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Effect of adding Sesame (*Sesamum Indicum L.*) Protein Isolate on the Bromatological, Microbiological, Sensory, and Textural Properties of a Frankfurt-Type Sausage

EVELYN MILENA FACIOLINCE BAENA[1], KELVIN RODRIGO NIÑO LOPEZ[2], PIEDAD MONTERO CASTILLO[3]*, KATHERINE PATERNINA SIERRA[4] and LUIS ALFONSO BELTRÁN COTTA[5]

[1, 2, 3, 4]Faculty of engineering, Programme: Food Engineering, University of Cartagena, Cartagena, Colombia.
[5]Faculty of Pharmacy, Federal, University of Bahia, Salvador, Brazil.

Abstract

This study aimed to evaluate the effect of adding sesame protein isolate (*Sesamum indicum L.*) on the bromatological, microbiological, sensory, and textural properties of a Frankfurt-type sausage. Sesame protein isolate (SPI) was obtained by isoelectric precipitation. Four percentages of SPI were used in the Frankfurt-type sausage samples: 0% (SS0), 2% (SS2), 4% (SS4) and 6% (SS6). The proximate composition of the sausages and SPI was determined. Microbiological, sensory (preference), and textural (TPA) properties were also studied. The results indicated that the protein content of the SPI was 88.02%. Regarding sensory acceptance, SS4 and SS6 obtained the highest scores in most parameters. On the other hand, SS6 showed higher results in terms of cohesiveness (4.04), elasticity (9.98), and chewiness (47.93). In conclusion, SPI can be used in meat products because it increases the bromatological parameters of sausages and improves the acceptance of some sensory parameters.

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Introduction

The food industry is transforming and reinventing itself daily to adapt to new consumer trends. These trends focus on creating functional products with high nutritional value, promoting health, and protecting natural resources in response to the demands of an ever-growing market. In this sense, it is important to emphasize that the meat industry plays a fundamental role in providing a wide range of products that meet the protein needs of consumers.

CONTACT Piedad Montero Castillo pmonteroc@unicartagena.edu.co Faculty of engineering, Programme: Food Engineering. University of Cartagena, Cartagena, Colombia

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In addition, it has experienced significant growth in recent years in terms of technology and innovation. It should be noted that this industry is expected to consume 333.9 million tonnes worldwide in 2022 alone.[1] However, the acquisition of raw materials by the meat industry is becoming increasingly complex due to the high cost of meat, which is the main element of this type of food. In addition, the consumption of red and processed meat has been linked to public health problems,[2,3] causing changes in the health of consumers, with cardiovascular diseases being one of the most common.[3,4,5]

Given the disadvantages mentioned above associated with the production and consumption of processed meat products, there is a need to reduce the production and purchase costs and improve the health impact of these products.

In this context, it is pertinent to note that plant proteins are considered an important source of essential nutrients due to their high protein content, consumer acceptance, availability of raw materials, and diversity, and their properties are of great interest to the food industry.[6] Thus, the use of plant proteins in sausage foods is presented as a viable option to reduce production costs,[7] improve the nutritional profile of the food, reduce the consumption of animal proteins 3 and minimize the losses (wastage) of water and fat during the production of this type of products.[8] These plant proteins are characterized as nutrients consisting mainly of incomplete essential amino acids and other non-protein substances.[5] The main sources of these proteins are oilseeds, cereals, leaves, pulses, and legumes, among which soybean and wheat gluten stand out for their wide use as extenders and partial or complete protein substitutes in the production of meat products.[7] Within the food processing industry, proteins of plant origin are mainly used in the form of isolated proteins, which are a combination of substances with a high protein content.[9]

Among the plant sources available for the extraction of the isolated protein, sesame stands out for its high production levels in Colombia, in addition to being considered an oilseed with a high nutritional value,[10] and a high protein content (20-25%).[11] Sesame protein has several functional properties such as solubility, water retention, stability, fat absorption, and emulsifying capacity. All these properties are of great technological importance in the production of meat products.[12] Despite the high production of sesame in the department of Bolivar, Colombia (868 tonnes) and the techno-functional advantages of its use, this product has been little studied in the food industry at the national level.[13, 14] For all these reasons, this work aimed to evaluate the effect of adding sesame (*Sesamum indicum L.*) protein isolates on the bromatological, microbiological, sensory, and textural properties of a Frankfurt sausage.

Materials and Methods

Sesame Protein Extraction

Sesame protein was isolated following the method described by Sharma,[15] with some modifications. Protein isolate was obtained from defatted sesame flour (0.5% fat). The sesame flour was mixed with deionized water (1:10 w/v ratio). After adjusting the mixture's pH to 11 with 1.0 M NaOH, it was stirred for 1 hour using a magnetic stirrer. Centrifugation of the mixture was carried out at 5000 rpm for 15 minutes at 20 °C. The pH of the soluble phase was then adjusted to 4.5 with 1.0 M HCl (to cause isoelectric precipitation of the protein). The suspension was again centrifuged at 5000 rpm for 15 min at 20 °C. The precipitate obtained was pH-adjusted to 7.0 using 1.0 M NaOH and dialyzed with deionized water at 4.0 °C for 12 hours. Finally, the isolated protein was lyophilized to obtain a dry flour, which was passed through an 80-mesh sieve.

Frankfurt Sausage Processing

To prepare the sausages, the formulation described in Table 1 was used, following the methodology described by Alirezalu,[16] with modifications. The beef, together with the salts and additives, was passed through the cutter for about 5 minutes to obtain a fine paste. SPI (0%, 2%, 4%, or 6%) was then added together with the other ingredients. The paste obtained was stuffed into synthetic casings of 20 mm diameter and approximately 1000 g of Frankfurt-type sausages were obtained. The sausages were ultimately cooked to an internal temperature of 75 °C and then exposed to a heat shock.

Table 1. Formulation of Frankfurt sausage with added SPI

Raw materials	SS0 (%)	SS2 (%)	SS4 (%)	SS6 (%)
Beef	35	33	31	29
Pork	25	25	25	25
Back fat	14	14	14	14
SPI	0	2	4	6
Water (ice)	20	20	20	20
Starch	6	6	6	6
Total	**100**	**100**	**100**	**100**
Salt	1.8	1.8	1.8	1.8
Phosphate	0.05	0.05	0.05	0.05
Eritorbate	0.005	0.005	0.005	0.005
Sausage flavor	1.4	1.4	1.4	1.4
Nitro salt	0.02	0.02	0.02	0.02

Proximal Composition

The percentages of moisture (952.08), protein (995.04), fat (948.15), ash (942.05), and fiber (962.09) of the SPI and the four formulations were determined according to AOAC.[17] The carbohydrate content was determined by difference.

Determination of Microbiological Characteristics

The following analyses were carried out to determine the microbiological characteristics of the sausages: mesophilic aerobes,[18] total coliforms,[19] Listeria monocytogenes,[20] Escherichia coli,[19] coagulase-positive staphylococci,[21] Salmonella spp[22] and Clostridium sulfite-reducing spores.[20]

Sensory Analysis

The panel assessed the acceptability of the four sausage samples on a 5-point hedonic scale, with descriptors ranging from 1 ("I dislike very much") to 5 ("I like very much"). Each sample was randomly labeled with a three-digit number before presentation. The evaluation was carried out in a ventilated area with good lighting and no extraneous odors. The untrained evaluation panel was composed of 50 people of both sexes, aged between 20 and 60 years, who were provided with an evaluation form.[23] The parameters evaluated were: colour, odor, taste, texture, and general acceptability.

Texture Profile Analysis (TPA)

The texture of four sausages was evaluated with a TA.XT2i texture analyzer (Stable Micro Systems Ltd, Surrey, England). For TPA, samples were cut into 2 cm cylinders and left for 1 h at room temperature in a polyethylene bag. The TPA parameters analyzed were hardness (N), cohesiveness, elasticity, chewability, and resilience. The texturometer conditions were a compression distance of 15.0 mm and a waiting time between compressions of 5 s using an SMSP-75 probe at a test speed of 2 mm/s and a post-test speed of 5 mm/s with a load cell of 50 kgf.

Statistical Analysis

Each experiment was performed in duplicate and analyses were performed at least in triplicate. An analysis of variance (ANOVA) was performed with the SPSS 25.0 program (SPSS Inc., Chicago, IL, USA) to analyze the effect of SPI addition at different percentages on the proximal composition, texture profile, microbiological properties, and sensory properties of a Frankfurt-type sausage. A Tukey test was applied to compare means, considering differences significant when $p < 0.05$.

Results and discussion

Bromatological Analysis of Sesame Protein Isolate and Frankfurt-Type Sausage

The results showed that SPI (Table 2) had a protein content of 88.02%. This percentage was obtained by alkaline extraction with a protein isoelectric point at pH 4.5, which technically classifies it as an isolated protein, as it exceeds the 65% required for this designation. Similar results were presented by Sharma,[15] Fathi,[24] and Achouri,[25] in obtaining SPI by alkaline extraction method, with percentages of 86.33%, 90.5%, and 94.6%, respectively. The differences may be related to the sesame variety used and to the different pH (4.5) used in the extraction process.

Table 2. Bromatological analysis of the SPI isolate and Frankfurt-type sausages with SPI added

	Moisture (%)	Protein (%)	Fat (%)	CHO (%)	Ashes (%)	Fiber (%)
SPI	6.87 ± 0.03	88.02 ± 0.01	0.40 ± 0.05	2.47 ± 0.03	2.23 ± 0.01	0.8 ± 0.01
SS0	66.05 ± 0.02[a]	13.74 ± 0.13[c]	15.09 ± 0.02[c]	3.08 ± 0.008[c]	2.72 ± 0.02[d]	0.005 ± 0.001[b]
SS2	63.05 ± 0.05[b]	14.98 ± 0.03[b]	16.42 ± 0.015[b]	3.10 ± 0.001[c]	2.83 ± 0.03[c]	0.018 ± 0.007[a]
SS4	60.00 ± 0.01[c]	16.84 ± 0.07[a]	16.77 ± 0.15[a]	3.38 ± 0.017[b]	2.98 ± 0.01[b]	0.019 ± 0.005[a]
SS6	59.41 ± 0.40[d]	16.86 ± 0.04[a]	16.97 ± 0.015[a]	3.66 ± 0.024[a]	3.06 ± 0.02[a]	0.02 ± 0.006[a]

Values are presented as mean ± standard deviation; distinct letters within the same column denote statistically significant differences ($p < 0.05$). SPI: sesame protein isolate; CHO: carbohydrate.

Regarding the proximal composition of the sausages, for the moisture parameter, all samples showed significant differences ($p < 0.05$).The moisture percentages ranged from 66.05% to 59.41%, with SS0 having the highest value of all. As shown in Table 2, there was an inverse relationship with the addition of SPI, where the samples that had greater addition of SPI presented lower moisture, which is related to the fact that the amount of substituted beef has a higher percentage of moisture (up to 75%) compared to SPI.[26] Similar results to the above were presented by Callejas,[27] who prepared sausages with soy protein isolate, obtaining values between 59.41 and 62.25% with percentages of protein isolate between 25 and 75%. The main difference between the results obtained could be attributed to the variation in the amount of protein isolate and/or extenders used in these studies (up to 75% protein isolate, with moisture percentages below 10%).

It is essential to mention that the percentages obtained for protein content comply with the minimum requirements established by NTC,[28] which states that this type of product must have a minimum of 10% protein. The protein percentages of the Frankfurt-type sausages ranged from 13.74% to 16.86%. Samples SS6 (16.86%) and SS4 (16.84%) had the highest protein values compared to samples SS2 and SS0 ($p < 0.05$). The results suggest that the SPI incorporated into the samples SS2, SS4, and SS6 directly influenced the final protein content of the treatments. The results obtained here are similar to those obtained by García,[29] who obtained a protein content of 16.77% in the sample with a concentration of 3.70% amaranth protein isolate, and a protein content of 16.44% in a Frankfurt sausage.

According to NTC,[28] the fat content of sausages must not exceed 90%. In this respect, the results of the treatments show that they were within the limits set by the standard. It was observed that the fat concentration in the samples increased as the added SPI content increased, with the treatments SS6 (16.97%) and SS4 (16.77%) showing the highest percentages. Similar behavior was observed for Mazón,[29] in mortadellas with added choco protein. When SPI was incorporated into the sausage samples, it could act as an emulsifier, stabilizing the water-fat emulsion. Proteins can trap fat droplets, leading to the formation of a three-dimensional network that allows lipids to be retained in the product.[30,31] This phenomenon of fat loss can occur during the cooking process.

Regarding the fat parameter, significant differences ($p<0.05$) were found between SS4 and SS6 concerning SS0 (15.09%) and SS2 (16.42%). The results obtained are similar to those obtained by Thirumdas,[33] who prepared Spanish sausages using six different protein sources (soya, beans, lentils, broad beans, and chlorella and spirulina), enriched with 3% protein according to the protein source, obtaining values between 13.81 and 15.09% fat content. On the other hand, García,[29] reported a fat content percentage of 22.47% in a Frankfurt sausage with amaranth protein isolate. However, the samples with the isolated protein did not differ from SS0 ($p>0.05$).

In terms of carbohydrate content, sample SS6 had the highest percentage with 3.66%. The samples showed statistically significant differences ($p < 0.05$), except between SS0 and SS2. The treatments with a higher content of SPI in their formulation (SS4 and SS6) had a higher percentage of carbohydrates. These findings are consistent with those reported by Thirumdas,[33] for fermented Spanish "chorizo" sausages with lentil protein isolate (4.72 ± 0.94%), which could be due to the type of product produced.

Statistically significant differences were evident in ash content ($p<0.05$). The samples with the highest SPI content were those with the highest percentage of ash. SS6 was the sample with the highest percentage (3.06%), followed by SS4 (2.98%), SS2 (2.83%) and SS0 (2.72%). According to Umaña,[31] high ash content is directly related to high nutrient content. These findings might be associated with sesame's high mineral content, including calcium (973 mg), phosphorus (629 mg), potassium (468 mg), magnesium (351 mg), iron (14.55 mg), and zinc (7.75 mg) (USDA, 2016).

The percentage of fiber in the four Frankfurt-type sausage formulations was also determined. No significant differences ($p > 0.05$) were found among samples SS2, SS4, and SS6. However, samples containing SPI showed statistical differences ($p < 0.05$) when compared to SS0. There was also a direct increase in fiber content in the formulations as a function of the percentage of protein added.

Microbiological Analysis

The microbiological results of the sausages are presented in Table 3. All samples were within the limits allowed by NTC.[28] The mesophilic aerobic count, total coliforms, Escherichia coli, coagulase-positive Staphylococcus aureus, and Clostridium sulfite-reducing spores were below the limits established by Colombian regulations. An upward trend in the number of mesophilic aerobic bacteria was noted as the SPI content in the formulations increased. These findings are in line with those reported by Das,[35] and Das,[36] who reported an increase in microbiological results with the addition of soy protein isolate (0-15%) in the formulation of nuggets and patties. This behavior is associated with an increase in moisture, pH, and nutrients suitable for microbial growth, which could have been provided by SPI. Pathogenic bacteria (*Listeria monocytogenes and Salmonella spp*) were not present in any of the samples. These results suggest the implementation of quality standards during product processing and the use of quality raw materials.[37]

Table 3. Microbiological results of Frankfurt sausages with the addition of SPI

Microbiological analysis	NTC 1325	Samples			
		SS0	SS2	SS4	SS6
Mesophilic aerobic count (CFU/g)	100.000	<60	<40	<90	<200
Total coliform count (CFU/g)	≤500	<10	<10	<10	<10
Escherichia coli count (CFU/g)	<10	<10	<10	<10	<10
Coagulase-positive *Staphylococcus aureus* count (CFU/g)	<100	<100	<100	<100	<100
Clostridium sulfite-reducing spore count (CFU/g)	≤100	<10	<10	<10	<10
Detection of *Listeria monocytogenes* (/25 g)	Absence	Absence	Absence	Absence	Absence
Detection of *Salmonella spp* (/25 g)	Absence	Absence	Absence	Absence	Absence

Sensory Analysis

The sensory results of the four Frankfurt sausage samples with SPI are shown in Table 4. The evaluators perceived a decrease in the color score as the concentration of SPI in the formulations increased, resulting in slightly darker sausages. It should be noted that the SPI obtained presented a dark brown color. The results obtained for the color parameter showed that SS0 (3.86) received the highest score from the sensory panel, close to "like".

This sample also showed a significant difference ($p<0.05$) from the samples with SPI. On the other hand, SS2, SS4, and SS6 did not show significant differences ($p>0.05$). Similar results were obtained by Ospina,[38] who added hydrolyzed potato protein to sausages and found that the samples with higher protein content had a darker color than the control sample.

Table 4. Results of sensory analysis of Frankfurt-type sausages

Samples	Color	Odor	Taste	Texture	General acceptability
SS0	3.86 ± 1.14^a	3.72 ± 0.90^a	3.68 ± 1.05^b	3.4 ± 0.79^b	3.8 ± 0.92^a
SS2	3.6 ± 1.16^b	3.84 ± 1.03^a	3.66 ± 0.98^b	3.93 ± 0.69^a	3.86 ± 0.67^a
SS4	3.36 ± 1.04^b	3.90 ± 1.09^a	4.26 ± 0.89^a	4.01 ± 0.66^a	3.98 ± 0.69^a
SS6	3.1 ± 1.24^b	3.8 ± 1.19^a	3.98 ± 1.05^a	3.75 ± 0.62^a	3.76 ± 0.89^a

Values are presented as mean ± standard deviation; distinct letters within the same column denote statistically significant differences ($p < 0.05$).

Regarding the odor attribute, there were no statistically significant differences ($p > 0.05$) among the treatments. However, it was found that as the percentage of SPI increased, a higher score was given to the odor the odor was rated higher. All samples were close to the "like" rating. It should be emphasized that samples with SPI contain volatile substances that contribute pleasant aromas and could be bet to the sausages.[27]

For the taste attribute, SS4 (4.26) and SS6 (3.98) were the samples that received the highest rating, showing significant differences ($p<0.05$) concerning SS0 and SS2. Samples SS4 and SS6 were in the "I like it very much" range. There was a greater preference for the treatments with a higher concentration of SPI, as this adds a characteristic sesame flavor. This is in line with what was reported by Sharma,[15] and Franco,[39] who indicated that the addition of sesame protein isolate adds a special characteristic flavor to the food matrix used.

For texture, samples with SPI showed significant differences ($p < 0.05$) compared to SS0. Treatments SS2 and SS4 were classified in the "I like it very much" category. The lower moisture content in the SPI samples could be associated with these scores and therefore they were better rated in this attribute. In addition, the use of isolated proteins can modify the consistency of meat products by forming firmer structures that can modify the rheological properties of meat emulsions.[40]

In evaluating overall acceptability, the samples showed no significant differences ($p > 0.05$). However, SS4 was the treatment with the highest acceptability rating of "I like it very much".

Texture Profile Analysis

The texture is a highly relevant parameter as food quality and consumer acceptance are related to it.[41,42] The TPA parameters—hardness, cohesiveness, elasticity, resilience, and chewiness—of the various Frankfurt sausage treatments are displayed in Table 5. For hardness, all treatments exhibited significant differences ($p < 0.05$), with SS6 recording the highest value. For the chewiness parameter, the results showed an increase with increasing SPI concentration, with statistical differences ($p<0.05$) between SS0 and SS4 concerning SS6.

Studies have found that incorporating protein isolates into meat products can enhance toughness and, as a result, increase chewiness, attributed to the water-holding properties of vegetable protein.[43] Similar results were presented by Akesowan,[44] who observed an increase in the chewiness parameter in different sausage formulations with soy protein isolate incorporation (0 and 2%). The increase in toughness and chewiness of sausages with SPI may be related to gel formation, which enhances protein-water interactions and protein-protein cross-linking effects.[45,46] Similarly, Canti,[47] reported that water-fat gel formation was induced by the gelling ability of plant protein by replacing animal protein with a combination of soy protein isolate and bean protein (0 - 1.72%) in sausage formulation.

Regarding the cohesiveness parameter, significant differences ($p < 0.05$) were observed between SS0 and SS4 in the results concerning SS6, the latter being the one with the highest value among the 3 treatments. It was observed that the addition of SPI led to an increase in this parameter. This could be due to the role of SPI as a binder, which makes the sausage components hold together better. Similar results were obtained by Kim,[48] when soy protein (0, 10, 20, 30, and 40%) was added to sausages, where cohesiveness increased due to the film-forming ability of soy protein.

Table 5. Texture profile of Frankfurt-type sausages with added SPI

Samples	Hardness (N)	Chewiness (N)	Cohesiveness	Elasticity	Resilience
SS0	53.84 ± 0.80^{c}	28.02 ± 0.81^{b}	3.42 ± 0.01^{b}	1.2 ± 0.01^{a}	6.82 ± 0.12^{b}
SS4	82.8 ± 0.75^{a}	20.91 ± 3.32^{b}	2.94 ± 0.25^{b}	1.19 ± 0.02^{a}	7.04 ± 1.01^{b}
SS6	77.37 ± 0.01^{b}	47.93 ± 7.01^{a}	4.04 ± 0.36^{a}	1.18 ± 0.01^{a}	9.98 ± 1.00^{a}

Values are presented as mean ± standard deviation; distinct letters within the same column denote statistically significant differences ($p < 0.05$).

In the elasticity parameter of the different treatments, there was a decrease as the SPI concentration increased. However, significant differences ($p > 0.05$) were not detected between the formulations. Revilla,[49] obtained similar results when replacing animal protein with texturized pea protein (0, 25, 50, 50, 75 and 100%). This decline in elasticity resulted from the vegetable protein's ability to retain water and fat, which could fill the interstitial spaces in the protein matrix of the feed.

For the resilience parameter of the different treatments, significant differences ($p<0.05$) were obtained between SS0 and SS4 concerning SS6, where this value increased with a higher concentration of SPI. The increase in the resistance of the different treatments may be due to the water-fat gelling capacity of the vegetable protein added to the diet and the capacity of the incorporated protein to form a more solid network structure as a result of cooking, resulting in greater water retention,[47] A similar behavior was shown by Toledo, 50, who indicated that the increase in resilience was due to higher energy absorption during and after compression of the texture analysis due to the starch-fat interaction in a meat smoothie with starch incorporation at different concentrations (5, 7.4 and 10%).

Conclusion

The isoelectric precipitation technique used to extract the SPI was suitable as the isolate had high protein content and low moisture, fat, carbohydrate, ash, and fiber content, in line with previous research. The addition of SPI to the sausages improved the protein and nutritional value. Although the addition of SPI resulted in a slight increase in mesophilic aerobic counts in all samples. However, the microbiological results remained within the Colombian standard. The presence of SPI was found to have a positive effect on consumer acceptance. Although SS6 and SS4 did not show significant differences, the latter showed the highest values for odor, taste, texture, and general acceptability. The color parameter of the different treatments decreased with the addition of the isolate, with SS0 having the highest acceptance. Similarly, the addition of SPI influenced the variations in the textural parameters of SS4 and SS6, increasing hardness, elasticity, and chewiness. Considering the results obtained, the addition of 4% SPI (SS4) showed the most beneficial effect on the meat product. It is proposed to investigate the incorporation of other vegetable protein isolates into sausage meat products to improve their properties and add value.

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Conflict of Interest

There is no conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author Contributions

- **Evelyn Milena Faciolince Baena:** conducting experiments, collecting and analyzing results.
- **Kelvin Rodrigo Niño Lopez:** conducting experiments, collecting and analyzing results.
- **Piedad Montero Castillo:** general supervision of the project and the research team, methodological design and data analysis.
- **Katherine Paternina Sierra:** Interpretation and processing of the data obtained, data analysis.
- **Luis Alfonso Beltrán Cotta:** critical revision of the manuscript, contributing to the improvement

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