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Effect of chestnut flour and probiotic microorganism on the functionality of dry-cured meat sausages.

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Abstract

The meat industry has made efforts to develop meat and meat products with functional ingredients to prevent the risk of disease and to promote health conditions. Therefore, the aim of the present work was to study the combined use of the probiotic strain, *Lactobacillus plantarum,* and potential prebiotic chestnut flour in Spanish dry-cured sausage (Longaniza de Pascua). Chestnut flour and the probiotic strain improved LAB counts on Longaniza de Pascua without modifying product flavour. Chestnut flour had a significant effect on pH decrease and residual nitrite values, but lipid oxidation values were increased. The symbiotic meat product could be considered a healthy matrix as a probiotic carrier.

1. Introduction

Functional foods play an important role by offering a new kind of healthy tool that promises specific effects related to particular bioactive components. The most commonly used functional ingredients are probiotic bacteria, prebiotic carbohydrates, multiple types of antioxidants and some lipids.

Nowadays, consumer demand for high-quality meat products is strong and growing. Such demand provides great opportunities for the meat industry, compelling said industry to strive in the research and production of healthier sausages (Rosmini, Frizzo, & Zogbi, 2008).

Sweet chestnut (*Castanea sativa Mill*.) is a native deciduous seasonal tree of Mediterranean countries that produces edible nuts. Chestnut is a good source of many bioactive compounds that have been associated with cancer and cardiovascular disease prevention as well as anti-inflammatory effects (Barreira, Ferreira, Oliveira, & Pereira, 2008). The main antioxidant compounds found in chestnuts and their by-products are phenolic acids, phenolic compounds, flavonoids, and tannins, which are the most abundant in accordance with different studies, particularly in the prevention of non-communicable diseases (Vasconcelos, Quideau, Jacquet, Rosa, & Ferreira-Cardoso 2010; Vázquez, Fernández-Agulló, Gómez-Castro, Freire, Antorrena, & González-Álvarez, 2012; Echegaray *et al*., 2018).

Prebiotics are defined as substrates used selectively by host microorganisms to produce a beneficial effect (ISAPP 2017).Indeed, Ozcan, Yilmaz-Ersan, Akpinar-Bayizit, & Delikanli (2017) have reported that chestnut flour could be considered as a good prebiotic because it contains oligosaccharides (non-digestible ingredients), which are fermented by probiotic bacteria such as *Bifidobacteria* and *Lactobacilli*.

There are advantages and disadvantages related to fermented meat matrices. On the one hand, they are suitable for transporting probiotic bacteria since they generally do not heat up, promoting the survival of probiotic bacteria in the gastrointestinal tract. On the other hand, bacteria viability is affected by the low A_w and pH values, as well as the high content of curing agents. Therefore, results are expected to be strain-dependent (De Vuyst, Falony, & Leroy, 2008; Agüero, Frizzo, Ouwehand, Aleu, & Rosmini, 2020). That is the reason why the incorporation of these ingredients into Longaniza de Pascua could represent an added value while improving its perspective as a healthy food.

In symbiosis, it is expected that prebiotic ingredients could promote probiotic survival in the product, in the gastrointestinal tract, and their growth in the colon (Grimoud *et al*., 2010). Therefore, the main goal of this work was to evaluate the effect of the combined incorporation of *Lactobacillus plantarum* and chestnut flour on the technological and functional properties of Longaniza de Pascua.

2. Materials and methods

2.1 Materials

Chestnut flour was purchased from a local market in Spain, being previously characterized by Fernández-López, *et al*. (2019b). The colour parameters of chestnut flour were L* 87.50, a* 1.15, b* 12.60, C* 12.66 and h* 84.79. The HPLC analyses on chestnut flour showed a total of 20 polyphenolic compounds, 15 of which were phenolic acids (mainly galic, ferulic and sinapic acids).

The meat (lean and fatty meat) was purchased in a local supermarket, and was transported under refrigerated conditions (4 ºC) and immediately processed at the IPOA Research Pilot Plant facility at Miguel Hernández University.

2.2 Longaniza de Pascua Manufacturing

The composition and production method of Longaniza was carried out according to the Longaniza de Pascua quality regulations of the Valencian Agro-food Institute. Longaniza de Pascua is a traditional product of the Valencian community (Spain) that has been recognized with its own distinctive quality (Resolución 10/09/2003 CV).They were manufactured in the IPOA Research Pilot Plant. The Longaniza mixture was prepared according to a traditional formula (only meat percentages add up to 100% and percentages of other ingredients are meat-related): pork lean meat (60%), pork back fat (40%), water (5%), salt (2%), glucose (0.02%), ascorbic acid (500 mg/kg), nitrite (100 mg/kg) and spices (0.2% black pepper and 0.01% anise). *L. plantarum* is a food-grade strain normally used by the food manufacturing industry. It was isolated, for research purposes only, from the Bioflora™ product (BIOSIDUS S.A), which is commercialized as a probiotic with sanitary certifications. The inoculum was made as previously described by Rubio, Jofré, Aymerich, Guàrdia, & Garriga, (2014) and its concentration was about 8.5 log CFU/g. The sausages were stuffed into natural lamb casings of 18 mm in diameter. Four batches were prepared: batch CL with 3% chestnut flour added; batch CPL with 3% chestnut flour and 8.5 log CFU/g *L. plantarum* added; batch PL with 8.5 log CFU/g *L. plantarum* added and batch control (L)(without chestnut flour and L. *plantarum*). Chamber drying conditions were as follows: 15±1° C and 75±2% relative humidity. After 5 d of drying, the Longanizas were considered ¨ready-to-eat¨ (30% weight losses). The small calibre of the sausage allows the required drying time to be shortened, quickly reaching slice ability. Shelf life conditions will be the object of a further study.

The moisture, residual nitrite level, microbiological (acid lactic bacteria and *L. plantarum*) and physico-chemical analysis was determined at 0, 1, 2, 3, 4, 5 d of dry-curing. For aerobic mesophilic bacteria (AMB), moulds and yeasts and *Enterobacteriaceae* determination samples were taken at 0, 2 and 4 d of dry-curing. For organic acids and sugars determination, samples were taken at 1, 3 and 5 d of dry-curing. Lipid oxidation, sensorial and texture determinations were run in the final product. All determinations were performed in triplicate, except the colour and texture determinations with 9 and 6 measurements, respectively. The studied replicates were in the same batch. Three productions were studied in three different times.

2.3 pH, A_w and color analysis

The pH of Longaniza de Pascua was measured directly using a Crison combination electrode probe (Cat. No. 52) connected to a pH-meter (model 510 Crison, Barcelona, Spain), according to Sayas-Barberá, Viuda-Martos, Fernández-López, Pérez-Alvarez, & Sendra, (2012). Water activity was determined with Novasina SPRINT TH-500 (Pfaffikon, Switzerland) at 25° C. The colour was studied in the CIELAB colour space using a Minolta CM-2600d (Minolta Camera Co., Osaka, Japan) spectrophotometer with illuminant D_{65}, 10° observer, SCI mode, 11 mm aperture of the instrument for illumination and 8 mm for measurement. Spectrally pure glass (Minolta CR-A51/1829-752) was placed between the samples and the equipment. The CIELAB coordinates determined were: lightness (L*), red/green (a*) and yellow/blue (b*), from which the magnitudes hue (h*) as arctan (a*/b*) (UNE 72-031, 1983), Chrome (C*) as

$[(a^*)^2+(b^*)^2]^{½}$ (UNE 72-031, 1983), and redness index values as (a*/b*) were calculated.

2.4. Microbiological analysis

A 10 g aliquot of each sausage sample was aseptically obtained and then homogenized with 90 mL of sterile saline (8.5 g NaCl/l deionized water (Merck)) in a Stomacher 400 (Colworth, London, UK) for 2 min. Aliquots were ten-fold serial diluted in sterile saline and plated. Microbial analysis was determined during 5 d of dry-curing as described below: lactic acid bacteria (LAB) were determined on MRS medium (Merck), incubated under anaerobic conditions at 37 °C for 72 h. *L. plantarum* was counted on *Lactobacillus plantarum* selective medium (LPSM) as described by Bujalance, Jiménez-Valera, Moreno, & Ruiz-Bravo (2006), which was incubated under anaerobic conditions at 37 °C for 72 h. Aerobic mesophilic bacteria (AMB) were determined using Petrifilm™, incubated at 35 °C for 48 h. Moulds and yeasts counts were obtained in Petrifilm™, incubated at 28 °C for 5 d. *Enterobacteriaceae* counts were determined in *Enterobacteriaceae* Petrifilm™ plates, incubated at 37 °C for 24 h.

2.5 Chemical analysis

Moisture (g water/100 g sample) was determined by drying a 3 g sample at 100-105 °C to constant weight (AOAC, 1999).

The residual nitrite level was determined by following ISO/DIS 2918 standards (ISO, 1975). The absorbance was read in an HP 8451 Array Diode (Hewlett Packard, Palo Alto, CA) setting in 520 nm, and results were expressed as mg $NaNO_2$/kg.

Lactic acid from Longanizas was determined by HPLC (Hewlett Packard, Palo Alto, CA) coupled with two detectors: DAD (set at 210 nm) and refractive index detector, as previously described Sayas-Barberá *et al*. (2012). Lactic acid standards were obtained from Supelco (Darmstadt, Germany). Peaks were identified by comparison with the retention time of standards, and quantified by regression formula obtained with these standards.

2.6 Lipid oxidation

Lipid oxidation was evaluated as a function of changes in thiobarbituric acid-reactive substances (TBARs), following the method described by Rosmini *et al.* (1996).

2.7 Texture profile analysis

The texture profile analysis (TPA) was performed with a Texture Analyser TA-XT2i (Stable Micro Systems, Surrey, England), according to Herrero *et al*. (2007).The texture profile parameters, hardness, cohesiveness, springiness and chewiness were determined.

2.8 Sensory evaluation

Testing was carried out by 47 panellists from the Miguel Hernández University, Alicante, Spain. A Quantitative Descriptive Analysis (QDA) and an acceptability analysis were carried out in the sensory laboratory at the Agri-Food Technology Department, according to international standards (ASTM, 1986; ISO 2007). A slice of Longaniza (2 cm long approximately) from each batch was served at room temperature with pieces of bread and water to clean the palate between samples.

Aspects by sample observation were determined, like *appearance overall assessment* (scored from dislike extremely to like extremely), *global colour* (scored from dislike extremely to like extremely) and *colour intensity* (scored from too light to too dark). On the other hand, aspects by sample taste were determined, like *taste overall assessment* (scored from dislike extremely to like extremely), *global flavour* (scored from dislike extremely to like extremely and scored from too weak to too strong), *saltiness intensity* (scored from imperceptible to too salty), *fattiness intensity* (scored from imperceptible to too fatty), *hardness intensity* (scored from dislike extremely to like extremely and scored from imperceptible to too hard), acidity intensity (from imperceptible to very acidic), and *juiciness intensity* (scored from dislike extremely to like extremely and scored from imperceptible to very strong).

Panellists evaluated the attributes of the acceptability test using a 9-point hedonic scale, varying from (1) "dislike extremely" to (9) "like extremely". A 5-point scale was used for QDA.

2.9 Statistical analysis

All collected data during the dry-curing process were evaluated by applying ANOVA, according to a two factorial design with repeated measurements in time. The data collected after the drying process (ready-to-eat) were analysed using a factorial ANOVA. The factors in both cases were chestnut flour levels (0% and 3%) and the probiotic strain-*Lactobacillus plantarum*- (0 log CFU/g and 8.5 log CFU/g). For all analyses, SPSS 24.0 for Windows software was used, with $P < 0.05$ representing a significant difference between means.

3. Results and discussion

3.1 Changes in pH, water activity (A_w) and moisture.

Chestnut flour affected pH values significantly by decreasing them ($p < 0.001$) (Table 1). This drop may be attributed mainly to chestnut flour due to the fact that it contains many organic acids, like gallic acid and malic acid (Gonçalves *et al*., 2010). Moreover, it could be due to microbial activity since microorganisms metabolize soluble sugars from the meat batter. On the other hand, a tendency to decrease pH by probiotic strain ($p = 0.065$) was observed (Table 1). It could be attributed to differences in lactic acid production by *L. plantarum* (Table 4). *L. plantarum* produces higher amounts of D-lactic acid and has a wider spectrum of fermentable carbohydrates than L*. sakei* and L. *curvatus*, other endogenous lactic acid bacteria (Signorini, 2006). The addition of lactic acid bacteria produces a drop in pH that tends to be corrected during drying by reaction of lactic acid with amino groups derived from protein degradation. The Longaniza de Pascua would have less ability to correct this initial acidity, compared to other sausages with a higher degree of maturation (Martínez, Bedia, Méndez & Bañón 2009). A trend between chestnut flour and probiotic strain interaction was observed (p = 0.071). Therefore, only the effect on the probiotic in the absence of chestnut flour was observed.

No significant effects were observed either due to chestnut flour or to the probiotic in Aw and moisture (Table 1).

3.2 Residual Nitrite Level

Nitrite in meat products inhibits the growth of *C. botulinum*, contributes to the development of flavour and colour (pink/red) in cured meat products and acts as an antioxidant against lipid oxidation (Beriain, Gómez, Ibáñez, Sarriés, & Ordóñez, 2018). However, finding a way to reduce residual nitrites has become a key issue for the food industry. This work has complied with current European regulations, which have established maximum levels of incorporation (Commission Decision (EU) 2018/702). In the present work, the incorporation of chestnut flour to Longaniza de Pascua produced a significant decrease in the residual nitrite level (p = 0.028) (Table 1). Andrée, Jira, Schwind, Wagner, & Schwägele, (2010) have reported that assuming an estimated addition of 80 to 100 mg nitrite/kg, only about 11 to 14% of the added nitrite will be found in the cured meat product. In this work, CL and CPL batches showed 2.92% and 2% of the added nitrite in the final product, respectively, whereas PL and L showed 10.92% and 11.56% of the added nitrite, respectively. This drop in CL and CPL means that residual nitrite levels could be explained due to the high reactivity of nitrite with the different bio-compounds present in chestnut flour, like polyphenols and flavonoids, considering that when the meat pH is around 6.0 or less the nitrite can be transformed into nitric oxide or nitrous acid, leading to polyphenol or endogenous substance reactions (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, (2010); Li, Shao, Zhu, Zhou, & Xu, (2013). In turn, caffeic acid and ferulic acid offer strong protection against the nitrite ion by preventing the formation of nitrosamines in foods, which could explain these results (Krishnaswamy, 2001). The nitrite is reduced to nitric oxide (NO) as soon as it is added to the meat formulation, quickly starting to react with myoglobin to form nitric oxide myoglobin. Residual nitrite levels will correspond to nitrite that has not reacted with myoglobin, allowing it to be available for other reactions in the organism (Fernández – López, Viuda-Martos, Lucas-González, Pérez-Álvarez, 2019a), such as the formation of carcinogenic nitrosamines. Therefore, the effect of chestnut flour on residual nitrite could be an interesting contribution in the formulation of healthier meat products. The probiotic addition did not have a significant effect on residual nitrites (Table 1).

3.3 Lipid oxidation

TBARS values increased significantly (p < 0.001) in batches with a presence of chestnut flour (CL and CPL), compared to PL and L (Table 1). This can be explained due to the fact that, under certain conditions (e.g., when iron is present), the phenolic antioxidants can initiate an auto-oxidation process and finally behave like pro-oxidants (León-González, Auger, & Schini-Kerth, 2015). According to the literature, there are elements like iron, which promote the formation of free radicals such as transition metal ions that change their state of valence by losing or gaining electrons (Garcez, Bordin, Peres, & Salvador, 2004). In the case where chestnut flour and probiotic strain are present (CPL), a tendency to increased lipid oxidation was observed (p = 0.051). However, in the presence of the probiotic strain (PL), TBARS values tend not to be increased (p = 0.069) when compared to the control (L). Therefore, a different behaviour of the probiotic strain in the presence of chestnut flour was shown, which could be explained due to the marked effect of chestnut flour on lipid oxidation. Another reason chestnut flour batches (CPL and CL) have a higher lipid oxidation could be the presence of less residual nitrite on them; therefore, they have less antioxidant capacity.

Independently of differences between batches, none exceeded 2 mg/kg of TBARS, since it could not be considered as a threshold for meat rancidity (Campo *et al*., 2006).

3.4 Changes in Colour

Results for lightness (L*), yellowness (b*) and hue (h*) indicated no significant differences due either to chestnut flour or to the probiotic strain (Table 2). As regards redness (a*), no effect was found by chestnut flour (Table 2). On the contrary, the probiotic strain caused a decrease in (a*) (p = 0.04) (Table 2). As expected, in this type of meat product the increase in redness throughout the dry curing process could be attributed to the formation of nitrosomyoglobin (Feiner, 2016). Subsequently, the redness began to decrease, probably due to the partial or total denaturation of nitrosomyoglobin caused by the production of lactic acid (Table 4). The interaction between two factors (CPL) tended to reach a deeper decrease than each factor individually.

Regarding C* values, the presence of the probiotic strain (p = 0.029) and the interaction between chestnut flour and the probiotic strain (p = 0.041) caused a significant decrease (Table 2). The C* value decrease indicates that the colour is less vivid and becomes duller (Hunt *et al.*, 2012). It is a magnitude that depends on the concentration of hem pigments (Pérez Álvarez *et al.*, 1999). Therefore, the decrease observed in coordinate a* is probably due to the effect of lactic acid on red components, which could explain the loss of saturation or drop in chrome values.

Regarding the h* values, as the curing process progresses both the control and the different treatments move towards red hues (Table 2).

An increase in redness index values (a^*/b^*) in all treatments throughout the process was observed, evidencing the typical redness of dry-cured meat products (data are not shown). No significant difference between treatments was found (L x CL p = 0.707; L x PL p = 0.510; L x PL x CL p = 0.441). These results show that the treatments did not affect the formation of the typical colour of dry-cured meat products.

3.5 Microbiological analysis

Table 3 shows Lactic Acid Bacteria (LAB) and *L. plantarum* counts log CFU/g during the Longanizas dry-curing process. These results are important since they allow a viability of probiotic bacteria during the manufacturing process, a condition that is recognized as essential for a functional food (Agüero *et al*., 2020; Pavli, Argyri, Chorianopoulos, Nychas, & Tassou, 2020). A significant improvement in LAB counts was observed due to the effect of chestnut flour (p = 0.026) and the probiotic strain (p < 0.001) on Longaniza de Pascua when compared to the control. An interaction between chestnut flour and the probiotic strain was observed (p = 0.014). In the case where chestnut flour and probiotic strain are added together (CPL), they achieve a higher LAB count than the CL batch and the control (L).

L. plantarum counts were not affected by chestnut flour. The characteristic *L. plantarum* colonies were easily distinguished from the rest of the lactic microbiota throughout the test in the LPSM medium. There were no *L. plantarum* counts observed in non-inoculated samples (CL and L) (Table 3).

There were no differences between CL and CPL batches in total LAB and *L. plantarum* counts. Therefore, it is concluded that no synergistic effect was observed between the probiotic strain and the chestnut flour. As the load of the probiotic strain remains stable during fermentation and drying, it is likely that the addition of high loads of probiotic has made it impossible to see a synergistic effect on its growth due to the chestnut flour. Other trials where low loads of beneficial strain are inoculated will be necessary to study this effect. Improving human health through modulation of the intestinal microbiota is an evolving strategy. Therefore, the amount of the probiotic microorganism found in the meat product together with the non-digestible fibre provided by the chestnut flour make a healthier product that could benefit the consumer.

Aerobic mesophilic bacteria (AMB) counts, *Enterobacteriaceae counts* and yeasts and moulds counts were not affected by chestnut flour and the probiotic strain (Table 3).

3.6 Texture analysis

The texture values observed in the present work were consistent with those reported by Herrero *et al*., 2007. The probiotic strain (p = 0.045, p = 0.001), the chestnut flour (p < 0.001), and their interaction (p < 0.001, p = 0.001) had significant effect on the increase of springiness and cohesiveness values, respectively. Chestnut flour and the probiotic strain did not have a significant effect on chewiness (Table 4). However, in the case of the interaction of two factors, a tendency to increase chewiness values was observed compare to CL. (Table 4). According to Sánchez-Zapata, Díaz-Vela, Pérez-Chavela, Pérez-Alvarez, & Fernández-López, (2013), cohesiveness and springiness increased when a fibre rich matrix was added into dry-cured sausages.

The increase observed in this work could be caused due the effect of the chestnut flour addition (composition and type of dietary fibre). On the other hand, under certain conditions lactic acid seems to improve the texture (Hu *et al*., 2019), affecting the functional properties of muscle proteins and leading to acid-induced gelation that could mainly explain texture development (Table 4).

A significant effect on hardness due to chestnut flour was observed ($p = 0.007$). The chestnut flour batches presented a decrease in hardness values compared to the control (L). The lowest hardness value was observed in the case where two factors were present (CPL). However, the probiotic strain (PL) did not have a significant effect on hardness (Table 4).This could be explained by the fact that due to the presence of chestnut flour in the matrix, the acid-induced gelation can be physically hindered, thus resulting in a softer slice ability.

3.7 Lactic acid values

LAB plays an important role in the formation of lactic acid by fermenting carbohydrates. After around 36–48 hours of fermentation, large amounts of lactic acid are produced (Feiner, 2016) .The lactic acid production contributes to the formation of texture, to the acid taste and the development of the curing colour.Table 4 presents the lactic acid values of sausages. In the present research, a tendency to produce more lactic acid by *L. plantarum* has been observed ($p = 0.055$), which may be due to a greater metabolic activity by probiotic bacteria. The L batch produced the lowest acid lactic amount compared with other batches (CL, CPL, PL).

3.8 Sensory analysis of final products

Fig. 1a and Fig. 1b show sensory scores of Longaniza de Pascua in the QDA and acceptability analysis, respectively. Sensory acceptance by potential consumers and the quantitative descriptive characteristics of Longaniza were not affected by the addition of a probiotic strain ($p > 0.05$). Similar results were shown by other authors (Pavli *et al.,* 2020, Coelho *et al.,* 2019). No attribute was affected by the addition of chestnut flour, except hardness intensity in QDA ($p = 0.005$). The addition of chestnut flour caused a significant increase of hardness intensity according to potential consumers compared to the control (L) and probiotic strain batch (PL), which were scored as ¨slightly soft¨. The CPL batch was scored as ¨correct¨, and CL was scored between ¨correct¨ and ¨slightly hard¨ (Fig. 1a).

Therefore, except for the hardness intensity, all attributes for CL, CPL, PL and L shown in Fig. 1a received a good description by the assessors with a perception of "Correct", which was assigned a value around 3.

In Fig. 1b, the appearance overall assessment, global colour, taste overall assessment, juiciness and global flavour showed an average acceptance of approximately 7.0 (moderately like). Hardness was scored as values between 5 and 6, representing¨neither like nor dislike ¨ and ¨like slightly¨, respectively. No difference between treatments was observed ($p > 0.05$).

Longaniza de Pascua could be a good alternative to carrier *L. plantarum* because consumers cannot perceive organoleptic differences, while also exhibiting satisfactory scores. On the other hand, chestnut flour was imperceptible in most attributes except in intensity hardness, which was still well qualified in the CPL batch according to consumer preferences. Therefore, in this way, chestnut flour and the probiotic strain provide added value to the product without significant changes in organoleptic quality.

4. Conclusion

The incorporation of chestnut flour and *Lactobacillus plantarum* into Longaniza de Pascua could represent a good alternative to provide some added value to such traditional meat products.

Among the improvements provided by both ingredients are: a tendency to produce greater amounts of lactic acid, which is an important contribution to the barrier technology in order to control undesirable microbiota while the texture is improved. Neither chestnut flour nor the probiotic strain have changed the flavour of the product.

As regards chestnut flour, it could be considered a healthy component of the dry-cured meat matrix since it reduces the presence of residual nitrite and is a source of dietary fibre and polyphenols.

Lactobacillus plantarum, a GRAS probiotic strain, has shown an excellent capacity to adapt to the sausage ecosystem studied with respect to low pH tolerance and low A_w conditions. Its presence is another factor that contributes to the development of healthy meat products.

Further studies are necessary to demonstrate the existence of symbiosis between chestnut, a prebiotic potential and the probiotic strain and, at some time, to study the strain viability and lipid oxidation of chestnut flour during storage.

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Figure legends

Fig.1a. Sensory scores (QDA) of Longaniza de Pascua formulated with Chestnut flour (3%) and/or L. *plantarum* at the end of the dry-curing process. *Different lowercase letters indicate a significant difference at the 5% level.

Fig.1.b.Sensory scores (Acceptability analysis) of Longaniza de Pascua formulated with Chestnut flour (3%) and/or L. *plantarum* at the end of the dry-curing process.

Table 1.

pH, A_w, Moisture, Residual nitrites and TBARS (mean ± standard deviation and values of statistical significance (*p*) of chestnut flour, probiotic strain and their interaction factors) of Longaniza de Pascua.

Type of sausage	pH	A_w	Moisture	R.N (mg/Kg)	TBARS (mg MA/Kg)
CL	5.63 ± 0.07[b]	0.93 ± 0.01[a]	46.56 ± 1.87[a]	27.74 ± 5.44[b]	0.27 ± 0.05[b]
CPL	5.63 ± 0.04[b]	0.92 ± 0.01[a]	44.07 ± 2.53[a]	26.38 ± 4.61[b]	0.37 ± 0.06[a]
PL	5.71 ± 0.03[b]	0.90 ± 0.02[a]	47.99 ± 2.39[a]	33.86 ± 5.00[a]	0.13 ± 0.02[c]
L	5.88 ± 0.02[a]	0.92 ± 0.01[a]	47.28 ± 2.87[a]	44.20 ± 5.34[a]	0.14 ± 0.02[c]
Factors			***p***		
C.F	$p < 0.001$	0.315	0.343	0.028	$p < 0.001$
P.S	0.065	0.179	0.717	0.329	0.069
interaction	0.071	0.662	0.512	0.506	0.051

C.F: Chestnut flour

P.S: Probiotic strain

R.N: Residual nitrite

Table 2.

Colour parameters (mean ± standard deviation and values of statistical significance (*p*) of chestnut flour, probiotic strain and their interaction factors) of Longaniza de Pascua.

Type of sausage	L*	a*	b*	h*	C*
CL	46.99 ± 0.63^{a}	6.09 ± 0.33^{a}	7.36 ± 0.39^{a}	49.09 ± 1.89^{a}	9.82 ± 0.41^{a}
CPL	45.88 ± 0.61^{a}	5.07 ± 0.19^{b}	6.37 ± 0.34^{a}	49.27 ± 1.80^{a}	8.33 ± 0.30^{b}
PL	46.27 ± 0.52^{a}	5.46 ± 0.26^{a}	6.70 ± 0.38^{a}	48.80 ± 1.94^{a}	8.88 ± 0.37^{a}
L	46.70 ± 0.45^{a}	5.53 ± 0.27^{a}	6.79 ± 0.29^{a}	50.16 ± 1.60^{a}	8.93 ± 0.32^{a}
Factors			***p***		
C.F	0.933	0.804	0.772	0.869	0.684
P.S	0.167	0.040	0.121	0.745	0.029
Interaction	0.541	0.070	0.203	0.672	0.041

C.F: Chestnut flour

P.S: Probiotic strain

Table 3.

Enterobacteriaceae, mould and yeasts, aerobic mesophilic bacteria (AMB), acid lactic bacteria (LAB) and *L. plantarum* counts (log CFU/g) (mean ± standard deviation and values of statistical significance (*p*) of chestnut flour, probiotic strain and their interaction factors) of Longaniza de Pascua.

Type of sausage	Log CFU/g (Mean ± standard deviation)				
	Enterobact.	Yeasts and Moulds	AMB	LAB	*L .plantarum*
CL	4.50 ± 0.15^{a}	3.71 ± 0.65^{a}	8.02 ± 0.05^{a}	7.43 ± 0.30^{b}	nd
CPL	4.26 ± 0.12^{a}	3.43 ± 0.69^{a}	7.33 ± 0.05^{a}	8.44 ± 0.04^{a}	8.67 ± 0.06^{a}
PL	4.30 ± 0.09^{a}	3.40 ± 0.65^{a}	7.18 ± 0.05^{a}	8.51 ± 0.06^{a}	8.60 ± 0.03^{a}
L	4.20 ± 0.13^{a}	3.34 ± 0.70^{a}	7.47 ± 0.05^{a}	6.22 ± 0.40^{c}	nd
Factors			***p***		
C.F	0.296	0.831	0.291	0.026	0.214
P.S	0.593	0.855	0.141	$p < 0.001$	$p < 0.001$
interaction	0.184	0.968	0.572	0.014	0.232

n=3

C.F: Chestnut flour

P.S: Probiotic strain

Enterobact: Enterobacteriaceae

nd: not detected

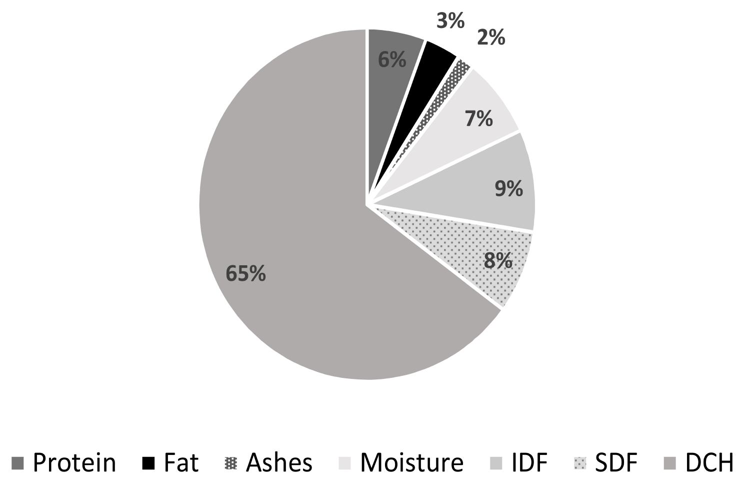
Table 4. Textural properties and Lactic acid values of Longaniza de Pascua (mean ± standard deviation and values of statistical significance (*p*) of chestnut flour, probiotic strain and their interaction factors)

Type of sausage	Springiness (mm)	Cohesiveness	Chewiness (N x mm)	Hardness (N)	Lactic acid mg/100g
CL	0.40 ± 0.04^{b}	0.43 ± 0.03^{b}	16.16 ± 0.48^{b}	40.41 ± 12.11^{ab}	176.95 ± 17.05^{a}
CPL	0.48 ± 0.05^{a}	0.51 ± 0.03^{a}	18.45 ± 0.32^{a}	38.45 ± 6.49^{b}	204.35 ± 36.86^{a}
PL	0.37 ± 0.02^{c}	0.41 ± 0.03^{b}	18.27 ± 0.04^{a}	49.38 ± 1.97^{a}	189.70 ± 26.08^{a}
L	0.40 ± 0.01^{b}	0.42 ± 0.02^{b}	19.14 ± 0.08^{a}	47.85 ± 8.04^{a}	134.76 ± 7.55^{b}
Factors			***P***		
C.F	P <0.001	P <0.001	0.573	0.007	0.170
P.S	0.045	0.001	0.262	0.909	0.055
Interaction	P <0.001	0.001	0.053	0.602	0.433

n=3

C.F: Chestnut flour

P.S: Probiotic strain

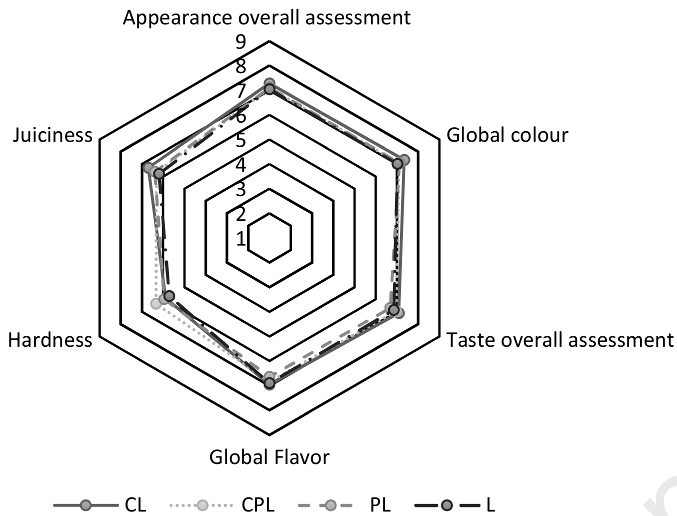


Chemical composition of chestnut flour.

IDF: Insoluble dietary fibre.

SDF: Soluble dietary fibre.

DCH: Digestible carbohydrate.


Appearance overall assessment
9
8
7
6
5
4
3
2
1
Global colour
Taste overall assessment
Global Flavor
Hardness
Juiciness
CL
CPL
PL
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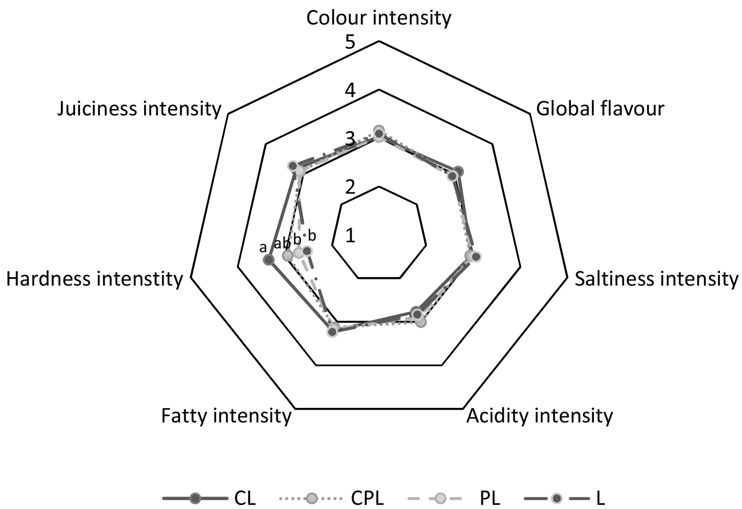
Colour intensity
Global flavour
Saltiness intensity
Acidity intensity
Fatty intensity
Hardness intenstity
Juiciness intensity
5
4
3
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1
a
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b
b
CL
CPL
PL
L

- The addition of chestnut flour reduces pH and residual nitrite in Longaniza de Pascua.
- *Lactobacillus plantarum* has adapted to the sausage ecosystem, therefore Longaniza de Pascua is a good alternative to carrier it.
- Chestnut flour improved LAB counts.
- Probiotic Longaniza de Pascua was successfully accepted by consumers.

Conflict of interest

There is not any conflict or competitive interest.