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EFECTO DE LA SUSTITUCIÓN PARCIAL DE CARNE POR HARINA DE SOYA SOBRE LAS PROPIEDADES FISICOQUÍMICAS, SENSORIALES Y MICROBIOLÓGICAS DE HAMBURGUESAS

EFFECT OF PARTIAL MEAT REPLACEMENT WITH SOY FLOUR ON PHYSICOCHEMICAL, SENSORY AND MICROBIOLOGICAL PROPERTIES OF BEEF BURGERS

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RESUMEN: En la actualidad el beneficio de cambiar la composición de productos cárnicos sin eliminar su matriz tradicional es reemplazar parcialmente la carne por ingredientes vegetales.Este estudio se propuso determinar el impacto que tiene la adición de harina de soya (Glycine max) en hamburguesas de res a través de análisis microbiológicos, fisicoquímicos y sensoriales. Se formularon cuatro tratamientos: uno de control sin harina de soya (C1S1B) y tres con 5% (C2S2B), 10% (C3S3B) y 15% (C4S4B). Cada uno fue examinado en tres ocasiones. Se establecieron los niveles de pH, grasa, proteína, fibra, cenizas y humedad; para procesar la información se emplearon la prueba de Tukey con α=0,05 y el análisis de varianza. Se llevó a cabo la valoración sensorial con 20 catadores y una escala hedónica de cinco grados para el color, el aroma, el gusto y la apariencia. El aumento de harina de soya generó diferencias importantes en las variables fisicoquímicas. El tratamiento con 15% mostró el aporte composicional más alto, con 3,31% de cenizas, 19,02% de grasa, 2,41% de fibra y 26,71% de proteína. No obstante, el tratamiento con un 10% fue el que tuvo la mayor aceptación sensorial, con una media total de 4.45/5. Este tratamiento mostró 8×10^6 UFC/g de aerobios mesófilos, 3×10^2 UFC/g de Escherichia coli, 8×10^3 UFC/g de Staphylococcus aureus y no hubo Salmonella en 25 g. En el análisis se consideraron estos valores como acordes con la NTE INEN 1338. Se concluye que la harina de soya altera en gran medida la composición de las hamburguesas y que el 10% es el porcentaje óptimo entre mejoramiento composicional y aceptabilidad.

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Palabras clave: hamburguesa; harina de soya; sustitución parcial; análisis proximal; evaluación sensorial

ABSTRACT: Partial replacement of meat with plant-based ingredients is an alternative for modifying the composition of meat products without eliminating their traditional matrix. This study aimed to evaluate the effect of incorporating soy flour (Glycine max) into beef burgers through physicochemical, sensory and microbiological analyses. Four formulations were prepared: a control without soy flour (C1S1B) and treatments containing 5% (C2S2B), 10% (C3S3B) and 15% (C4S4B), analyzed in triplicate. Moisture, ash, fat, fiber, protein and pH were determined, and the data were processed by analysis of variance and Tukey's test at α=0.05. Sensory evaluation was performed by 20 panelists using a five-point hedonic scale for color, odor, flavor and appearance. Increasing soy flour caused significant differences in the physicochemical variables. The 15% treatment contained 3.31% ash, 19.02% fat, 2.41% fiber and 26.71% protein, showing the greatest compositional contribution and the lowest fat content among treatments. However, the highest sensory acceptance was observed for the 10% treatment, with an overall mean score of 4.45/5. This treatment showed 8 × 10^6 CFU/g of mesophilic aerobes, 3 × 10^2 CFU/g of Escherichia coli, and 8 × 10^3 CFU/g of Staphylococcus aureus, with an absence of Salmonella in 25 g values interpreted in the study as compliant with NTE INEN 1338. It is concluded that soy flour significantly modifies the composition of the hamburger and that the 10% level offers the best balance between compositional improvements and acceptability.

Keywords: beef burger; soy flour; partial replacement; proximate analysis; sensory evaluation

INTRODUCTION

Processed meat products constitute a technological matrix susceptible to reformulation through the incorporation of plant ingredients that act as extenders and modify their composition. In the case of minced meat used to make hamburgers, it can be combined with permitted ingredients and seasonings, as long as the product meets the composition and safety requirements established for its category. The incorporation of soy is of interest due to its protein, lipid, carbohydrate and fiber content, in addition to the diversity of technological applications described for this legume and its derivatives (1-3).

Soy (Glycine max) is characterized by having a high protein content in addition to providing lipid fractions and carbohydrates that can influence the properties of the food in which it is incorporated. The use of soy flour as a partial meat substitute has been previously evaluated in different meat products. Odiase et al. (2013) studied the incorporation of whole soy flour at levels of 10, 15, 20 and 25% in meatballs made with beef, showing that intermediate levels of inclusion, particularly 10 and 15%, presented greater sensory acceptance. Similarly, Cho et al. (2017) evaluated the incorporation of 5% defatted soy flour with different particle sizes in hamburgers, analyzing its physicochemical and sensory properties; Their results demonstrated that the use of superfine flour allowed maintaining a general acceptability comparable to the control

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treatment and reducing cooking losses. These antecedents show the technological potential of soy flour in meat products and support the need to continue studying levels of substitution that allow maintaining the acceptance of the product. In this sense, it is pertinent to evaluate the use of non-textured soy flour at intermediate levels of beef substitution through a comprehensive approach that jointly considers the physicochemical composition, sensory acceptance and microbiological quality of the product. In this context, the nutritional characteristics of soy flour justify its evaluation as a partial substitute for meat, particularly due to its contribution of protein and fiber and its potential influence on the final composition of the product. The literature compiled in the research also describes soy as an ingredient used in the production of foods with a high protein content and in various agro-industrial applications (19,20).

The use of protein sources of plant origin in meat products has been studied with different raw materials. García evaluated pigeon pea flour in low-fat hamburgers and reported modifications in the composition and technological properties of the product (4). Solórzano studied the substitution of beef for textured soy vegetable protein in a meat system, finding that a substitution of up to 9% could improve nutritional quality and maintain adequate sensory acceptance (5). In hamburgers, Beltrán analyzed the partial substitution of beef for soy meat and observed that a 40% substitution affects the acceptance of attributes such as flavor, smell and color (6). Soy by-products, such as okara, have also been evaluated in sausages, with favorable results from a nutritional and sensory point of view (7).

Consumer acceptance is a critical component in these types of reformulations. A compositional improvement does not necessarily translate into greater preference, because the incorporation of vegetable proteins can alter color, aroma, flavor, texture or appearance. Studies with preparations based on soy and other plant ingredients show that the sensory response depends on the formulation and the level of incorporation (8). Similarly, Lema and Majin evaluated hamburgers enriched with vegetable proteins and showed that the composition of the product changes according to the percentage of substitution (9). For this reason, the selection of a treatment must simultaneously consider physicochemical outcomes and acceptability.

In addition to the composition and sensory response, microbiological safety is an essential requirement. The NTE INEN 1338 establishes criteria applicable to raw meat products and was used in the original study as a reference to interpret the quality of the product (10). The microbiological evaluation of the sensorially preferred treatment included mesophilic aerobes, Escherichia coli, Staphylococcus aureus and Salmonella (23), with the purpose of verifying that the selected formulation remained within the limits considered in the research.

From this framework, the central problem is to determine what level of substitution with soy flour allows obtaining a hamburger with a favorable physicochemical profile and adequate sensory acceptance. The established experimental hypothesis was that partial substitution with soy flour influences the physicochemical properties of the hamburgers. Consequently, the objective of this article was to synthesize and analyze the effect of three levels of soy flour substitution (5%, 10% and 15%), compared to a

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control treatment without substitution (0%), on humidity, ash, fat, fiber, protein and pH; compare the acceptability of treatments by color, odor, taste and appearance; and present the microbiological condition of the treatment with greater sensory acceptance. This integration allows us to identify both the treatment with the best nutritional composition and the formulation with the greatest sensory acceptance, two criteria that do not necessarily coincide and that are relevant for the development of a reformulated meat product.

MATERIALS AND METHODS

An experimental investigation was developed with four beef burger formulations. The C1S1B treatment corresponded to the control without soy flour; C2S2B added 5%; C3S3B, 10%; and C4S4B, 15%. In all formulations, 4.98% of seasonings remained constant, distributed in 2.37% onion powder, 0.47% parsley, 0.23% pepper and 1.90% salt. The proportion of meat was 95.01%, 90.26%, 85.02% and 80.02%, respectively. Physicochemical analyzes were performed in triplicate.

For each formulation, 200 g hamburgers were made, obtained by homogeneously mixing ground beef, soy flour and seasonings; They were subsequently packed in polypropylene bags and stored at 4 °C until analysis. Physicochemical determinations of humidity, ash, fat, fiber, protein and pH were carried out in triplicate (n = 3) in each treatment. The sensory evaluation was carried out with 20 panelists using a five-point hedonic scale for color, smell, flavor and appearance. The microbiological analysis was applied only to the treatment with greater sensory acceptance.

Table 1. Hamburger formulations with partial substitution of soy flour.

Ingredient	C1S1B Control	C2S2B 5%	C3S3B 10%	C4S4B 15%
Beef (%)	95,01	90,26	85,02	80,02
Soy flour (%)	0,00	5,00	10,00	15,00
Onion powder (%)	2,37	2,37	2,37	2,37
Parsley (%)	0,47	0,47	0,47	0,47
Pepper (%)	0,23	0,23	0,23	0,23
Salt (%)	1,90	1,90	1,90	1,90

Source: synthesis of the reported formulations.

The beef was purchased previously ground and, along with soy flour and seasonings, was subjected to reception and quality control. The ingredients were weighed on a ME204 digital scale and mixed in a food processor until a uniform mass was obtained. Subsequently, 200 g portions were manually formed using a plastic hamburger mold, packed in high-density polypropylene bags, and stored at 4 °C until analysis. The original study did not report the particle size of the meat or specific values of time and temperature during mixing, so these parameters could not be specified

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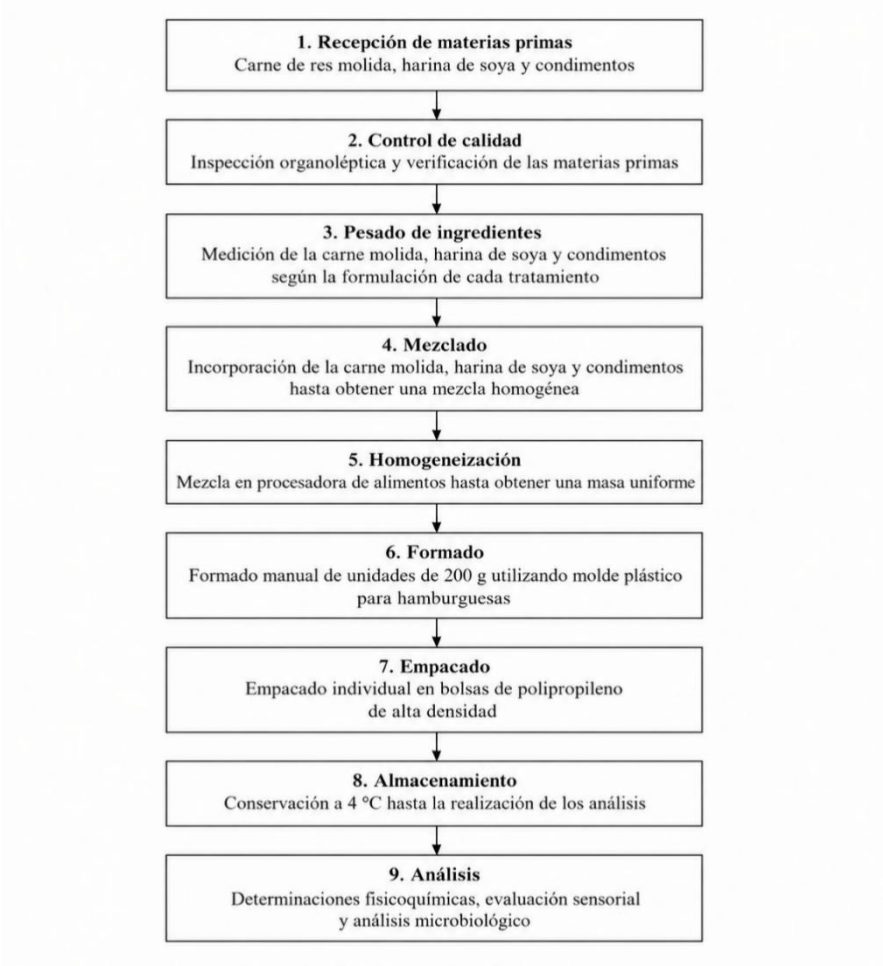


Figure 1. Flow diagram for making hamburgers with soy flour.

For the physicochemical analyzes the following were included: humidity, ash, fat, protein, crude fiber and pH. Humidity was determined by mass loss due to oven heating, using the NTE INEN 0777 method and the analytical procedure described by AOAC (11). The ashes obtained at 550 ± 10 °C until constant weight, according to NTE INEN 786; The fat was determined by extraction with hexane and evaporation of the solvent, following the NTE INEN 778 regulations; The protein was estimated by nitrogen determination, acid digestion, alkalinization, distillation and titration, according to NTE INEN 781; crude fiber instead using the MAL-50/Pearson method; and the pH in accordance with NTE INEN 783. and all of this for the meat product was taken as a reference by NTE INEN 1338 (10), in this way all analyzes are carried out based on current and regularized regulations and procedures.

The sensory evaluation was carried out with a semi-trained panel of 20 Agroindustrial Engineering students, who had previously participated in sensory evaluations. Each treatment was rated on color, smell, taste, and appearance using a five-point hedonic scale: 1, “I really dislike it”; 2, “I dislike it”; 3, “I neither like nor dislike”; 4, “I like it”; and 5, “I like it a lot.” The results were tabulated and represented using a radial graph, and the overall mean of the four attributes was used only as a descriptive measure of acceptance. It was carried out by randomizing the samples with control and balancing

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of the order of presentation. It should be noted that no inferential statistical analysis was applied to the sensory data.

This evaluation was not intended to microbiologically compare the treatments, but rather to verify the microbiological quality of the selected formulation, which is why it was applied only to the treatment with the best sensory evaluation where mesophilic aerobes were analyzed using NTE INEN 1529-5 (13), Escherichia coli using AOAC 991.14 with the use of Petrifilm type plates (14), Staphylococcus aureus using NTE INEN 1529-14 (15) and Salmonella through NTE INEN 1529-15 (16). The work reported the use of Nissui Compact Dry Discs plates. Microsoft Excel 2019 and InfoStat version 6.2 were used for the statistical analysis of the physicochemical variables. The data were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparison test, with a significance level of $\alpha = 0.05$.

Regarding storage, once prepared, the burgers were individually packaged in high-density polypropylene bags and kept refrigerated at 4 °C until analysis. The physicochemical and sensory determinations were carried out within 24 hours after preparation, keeping the samples under refrigeration during this period.

RESULTS

The results showed a consistent response of the composition of the hamburgers to the increase in the percentage of soy flour. In all the physicochemical variables evaluated, significant differences between treatments were reported. For humidity, fiber, fat, protein and pH, the analysis of variance presented values of $p<0.0001$; for ashes $p=0.0002$ was obtained. Consequently, the study rejected the null hypothesis and accepted that partial substitution with soybean flour significantly influences the physicochemical properties of the product.

The values obtained in the proximal analyzes carried out on the ground beef that was used as raw material in the preparation of the hamburger, presented averages of 58.15% humidity, compared to the humidity presented in the soy flour which was 4.38%, an expected result because soy flour is a dehydrated product, which favors its conservation and incorporation in the formulation, with regard to The ash values obtained 2.22% of the mineral fraction in the soy flour, higher than the 0.43% obtained in the meat raw material, indicating that the soybeans contribute a greater amount of minerals to the formulation.

Regarding the protein values, the meat material presented 21.80% and 1.83% fiber while the soy flour presented 27.01% protein and 2.15% fiber, in this way it can contribute with an important protein content and improve the fiber value in the final product, increasing its nutritional value.

Regarding the fat percentage of the ground beef, it was 19.10%, while the soy reached 19.10%, although the value is lower than that of beef, it does constitute a relevant source of fat from a nutritional point of view, since using it as a replacement ingredient will maintain a considerable contribution of fat in the final product. With regard to the pH values, there is no major difference since the pH of the meat matrix was 6.57. and

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for soy flour it was 6.73%, indicating that both ingredients have similar acidity conditions.

Table 2. Physicochemical means reported in Tukey tests by treatment.

Process	Moisture %	Ash %	Fiber %	Fat %	Protein %	pH
C1S1B 0%	64,36	2,53	1,83	27,70	21,80	6,60
C2S2B 5%	60,04	3,09	2,04	23,54	23,41	6,68
C3S3B 10%	56,98	3,22	2,27	19,83	25,27	7,04
C4S4B 15%	55,24	3,31	2,41	19,02	26,71	7,07

ANOVA: moisture, fiber, fat, protein and pH, p<0.0001; ashes, p=0.0002. Means taken from Tukey tables from the original study.

Moisture showed a decrease as the percentage of substitution in the formulations increased, observing a reduction in the value of the parameter between the control sample and the 15% substitution by 9.12 percentage points. This behavior was one of the most notable changes in the product and may be related to the lower water content that soy flour has compared to meat matter.

Regarding the ash content, it was observed that this parameter increases gradually as the percentage of substitution was increased, showing a difference of 0.78 percentage points between the control and 15%. In this case, the Tukey test separated the four levels, showing a gradual and significant increase. The result of the fiber analysis shows that the value increased, because soy flour has a higher fiber content, with 0.58 percentage points being the difference between the control and the 15% formulation.

The fat content values indicated a decrease of 8.68 percentage points between the control treatment and the 15% treatment; this result may be related to the lower fat content of the soy flour compared to the meat mixture. Protein is one of the parameters of greatest interest and relevance since it contributes to the nutritional value of the product and also participates in technological characteristics such as water retention and structure formation during processing, so going from 21.80% in the control sample to 26.71% in the 15% substitution represents approximately a 22.5% relative increase with respect to the initial value.

Considering the physicochemical variables together, C4S4B presented 3.31% ash, 19.02% fat, 2.41% fiber and 26.71% protein. Due to these characteristics, the original study identified it as the best treatment from the point of view of nutritional and compositional contribution. However, the sensory response showed a different pattern. The control obtained average ratings of 4.0 for color, 4.2 for odor, 4.2 for flavor, and 4.0 for appearance, with an overall descriptive mean of 4.10/5. C2S2B achieved 3.6 for color, 4.4 for smell, 4.8 for flavor and 4.2 for appearance, equivalent to 4.25/5. C3S3B obtained 4.2 in color, 4.8 in smell, 4.6 in flavor and 4.2 in appearance, reaching the highest overall average, 4.45/5. C4S4B presented 3.8 in color, 4.0 in smell, 4.0 in flavor and 3.8 in appearance, with an average of 3.90/5.

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Table 3. Descriptive sensory evaluation of the four formulations.

Treat.	Color	Odor	Taste	Appearance	Medium
C1S1B 0%	4,0	4,2	4,2	4,0	4,10
C2S2B 5%	3,6	4,4	4,8	4,2	4,25
C3S3B 10%	4,2	4,8	4,6	4,2	4,45
C4S4B 15%	3,8	4,0	4,0	3,8	3,90

Note. Hedonic scale from 1 to 5. The global average was calculated from the four attributes as a descriptive synthesis.

These data show that the maximum incorporation of soy did not coincide with the maximum sensory acceptance. Although C4S4B was higher in protein and fiber and recorded the lowest fat, its sensory evaluation was lower than that of C2S2B and C3S3B, and even slightly lower than that of the control. The 10% formulation achieved the best combined ratings, especially in smell, with 4.8/5, and flavor, with 4.6/5. In the interpretation of the work, this result made C3S3B the sensory preferred treatment.

The microbiological analysis was carried out exclusively in C3S3B as it is the most widely accepted treatment. 8×10^6 CFU/g of mesophilic aerobes, 3×10^2 CFU/g of Escherichia coli and 8×10^3 CFU/g of Staphylococcus aureus were reported; Salmonella was absent in 25 g. The study compared these values with the intervals used to interpret NTE INEN 1338 and concluded that the treatment was within the permitted limits. Therefore, the sensorially selected formulation also presented a microbiological condition considered acceptable within the framework of the research.

Table 4. Microbiological results of the C3S3B treatment (10% soybean flour).

Analysis	Unit	Minimum reported	Maximum reported	Result
Mesophilic aerobes	CFU/g	1.0×10^6	1.0×10^7	8×10^6
Escherichia coli	CFU/g	1.0×10^2	1.0×10^3	3×10^2
Staphylococcus aureus	CFU/g	1.0×10^3	1.0×10^4	8×10^3
Salmonella	in 25 g	Absence	Absence	Absence

Note: interpretation of results as in accordance with NTE INEN 1338.

The integration of results allows two selection criteria to be differentiated. If the objective focuses on maximizing protein and fiber and reducing fat, the C4S4B treatment, with 15% soy flour, showed the most favorable response. If sensory acceptance is simultaneously considered, C3S3B, with 10%, presented a better balance: its protein was 25.27%, its fiber 2.27% and its fat 19.83%, clearly improved values compared to the control, while its global sensory average was the highest. This difference between compositional performance and sensory preference is the central finding of the study and supports the need to value both components when selecting the level of substitution.

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DISCUSSION

The results obtained provide relevant evidence on the potential of soy flour as a partial substitute for beef in hamburgers, particularly by identifying a favorable trend of acceptance for the formulation with 10%. However, the sensory evaluation was analyzed descriptively, so the observed differences represent preference trends and not a statistically demonstrated superiority. Likewise, the microbiological analysis was carried out only on the most widely accepted formulation with the purpose of verifying its hygienic-sanitary condition. Although this approach does not allow establishing microbiological comparisons between treatments, it allowed us to complement the evaluation of the selected formulation and provide preliminary evidence for subsequent validation studies.

It is clear that the main differences between studies are based on parameters such as matrices, raw materials and substitution percentages; However, the evidence supports that the incorporation of plant ingredients positively modifies the composition of meat products.

The changes in the composition of the hamburgers show the effect of soy flour on the meat matrix. The increase in protein and fiber can be attributed to the composition of this legume and coincides with what was pointed out by Gallegos, Ridner and Riaz about its nutritional contribution and potential as an ingredient in foods (1,2,18). In turn, the decrease in fat and humidity suggests that the partial substitution of meat modified the balance of the components of the formulation, a behavior similar to that described in meat products made with vegetable extenders (4,9). Together, these results support the potential of soy flour as an ingredient for the reformulation of meat products, although its incorporation must be balanced with the sensory characteristics of the product.

Solórzano denotes that an intermediate level of textured vegetable protein provides a favorable sensory response to higher or lower formulations of the percentages treated (5). Taken together, the data indicate the existence of a balance point between two important aspects: the improvement in the composition and the intensity with which the sensory characteristics are seen once the soy is added.

The microbiological result of C3S3B was interpreted in the investigation as in accordance with NTE INEN 1338 (10). The absence of Salmonella and the reported counts for mesophilic aerobes, Escherichia coli and Staphylococcus aureus supported the selection of the treatment from a safety perspective. However, the microbiological evaluation was carried out only in the sensorially preferred treatment, so the data do not allow us to compare the effect of the percentage of soy flour on the microbial load of the four formulations.

It is necessary to consider that the sensory evaluation is descriptive, that is, through the use of averages and radial graphs, the data obtained is shown, where an inferential test of significant acceptance differences is not reported. This limitation was not an obstacle to the observed preference for C3S3B, but it does restrict the scope of the conclusion at the sensory level. Likewise, the study focused on proximal composition, pH, acceptance and microbiology of the selected treatment; Instrumental measurements of texture,

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cooking performance or storage stability of the product were not included. In future evaluations, these variables would allow the technological feasibility of the formulation to be characterized in greater depth.

Taken together, the results show a clear relationship between the level of substitution and the compositional properties of the hamburgers, accompanied by a favorable sensory response at intermediate levels. While a greater incorporation of soy flour favored physicochemical changes, the intermediate treatment showed the best balance between nutritional contribution and sensory acceptance. These findings support its potential as a viable formulation for future validation and development studies.

CONCLUSIONS

The partial replacement of beef with soy flour significantly modified the physicochemical properties of the burgers. By increasing the incorporation from 0 to 15%, moisture decreased from 64.36% to 55.24% and fat from 27.70% to 19.02%, while fiber increased from 1.83% to 2.41% and protein from 21.80% to 26.71%. The C4S4B treatment, with 15% soy flour, presented the highest compositional contribution according to the criteria considered in the research.

The sensory response showed that a greater substitution of meat for soy flour did not necessarily imply greater acceptance. The treatment with 10% substitution presented the best overall rating (4.45/5), with a favorable response in color, smell, flavor and appearance, which allowed it to be identified as the formulation with the best sensory balance. Likewise, this treatment was subjected to microbiological evaluation and its results were interpreted in accordance with the criteria established in NTE INEN 1338, highlighting the absence of Salmonella in 25 g of sample.

Altogether, the results show that the incorporation of soy flour constitutes a viable alternative for the partial replacement of beef in hamburgers. The treatment with 10% presented the best balance between composition and sensory acceptance, while 15% showed the greatest compositional contribution. These findings support the technological potential of soy flour in the reformulation of meat products. Future studies may expand these results through sensory inferential analysis, texture evaluation, cooking performance, storage stability and microbiological characterization of all formulations.

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