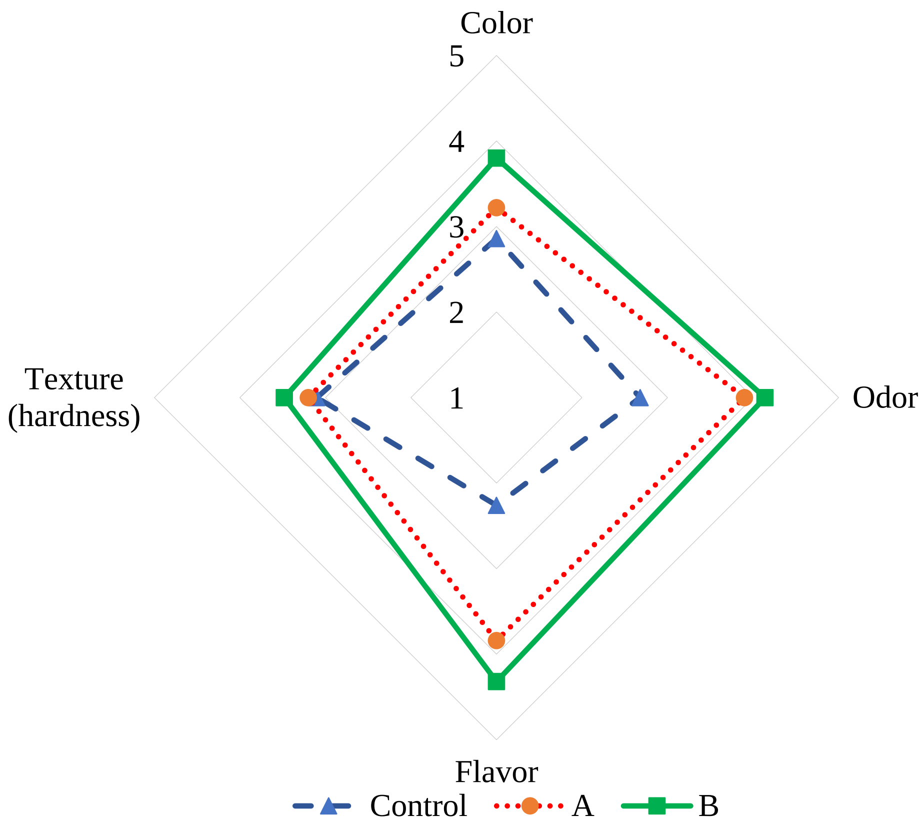


Figure 2. Sensory analysis of the fermented butifarras. Data represents mean values (n = 50). Scale category, 1–5.

4. Conclusions

Fermented butifarra with appropriate physico-chemical and microbiological properties was obtained for the first time. The incorporation of inulin increased the instrumental hardness of the butifarra; however, this property was not different from that of the control butifarra according to the sensory evaluation. The best sensory properties evaluated were those of the butifarra fermented with 7.5% inulin, indicating that inulin potentiated the sensory attributes, making it suitable for use in fermented meat formulations on an industrial scale. It can be stated that the addition of inulin to fermented sausages is possible.

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