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Effect of lyophilized porcine plasma and Zaragoza bean (*Phaseolus vulgaris*) flour on the technological and sensory quality parameters of frankfurter-type sausages

Efecto del plasma porcino liofilizado y la harina de frijol Zaragoza (*Phaseolus vulgaris*) sobre los parámetros de calidad tecnológica y sensorial de las salchichas tipo Frankfurt

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Keywords: Acceptability, functional properties, meat products, protein, texture profile.

Resumen: Las tendencias en alimentos saludables han influido en el sector cárnico en general, por lo que los productores están respondiendo a estas necesidades mediante el uso de ingredientes funcionales para complementar los beneficios de la proteína animal. En este sentido, el objetivo de este estudio fue evaluar el efecto de la incorporación de plasma porcino liofilizado y harina de frijol Zaragoza (Phaseolus vulgaris) sobre la calidad de salchichas tipo Frankfurt en diferentes concentraciones. Se utilizó un diseño factorial 2x2, donde las variables independientes fueron el contenido de plasma liofilizado y la cantidad de harina de frijol Zaragoza. Las variables dependientes fueron aceptabilidad, composición proximal, textura, pérdidas por cocción y capacidad de retención de agua. T4 (1,5 % de plasma y 5 % de harina) obtuvo la mayor aceptabilidad por su composición proximal (20,41 % de proteína, humedad 62,42 %, grasa 10,12 % y cenizas 4,76 %). A su vez, este tratamiento obtuvo mejores características texturales que el testigo, menores pérdidas de cocción (2,02 %) y mayor rendimiento (98,18 %) y capacidad de retención de agua (8,8 %). Lo anterior se atribuyó posiblemente a las propiedades funcionales del plasma y la harina. Los resultados de este estudio demuestran que es posible la incorporación de plasma porcino y harina de frijol Zaragoza en productos cárnicos como las salchichas tipo Frankfurt sin tener un efecto negativo en la aceptabilidad. Asimismo, se confirmó el impacto positivo en la aceptabilidad, composición proximal, textura, pérdidas por cocción y capacidad de retención de agua de estos productos cárnicos.

²³ Palabras clave: aceptabilidad, propiedades funcionales, productos cárnicos, proteína, perfil de textura.

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Introduction

In Colombia, the consumption of protein-rich foods, especially those of an animal origin, has been limited by high costs (Torres González et al., 2016). The World Health Organization (OMS, 2014) has estimated that approximately 160 million children suffer from moderate or severe malnutrition worldwide. According to the National Survey of the Nutritional Situation (ICBF, 2010), problems related to food consumption were identified, with more than a third of the population having a low protein intake.

Given the increase in the world population, the low availability of proteins needed for consumption has made it necessary to search for alternative sources that contribute to producing high protein foods. The reassessment of blood plasma, traditionally considered waste in the meat industry, has been promoted in recent years (Julio et al., 2015; Montero et al., 2015) because it can be incorporated into food as an essential source of protein-(Álvarez et al., 2018; Kim et al., 2017; Parés et al., 2014; Stader et al., 2019).

On the other hand, lyophilized porcine plasma, with approximately 70 % protein (Stader et al., 2019), can be used as a functional ingredient in the food industry due to its solubility, gelation, emulsification properties, and high water retention capacity (WRC). It is an ingredient that can improve the yield and stability of the final product, mainly used in the meat industry (Benítez et al., 2002; Jin et al., 2020; Julio et al., 2015; Polo et al., 2014).

Other ingredients increase the nutritional and functional characteristics of sausages, such as legumes, because of their high protein content and starch content, along with Zaragoza, *Phaseolus vulgaris* L. (Fabaceae), a grain legume that has a substantial impact on popular food culture from countries such as Mexico, Peru, Argentina, and Colombia (Granito et al., 2009; Sangronis et al., 2004).

However, the nutritional contribution of bean flour combined with the innovation of new markets has resulted in increased use as an additive or raw material in food compounds because of its nutritional and functional characteristics as a thickening agent and stabilizer (Drago et al., 2017; Marrugo et al., 2018).

Trends in healthy foods have influenced the meat sector in general, so producers are catering to these needs using functional ingredients to complement the benefits of animal protein. Therefore, this research aims to evaluate the effect of adding lyophilized porcine plasma and Zaragoza bean flour on Frankfurt sausage's technological and sensory quality parameters to find alternative substitutes for extenders and incorporate a vital source of functional characteristics into the range of meat products.

Materials and methods

Raw materials

We used beans (*P. vulgaris*) to obtain the flour. The lyophilized porcine plasma was obtained from the company TECNAS SA, and the raw meat material and other inputs were from distributors of chemical inputs and supermarkets in Cartagena de Indias (Colombia).

Experimental design

A 2x2 factorial design was used, whose independent variables were the lyophilized plasma content and the amount of bean flour, and dependent variables were acceptability, bromatological characteristics, texture, cooking losses, and WRC. The entire experiment was done in triplicate.

Obtaining the Zaragoza bean flour

The bean flour was made following the methodology established by Torres-González et al. (2016). The Zaragoza beans were selected considering health (whole and without weevil bites) and dried in an electric oven (HE 2652, Challenger, Colombia) at a temperature of 60 °C for 12 h. Then, they were ground in a traditional mill and passed through a No. 40 mesh screen to obtain a fine flour.

Formulating and preparing the sausages

Four treatments were formulated with the incorporation of lyophilized porcine plasma and Zaragoza bean flour (table 1).

	T1	T2	T3	T4	CT
Ingredients and additives	(% p/p)				
Beef	30	30	30	30	30
Pork meat	30	30	30	30	30
Dorsal pig fat	14	14	14	14	14
Nitrated salt	0.3	0.3	0.3	0.3	0.3
Phosphates	2.0	2.0	2.0	2.0	2.0
Sodium chloride	1.0	1.0	1.0	1.0	1.0
Seasoning for sausages	0.6	0.6	0.6	0.6	0.6
Ice	22	22	22	22	22
Total	100	100	100	100	100
Lyophilized Plasma	1.0	1.5	1.0	1.5	0
Zaragoza bean flour	3.0	3.0	5.0	5.0	0
Wheat flour	0	0	0	0	4

Table 1. Formulation of sausages

Note. *Percentage calculated on the total weight of the pulp Source: Elaborated by the authors

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Montero et al.'s (2015) methodology was followed to prepare the sausage. We weighed the ingredients according to the percentages indicated in Table 1. The beef and pork were ground to a paste with half of the ice and plasma in a food processor (5 mm thick disc grinder), subjecting the mixture to a curing process (common salt and nitrated salt) for 30 minutes at a refrigeration temperature of 4 °C. This paste was added to the cutter (Mainca, model CM14) with the fat, the other half of the ice, the Zaragoza bean flour, and the rest of the ingredients from Table 1 to obtain a suitable fine paste emulsion, avoiding breakage.

The paste was embedded in resistant synthetic covers and blanched in water up to an internal temperature of 75 °C for 15 min. Subsequently, the thermal shock was carried out in ice water at a temperature of 4 °C until reaching a temperature of 26 ± 1 °C at its midpoint. The samples were stored in refrigeration at 4 °C until the corresponding analyses. Four treatments were formulated with the addition of Zaragoza bean flour and lyophilized porcine plasma; the ingredients used are shown in Table 1. The percentages of lyophilized porcine plasma and Zaragoza bean flour were established not to alter the sensory properties of the samples.

Evaluating the acceptability of the sausages

The sensory evaluation of the different treatments was carried out with 50 untrained tasters, aged between 19 and 48, female and male, using acceptance tests. All were regular consumers of sausages. Samples (4-mm-thick slices) were served at room temperature (23-25 °C) in white plastic dishes, providing water for mouth rinsing. Samples and their containers were identified with random three-digit codes. To avoid distortions in the first position and carryover effects, the order of presentation followed a Williams design for five samples, and they were presented monadically (Colomer Sellas et al., 2021).

The five-point hedonic scale was used for each evaluated parameter (odor, color, flavor, and general acceptability) in every treatment: one (1) was dislike very much, two (2) was dislike slightly, three (3) was neither like nor dislike, four (4) was like slightly, and five (5) was like very much. A score equal to or greater than four was established as a pattern of acceptability, similar to Julio et al. (2015) and Torres-González et al. (2016), evaluating the treatment with the most sensory acceptability for bromatological and textural characteristics and WRC.

Proximal composition

This analysis was carried out only on the treatment with the best acceptability among the tasters, comparing it with a control sample. The moisture (AOAC 925.10), ash (AOAC 923.03), protein (AOAC 976.05), and fat (AOAC 920.85) content were measured following the AOAC methodology (2012).

Texture profile analysis (TPA)

Following the methodology proposed by Montero et al. (2015), approximately 1.5 cm-thick slices of sausage were cut and allowed to stand for one hour at room temperature inside a polyethylene bag to avoid moisture loss. Uniaxial double compression was performed at 75 % deformation (normal stress) and head speed of 1 mm/s with a waiting time of five seconds between compressions. A Texturometer brand TA.TX2i[®] Plus Stable Micro System was used with the Texture Expert Exceed program version 2.64. From the data obtained by the software, we obtained the following textural parameters: hardness (kg/g or N), adhesiveness (N), chewiness (kg), and cohesiveness (dimensionless).

Determining cooking losses

The methodology established by Pacheco Pérez et al. (2011) was followed by calculating the weight difference of the samples before and after cooking(Equation (1)):

$$\% MC = \frac{(m_1 - m_2)}{m_1} \times 100 \tag{1}$$

Where $m_1 = mass$ of the sample before cooking (g), $m_2 = mass$ of the sample at the end of cooking (g), % MC = losses from cooking.

Then the yield was determined with Equation (2):

$$\% R = 100 \% - \% MC$$
 (2)

Determining the Water Retention Capacity (WRC)

The WRC was quantified by placing 1 g of sample in a centrifuge tube and adding an excess of water (10 ml). The mixture was stirred for five minutes. The tubes were centrifuged at 3200 rpm after being maintained at 24 °C for 30 minutes. We used Equation (3) to measure the volume of water retained (Torres-González et al., 2016).

Statistical analysis

Data were presented as the mean ± standard deviation. The different regressions were calculated with the statistical program STATGRAPHICS Centurion XVI.I® in Windows 10. We performed Pearson's correlation coefficient analysis between the factors (% lyophilized plasma and % Zaragoza bean flour) and the sensory

parameters (color, odor, flavor, and general acceptability) with the XLSTAT statistical software version 2021.4.1.1212. The data were subjected to a two-way factorial analysis of variance (ANOVA) at a significance level of 95 % (p < 0.05). We compared treatment differences using the least significant difference (LSD) test, indicating significant differences with a 5 % probability.

Results and discussion

Evaluating the acceptability of the sausages

The sausage acceptability results with the incorporation of lyophilized porcine plasma and Zaragoza bean flour in terms of odor, color, flavor, and general acceptability are shown in Table 2, whose values represent the panelists' average ratings.

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	Treatments	Color	Odor	Flavor	General acceptability	
	T1	$3.5 \pm 0.2^{\circ}$	3.3 ± 0.2^{b}	$3.7 \pm 0.1^{\circ}$	$3.5 \pm 0.2^{\circ}$	
	T2	$3.7 \pm 0.3^{\circ}$	3.6 ± 0.2^{b}	$3.3 \pm 0.1^{\circ}$	$3.7 \pm 0.1^{\circ}$	
	T3	4.5 ± 0.3^{b}	4.3 ± 0.3^{a}	4.2 ± 0.3^{b}	4.2 ± 0.1^{b}	
	T4	4.7 ± 0.1^{a}	4.7 ± 0.1^{a}	4.8 ± 0.1^{a}	4.8 ± 0.1^{a}	

Table 2. Acceptability of frankfurter sausages

Note. Five-point hedonic scale where one (1) was dislike very much and five (5) like very much. Different letters in the same column indicate statistically significant differences (p < 0.05).

Source: Elaborated by the authors

The T3 and T4 treatments had significant differences (p < 0.05) for the parameters color and odor from the rest of the treatments; this could have been due to the same flour content in both treatments (5%). However, T4 had higher values than the other treatments for taste and general acceptance, with statistically significant differences (p < 0.05). These results coincide with those of Benítez et al. (2008), who formulated a biscuit based on cassava flour and bovine plasma, finding more exceptional positive results for taste.

The incorporation of lyophilized porcine plasma and Zaragoza bean flour did not negatively affect the perception of the panelists for color, odor, or flavor in the different treatments since both T1 and T2, despite obtaining a score lower than 4, scored a perception of not liking or disliking. It allowed us to state that the addition of Zaragoza bean flour and plasma did not negatively affect the organoleptic quality of this type of meat product. These results coincided with Isaza et al. (2010), who incorporated 10 and 20 % of blood plasma in a sausage-type meat product and reported good acceptance by the panelists.

In similar studies, Benítez et al. (2008) reported sensory acceptability higher than 91, 86, and 79 % for color, flavor, and texture, respectively, in a cookie formulated with cassava flour and bovine blood plasma. Likewise, Torres-González et al. (2016) obtained an excellent general acceptance of sausages made with a 5 % incorporation of Lens culinaris flour. In previous studies on the effect of commercial (T2) and own (T3) lyophilized porcine plasma (control, 10 g/kg; T2, 10 g/kg; T3) on the physicochemical properties of emulsion pork sausages, Kim et al., (2017) did not detect an apparent animal odor but did find a difference in the taste parameter compared to the other samples.

Also, Marrugo-Ligardo et al. (2017), in their study on the functional properties of white variety bean starch (*P. lunatus*) in a sausage, showed that all groups of sausages received an average score higher than 3.69 in the sensory evaluation. It should be noted that most of the studies on the general acceptance of sausages with the addition of porcine plasma like the one presented in this work indicate the feasibility of using blood by-products in meat formulations, such as mortadella, liver pate (Hamzeh et al., 2016), veal sausages (Martinez & Machado, 2016), and chicken sausages (Oro et al., 2018).

According to the values obtained by Pearson's correlation coefficient, we found that Zaragoza bean flour had a high influence (strong correlation) on all sensory parameters (color, odor, flavor, and general acceptability) since the values of Pearson's correlation coefficient were 0.915, 0.905, 0.864 and 0.876, respectively. As for the % lyophilized plasma, a weak correlation was found with the sensory parameters evaluated. The coefficients were between 0.086 and 0.389.

Proximal composition

The best treatment (T4) is obtained with the incorporation of 1.5 % lyophilized porcine plasma and 5 % Zaragoza bean flour, as compared with a control sausage (CT) (Table 3).

Parameter	Moisture (%)	Fat (%)	Protein (%)	Ashes (%)
СТ	60.65 ± 0.02^{b}	13.01 ± 0.07^{a}	19.03 ± 0.06^{b}	4.44 ± 0.02^{b}
T4	61.52 ± 0.03^{a}	10.03 ± 0.04^{b}	20.48 ± 0.08^{a}	4.75 ± 0.01^{a}

Table 3. Proximal	composition of the T4 sausage	e and a control CT
	composition of the 11 budbug	c una a control C1

Note. Different letters in the same column indicate statistically significant differences (p < 0.05).

Source: Elaborated by the authors

T4 had better proximal composition than CT, with statistically significant differences in all the evaluated parameters (p < 0.05) and a higher content of protein (20.48 %), ashes (4.75 %), and moisture (61.52 %), but a lower fat content (10.03 %) than the

control sample. These results coincide with those of Zapata and Pava (2018), who, in their study on sausages made with tilapia fillet and quinoa flour, reported a higher content of protein (18.13 %), moisture (61.97 %) and ash (2.0 %), and lower fat values (2.68 %) than the control sample. Also, Jin et al. (2020) reported in treatments with the same percentage of plasma addition (1.5 %) as in the present study, lower protein content (16 %), higher moisture (67.02 %), and a similar percentage of fat and ash, 10.11 % and 4.12 %, respectively.

Similarly, Aslinah et al. (2018) found that meatball samples' moisture and protein contents increased significantly, while fat decreased with *Vigna angularis* bean flour. Also, research such as Dzudie et al.'s (2002) has reported increased moisture content in beef sausages with the incorporation of bean flour. Montero et al. (2015) and Torres-González et al. (2016) confirmed a lower fat content in sausages with the incorporation of blood plasma and sesame paste (14.3 %) and sausages with *Lens culinaris* Medik (Fabaceae) flour as an extender (16.13 %).

For protein content, T4 had a higher percentage concerning the control sample, attributed to the protein content of the lyophilized plasma and the Zaragoza bean flour present in T4, higher results than those obtained by Hurtado et al. (2011) and Hurtado et al. (2012). They used porcine blood plasma in frankfurter sausages, with 11.32 % and 11.67 %, respectively. Also, the results obtained in the present study are higher than those reported by Morales (2018) and Torres-González et al. (2016), who obtained a protein content in sausages with flour from *Lens culinaris* and *Prosopis juliflora* (Sw.) DC (Fabaceae) of 18.3 % and 16.63 %, respectively.

Marrugo-Ligardo et al. (2017) obtained protein percentages of around 17.64 % for treatments with 5.25 % white variety *P. lunatus* starch. Finally, the ash content in T4 was higher than in the control sample, possibly because of the ash content provided by the lyophilized plasma and the bean flour in this treatment, whose value was even higher than those reported by Torres-González et al. (2016) (3.71 %) and Marrugo-Ligardo et al., (2017) (4.32 %).

Texture profile analysis (TPA)

The T4 and the control treatment CT results for the analyzed variables texture, hardness, adhesiveness, cohesiveness, and chewiness profile are shown in Table 4.

Parameters	Hardness (N)	Adhesiveness (N)	Cohesiveness (dimensionless)	Chewiness (kg)
T4	14.6083 ± 0.2864^{a}	-0.134 ± 0.0202^{b}	0.822 ± 0.011^{b}	1.3293 ± 0.0368ª
СТ	13.0925 ± 0.1249 ^b	-0.0570 ± 0.0171^{a}	0.853 ± 0.012^{a}	1.0720 ± 0.1450^{b}

Table 4. Parameters of TPA texture profile of the T4 sausage and a CT control

Note. Different letters in the same row indicate statistically significant differences (p < 0.05). Source: Elaborated by the authors

One of the most critical factors determining quality is hardness (Torres-González et al., 2016). The sausage made with Zaragoza bean flour had higher hardness than the control sausage; these results coincide with those of Kim et al. (2017), who reported that the sausages with the content of lyophilized porcine plasma (1%) exhibited higher hardness while the control showed lower hardness during the five weeks of storage. For their part, Zapata and Pava (2018) reported higher hardness for sausages with quinoa added than their control, stating that the hardness and cutting strength of the sausages increased with the addition of quinoa flour. Torres-Gonzalez et al. (2016) and Marrugo-Ligardo et al. (2017) reported higher hardness in their sausages with flour made from *L. culinaris* (29.97 N) and modified *P. lunatus* starch (25.7 N) than in their control.

When flour is added, the emulsion structure has more free charges, favoring adherence to the walls of other objects, states Albarracín et al. (2010) in their study on a sausage with common bean flour (*Phaseolus vulgaris*) as an extender. The adhesiveness values were negative, indicating that the sausages' texture was sticky or adhesive, so when the product is consumed, it adheres to the palate and is hard to remove. It may have been due to the greater amount of carbohydrates contributed by Zaragoza bean starch (Marrugo-Ligardo et al., 2017). The adhesiveness of the sausage with Zaragoza was within the range reported by other authors (Granados et al., 2013; Marrugo et al., 2017), with a significant difference (p < 0.05) that was higher than the control sausage, attributed to the differences in the moisture content of both products.

It should be noted that, among the functionalities of legume proteins, the properties that depend on the type of protein-protein interaction, such as adhesiveness, stand out. This behavior that the consumed product adheres to the palate and a greater force is needed to remove it could be due to the inclusion of Zaragoza flour in the

treatments and, therefore, to the type of protein-protein interaction of this legume (Guerrero et al., 2003, Petridis et al., 2010).

These results coincide with those reported by Torres-Gonzalez et al. (2016), who reported less cohesiveness in their sausages with *L. culinaris* (0.28) than in their control (0.32). Thus, less force was required to break the bonds of the structure belonging to the sausage matrix elaborated in this research, results that agree with Ayandipe et al. (2020). Their study on high-quality cassava and coconut composite flour combination as filler in chicken sausages reported that cohesiveness decreased as coconut flour increased, which could be due to the fiber content of the chicken sausages.

In this research, the chewiness of the sausage made with Zaragoza bean flour and lyophilized plasma (T4) was higher than the control sausage. Similar results were reported by Aslinah et al. (2018), who reported that a higher amount of adzuki bean (*Vigna angularis*) flour could contribute to higher protein content and starch properties in meatballs with greater hardness and chewability. According to Hayes et al. (2005), when the protein content of a meat product increases, its hardness increases, and so does its chewiness. During the cooking of the sausages, the animal and vegetable proteins and the flour formed a gel matrix that improved the hardness and chewiness of the sausages (Gao et al., 2015).

Cooking loss results, performance, and WRC of T4 and CT are shown in Table 5. The values found for both treatments had the same performance pattern. The lowest losses from cooking and higher yields were obtained with T4concerning the control, with a significant difference between these treatments (p < 0.05), indicating that the incorporation of lyophilized plasma and Zaragoza bean flour significantly affects cooking yield and cooking loss. The results presented in this research can be attributed to the starch and protein content (due to Zaragoza flour and plasma) in the sausage, which forms gels during cooking, contributing to more significant water and lipid retention in this product (Feiner, 2006). Leonard et al. (2019), in their study on the incorporation of roasted lupine *Lupinus angustifolius* L. (Fabaceae) flour in veal sausage, found that the inclusion of lupine flour increased the stability of the meat emulsion and decreased cooking losses.

It should be noted that the results obtained from cooking and yield losses in the present study are better than those obtained by Isaza et al. (2010) and Julio et al. (2015), who reported sausages formulated with bovine blood plasma with yields up to 94.35 % and sausages with hydrated bovine plasma incorporation with 97.59 % yield and 2.40 % cooking losses. Other authors have indicated the same behavior in making their meat products (Hernández & Güemes, 2010; Montero et al., 2015).

Properties	Cooking losses (%)	Yield (%)	WRC
T4	$2.02 \pm 0.10^{\mathrm{b}}$	98.18 ± 0.10^{a}	8.80 ± 0.17^{a}
СТ	$2.74\pm0.08^{\rm a}$	97.86 ± 0.12^{b}	5.80 ± 0.07^{b}

Table 5. Cooking losses, yield, and WRC of T4 and CT

Note. Different letters in the same row indicate statistically significant differences (p < 0.05). Source: Elaborated by the authors

For their part, Álvarez et al. (2018) found that plasma at a replacement level of 20 % of the total protein content in sausages notably improved the WRC and the cooking loss, which means that plasma proteins can retain more water. Plasma has been used to substitute for polyphosphates in sausages (as a water absorption agent).

Non-meat ingredients affect the WRC through electrostatic repulsion and the pH of meat products. Both water and other non-meat ingredients interact with the muscular structure of meat, altering the protein functionality and yield of the final product, which may be due to the affinity with water that this type of flour may have (Albarracín et al., 2010; García et al., 2012) and to its fiber content, which reduces water loss during cooking (Ponnampalam et al., 2017). The results of the present study were like those of Zapata and Pava (2018), who reported a higher WRC in sausages formulated with red tilapia fillet and quinoa flour (17.19) than in their sample control (14.57); however, these results were higher than those of this study.

Conclusions

Incorporating lyophilized porcine plasma and Zaragoza bean flour in meat products such as Frankfurter-type sausages does not harm acceptability. It should be noted that T4 (1.5 % plasma and 5 % flour) presented a high protein content and low-fat content, with the best behavior in texture and firmness. Additionally, this research reflected that incorporating blood plasma and Zaragoza bean flour decreased cooking losses and increased yield and WRC. These ingredients can contribute to the meat products industry. Future studies should increase the incorporation of porcine plasma and bean flour in meat products as a possible fat and protein substitute while evaluating the effect on other parameters such as microstructure and instrumental color.

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