Effect of probiotic Lactiplantibacillus plantarum and chestnut flour (Castanea sativa mill) on microbiological and physicochemical characteristics of dry-cured sausages during storage

N. Sirini, R. Lucas-González, J. Fernández-López, M. Viuda-Martos, J.A. Pérez-Álvarez, L.S. Frizzo, M.L. Signorini, M.V. Zbrun, M.R. Rosmini

PII: S0309-1740(21)00267-9

DOI: https://doi.org/10.1016/j.meatsci.2021.108691

Reference: MESC 108691

To appear in: *Meat Science*

Received date: 3 May 2021

Revised date: 1 October 2021

Accepted date: 4 October 2021

Please cite this article as: N. Sirini, R. Lucas-González, J. Fernández-López, et al., Effect of probiotic Lactiplantibacillus plantarum and chestnut flour (Castanea sativa mill) on microbiological and physicochemical characteristics of dry-cured sausages during storage, *Meat Science* (2021), https://doi.org/10.1016/j.meatsci.2021.108691

Effect of probiotic *Lactiplantibacillus plantarum* and chestnut flour (*Castanea sativa Mill*) on microbiological and physicochemical characteristics of dry-cured sausages during storage

N. Sirini[a], R. Lucas-González[b], J. Fernández-López[b], M. Viuda-Martos[b], J.A. Pérez-Álvarez, L.S[b]. Frizzo [a,c], M.L Signorini [c,d], M.V Zbrun[a,c], M.R. Rosmini[c].

[a]Laboratory of Food Analysis ¨Med. Vet R. Dalla Santina¨, Institute of Veterinary Science (ICiVet Litoral), National University of the Litoral - National Council of Scientific and Technical Research (UNL/CONICET), Esperanza, Province of Santa Fe, Argentina.

[b]IPOA Research Group, Agri-Food Technology Department, Centro de Investigación e Innovación Agroalimentaria y Agroambiental de la Universidad Miguel Hernández de Elche (CIAGRO-UMH), Ctra. de Beniel km 3.2, 03312 Orihuela (Alicante), Spain

[c]Department of Public Health, Faculty of Veterinary Science, National University of the Litoral, Esperanza, Province of Santa Fe, Argentina

[d]Instituto de Investigación de la Cadena Láctea (Idical CONICET – INTA). Ruta 34 km 227, Rafaela, Province of Santa Fe, Argentina.

Abstract

The effect of chestnut flour (*Castanea sativa Mill*) on *L. plantarum* viability and physicochemical characteristics in a dry-cured sausage (Longaniza de Pascua) during storage is discussed. Four batches were prepared: CL with 3% chestnut flour added; CPL with 3% chestnut flour and 8.5 log CFU/g *L. plantarum* added; PL with 8.5 log CFU/g *L. plantarum* added and L, the batch control. The sausages were stored at 4 °C and 20 °C, and vacuum packed for 43 d. *L. plantarum* viability was affected by storage time (P< 0.001). However, higher *L. plantarum* counts at the final of storage were reached due to chestnut flour addition (*P*< 0.001). At room storage, chestnut flour caused a higher increase in TBARS values (P= 0.022). Nevertheless, all lipid oxidation treatments were in the range of accepted values at the sensory detection level. In conclusion, Longaniza de Pascua can be kept at 4 °C or 20 °C for 43 d without causing any rancidity problems.

Keywords: dry cured sausage, Longaniza de Pascua, *Lactiplantibacillus plantarum*, Chestnut flour, Probiotics, viability.

1. Introduction

Nowadays, the meat industry is focusing on producing meat products with potential health benefits by using probiotic microorganisms (Agüero, Frizzo, Ouwehand, Aleu, & Rosmini, 2020; Sirini *et al*., 2021). Much attention has been paid to developing meat products with functional ingredients to promote health conditions and prevent the risk of diseases (Barone *et al*., 2021). Nonmeat ingredients have been commonly used in meat products to reduce costs, improve functionality and give added value to those products. These ingredients could include probiotics, prebiotics, vegetable proteins, dietary fibres, herbs and spices, thus increasing nutritional value and providing benefits to human health (Zhang *et al*., 2010; Gullón *et al*., 2020).

Longaniza de Pascua, a traditional dry-cured sausage from the Mediterranean area (Martín-Sánchez *et al*., 2014) with a small diameter (18-22 mm), is usually consumed as a snack. This dry-cured sausage is an intermediate moisture food (water activity <0.900) that can be stored at room temperature (Sayas-Barberá, Viuda-Martos, Fernández-López, Pérez-Alvarez, & Sendra, 2012). In addition, from a scientific point of view, it is considered an excellent dry-cured model system due to its easy elaboration process (4-8 days), and it can be an excellent delivery system of healthy ingredients in meat products (Sayas-Barberá *et al.,* 2012; Sánchez-Zapata, Díaz-Vela, Pérez-Chabela, Pérez-Alvarez & Fernández-López, 2013). Probiotic bacteria are successfully used in the production of many products; however, the commercial application of probiotic microorganisms in dry fermented meat products is not yet common (Agüero *et al.*, 2020). Controlling probiotic viability is necessary to ensure a beneficial daily dose for the host. The meat industry is developing strategies to incorporate different ingredients that could protect probiotics from adverse environment and storage conditions, limiting the effect of factors that tend to decrease or eliminate their viability in meat products (Burgain, Gaiani, Linder & Scher *et al*., 2011).

Viable probiotic counts in the final product are required to be at least 10^6-10^7 cfu mL^1 to offer health benefits to consumers (Shori, 2015). However, the minimal dose is dependent on several factors, such as the individual person, the strain and the food product concerned.

In recent years, consumers have been interested in chestnut (*Castanea sativa Mill*.) because of its nutritional qualities and potentially beneficial health effects. Chestnuts and their by-products contain antioxidant compounds such as phenolic acids, flavonoids and tannins (Diaz Reinoso *et al*., 2012). On the same line, it has been reported that chestnut flour could be used as a good prebiotic due to the fact that it contains non-digestible ingredients such as

oligosaccharides, which could be fermented by probiotic bacteria like *lactobacilli* and *Bifidobacteria* both in food and in the gut (Grimoud *et al*.,2010; Ozcan, Yilmaz-Ersan, Akpinar-Bayizit & Delikanli, 2017). The development of functional meat products capable of providing indigestible carbohydrates with the ability to provide optimal amounts of substrate for the nutrition and development of colon bacteria is among the pending tasks of the meat industry.

Meat matrices are favourable for lactic acid bacteria (LAB) growth and development. LAB have shown good adaptation to the environment during sausage fermentation, which is associated with a rapid growth rate and acidification. In addition, Rebucci *et al.* 2007 have proved the suitability of selected probiotic bacteria to be used as probiotic cultures in fermented sausages. Therefore, the *L. plantarum* probiotic could survive in Longaniza de Pascua, thus reaching consumers in a considerable dose. The presence of chestnut flour in the formulation could improve survival during storage.

The shelf life of fermented meat products is limited by physical and chemical spoilage, and said spoilage may be related to problems with the development of fermentative microbiota. Due to the fact that this spoilage could affect probiotic survival, the interaction between the meat matrixes and those new ingredients must be analysed (Sirini *et al.,* 2021). Therefore, the objective of this work was to evaluate *L. plantarum* viability and physicochemical characteristics in a dry-cured sausage (Longaniza de Pascua) with added chestnut flour.

2. Materials and methods

2.1 Materials

Chestnut flour was purchased from a local market in Orihuela, Spain. It was previously characterized by Fernández–López, Viuda-Martos, Lucas-González, & Pérez-Álvarez, (2019). The meat (lean and fatty meats) was purchased in a local supermarket, transported in refrigerated conditions (4 °C) and immediately processed at the IPOA pilot plant facility at Miguel Hernández University (Orihuela, Spain).

2.2 Longaniza de Pascua manufacture

Longaniza de Pascua was manufactured in the IPOA Research Pilot Plant (Orihuela, Spain). The elaboration process and technological conditions were established as described by Sirini

et al. (2020). The microorganism used as probiotic was *L. plantarum*, a food-grade strain normally used as a probiotic by food manufacturers. It was isolated, for research purposes only, from the Bioflora™ product (BIOSIDUS S.A), which is commercialized as a probiotic with sanitary certifications.

A pure overnight culture of *L. plantarum* was inoculated at 1% in 150 ml of MRS broth and incubated at 37 °C for 24h in aerobiosis. The 150 ml broth was then centrifuged at 5000 xg for 10 min at 4 °C and washed with PBS. The pellet was mixed with 150 ml of a sterile 25% w/v solution of maltodextrin and whey protein isolate. This solution forms the wall material of the microcapsules that present suitable technological properties, giving rise to the suspension that will be spray dried under controlled conditions. For this, a laboratory spray dryer (Mini Spray Dryer ADL311S, Yamato, Japan) was used. The best drying conditions were previously determined (Inlet temperature: 160 °C, Outlet temperature: 65 °C), which did not affect bacterial viability. The probiotic powder obtained was vacuum packed and transferred from Argentina to Spain. The probiotic strain was encapsulated to be transferred to the pilot plant where the present test was carried out. The confirmation of identity at the strain level was carried out using PFGE. Then, the strain was reconstituted at 25% with physiological solution (0.85%). The *L. plantarum* strain was re-isolated in the MRS agar. The inoculum was made as previously described by Rubio, Jofré, Aymerich, Guárdia, & Garriga (2014). Briefly, the strain was grown overnight in de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37 °C in aerobiosis, harvested by centrifugation at 9,600 x*g* for 10 min at 6 °C, washed and re-suspended in saline solution (0.85% NaCl) and stored at −80 °C with 20% of glycerol until further use. Before freezing, a sample of the inoculum was extracted for enumeration, which was performed by plate counting on MRS agar. The stock culture had 10 Log UFC/ml. The amount of microorganisms adjusted to final concentration in meat batter was 8.5 log CFU/g. Four batches were prepared: CL batch with 3% chestnut flour added; CPL batch with 3% chestnut flour and 8.5 log CFU/g L. plantarum added; PL batch with 8.5 log CFU/g *L. plantarum* added and L batch control. After 5 d of drying, Longaniza de Pascua was considered “ready-to-eat”. Chamber drying conditions were as follows: 15±1° C and 75±2% relative humidity (Tarré, Noain, Navarre, Spain). Day 5 of drying corresponds to day 0 of this experiment.

2.3 Storage experimental design

The resulting products were stored at 4 °C and 20 °C, and vacuum packed for 43 d. Vacuum-packed pouches are made of polyethylene and polyamide laminate of water vapor permeability 1.1 g/m2/24 h at 23 °C, nitrogen permeability 10 cm3/m2/24 h at 23 °C, carbon dioxide permeability 140 cm3/m2/24 h at 23 °C, and oxygen permeability 30 cm3/m2/24 h at 23 °C (Fibran, Girona, Spain). Samples were preserved at a controlled temperature of 20 °C in a Refrigerated cabinet HOT-COLD GL-2101507 (Selecta S.A., Abrera, Barcelona, Spain); in addition, samples were preserved at 4 ± 1°C in a commercial refrigerator with the objective of simulating the true way in which consumers preserve sausages throughtout the study. Samples of all treatments were analysed at 0, 15, 22 and 43 d of storage to perform the corresponding analysis. For this type of product, 43 days is the established time of commercial useful life. All determinations were performed in triplicate, except for colour with nine measurements. Production was repeated 3 times with the same raw material. The trial plan is a completely randomized design (n= 3).

2.4 Determination of pH

The pH value was determined at room temperature using a pH metre (510 Crison, Barcelona, Spain) equipped with a puncture electrode (Hach puncture electrode probe 5233). The measurement was made directly and taken three times by changing the electrode insertion place each time.

2.5 Microbiological analysis

Microbiological analyses were established as described by Sirini *et al*. (2020). LAB counts were determined using Deman, Rogosa, and Sharpe agar (MRS) after 72h at 37 °C in anaerobiosis, and *L. plantarum* counts were performed using *Lactobacillus plantarum* selective medium (LPSM), 37 °C for 72 h in anaerobiosis, as described by Bujalance, Jiménez-Valera, Moreno, & Ruiz-Bravo (2006). Enumeration results were signified as log CFU (colony forming units)/g sausage sample.

2.6 Evaluation of lipid oxidation

Lipid oxidation was evaluated as a function of changes in 2-Thiobarbituric acid reactive-substances (TBARs) in storage, following the method by Rosmini *et al.* (1996).

2.7 Colour measurement

The CIELAB coordinates determined were lightness (L*), red/green co-ordinate (a*) and yellow/blue co-ordinate (b*), from which the psychophysical magnitudes hue (h*) and chrome (C*) were calculated (UNE 72-031, 1983). A Minolta CM-2002 (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 was used. Determinations were assessed at 0, 15, 22, and 43 days of storage (at both storage temperatures). Spectrally pure glass (Minolta CR-A51/1829-752) was placed between the samples and the equipment. The American Meat Science Guidelines for colour evaluation were followed (Hunt *et al.,* 2012), and infinite solid and background were obtained following the methodology described by Sánchez-Zapata *et al*. (2011). The total colour difference (ΔE *) between each treatment (CL, CPL, PL) and traditional Longaniza de Pascua (L) was determined by equation number 1 (Hunt *et al*., 2012):

$$\Delta E^* = [(\Delta L^*)\,2 + (\Delta a^*)\,2 + (\Delta b^*)\,2]\,½ \quad (1)$$

2.8 Genomic DNA preparation

Strains isolated from the pharmaceutical product from the probiotic powder and from *L. plantarum* inocula used in Longaniza de Pascua formulations (CL and CPL) were taken. Regarding inocula samples, strains at 0 d and 43 d at room storage (20°C) were selected. All recollected samples were incubated in LPSM medium at 37 ° C in anaerobiosis. The protocol described by Gosiewski & Brzychczy-Wloch (2015) was used with modifications. Grown colonies were re-suspended in 3 ml of NaCl 0.85% (Biopack, Buenos Aires, Argentina). Optical density (OD) was measured at 600 nm, adjusting the concentration of cell suspensions to OD 2.0 ± 0.2. A 200 µl volume of adjusted cell suspensions was transferred to 1.5 ml microtubes. Plugs were made according to Blajman *et al*. (2015). The digestion of *L. plantarum* plugs was carried out with restriction enzyme *Sfi*I (Thermofisher) (incubation time 5 h at 50 °C). Electrophoresis was carried out at 4.5 V/m (initial orientation change 1.0 s, final orientation change 20 s) for 26 h at 14 °C.

2.9 Statistical analysis

All collected data during the storage process were evaluated by applying ANOVA, according to a four-factorial design (2x2x2x4) with batch of production as a completely random effect. The factors were chestnut flour (0% and 3%), probiotic *L. plantarum* strain (0 log CFU/g and 8.5 log CFU/g), storage temperature (room temperature at 20 °C and cold temperature at 4

C) and time of storage (vacuum packaged) on 0, 15, 22 and 43 d. For these analyses, InfoStat (Universidad Nacional de Córdoba) for Windows software was used, with P <0.05 representing a significant difference between means.

3. Results and discussion

3.1 pH

Longaniza de Pascua is a low-acid fermented meat product (final pH, 5.3 to 6.2). The final pH values obtained in this research work were in accordance with the findings on other studies on low-acid fermented sausages (Aymerich, Martin, Garriga, & Hugas, 2003; Casaburi *et al*., 2007). A decrease in pH values is frequently reported in fermented sausages during storage (Papadima & Bloukas 1999; Kim *et al*., 2012; Dong *et al*., 2020). The pH decrease could be related to an accumulation of lactic acid as a result of a carbohydrate breakdown during fermentation. Nevertheless, this decrease tends to be corrected during drying by the buffering effect on organic acids produced by the reaction of lactic acid with amino groups from protein degradation (Ordóñez, Hierro, Bruna & Hoz, 1999; Ruiz-Moyano *et al*., 2011). Fig. 1a and Fig.1b show pH changes during 43 d at room and cold storage, respectively. Although sugars were added to the sausage mixture during production, the sausage pH did not continuously decrease in storage for any treatments. Other authors found similar slight increases (Casaburi *et al*., 2007; Jin *et al*., 2018; Hilbig, Hartlieb, Herrmann, Weiss, & Gibis, 2020). The pH values were affected by chestnut flour and the probiotic strain interaction (P< 0.001) over 43 d of storage. Samples without *L. plantarum* (L and CL) tended to suffer a strong increase in pH during the first days of storage, whereas in the case of samples with *L. plantarum* (PL and CPL), this increase occurred more slowly (P= 0.068). This could be owing to the existence of autochthonous lactobacilli in matrix meat with low acidifying power (Casaburi *et al*., 2007). Besides, the small diameter of Longaniza de Pascua and the process temperature used (15 ±1 °C) may not have favoured a true fermentation process. Therefore, the pH profile is consistent with the decrease in LAB and the release of peptides, amino acids and ammonia as a result of the proteolytic activity of microorganisms (Gao *et al*., 2013). In addition, chestnut flour causes less pronounced increases in pH regardless of the presence of the probiotic (P< 0.001). This could be explained by lactate, which can be converted to lactic acid, being primarily produced from added carbohydrates. In case of carbohydrates as a potential prebiotic (chestnut flour), the metabolic capacity to form acid from dietary sugars differed significantly between probiotic strains, as shown by Hedberg, Hasslöf, Sjöström,

Twetman & Stecksen-Blicks (2008). It could be inferred that the *L. plantarum* strain used in this work would have the ability to metabolize the carbohydrates present in chestnut flour. Further research work should be carried out to verify this possibility.

According to Leistner & Roedel (1975), if these products have pH $\leqslant$5.2 and an $a_w \leqslant 0.95$ or only pH <5.0 or a_w <0.91, then they are considered "shelf stable meat" and need no refrigeration. Therefore, their shelf-life is often not limited by bacteria but by chemical or physical spoilage. In this context, it should be noted that lowering the pH is a major barrier against pathogens during the fermentation stage and until the time slice consistency is achieved. Due to the small diameter of Longaniza de Pascua and the "quick" drying process after stuffing, the main barrier against pathogens seems to be the a_w values decrease. All treatment a_w values were determined in previous studies during sausage drying by providing the safety of the product (CL: 0.860; CPL: 0.839; PL: 0.765 and L: 0.876) (Sirini *et al*., 2020). On the other hand, storage temperature did not affect pH values during the storage period (*P*= 0.397).

3.2 Microbiological analysis

Lactic acid bacteria are microorganisms commonly found in fermented sausages and are subject to a great variability of species depending on the region in which the products are manufactured. Aquilanti *et al*. 2016 reported that *L. plantarum* is a species that is not characteristic of these meat foods, whereas other authors reported that *L. plantarum* has been frequently isolated in fermented sausages, but not as a dominant microorganism (Drosinos *et al*., 2005; Cocolin *et al*., 2011; Nediani *et al*., 2017). In this research work, the inoculated *L. plantarum* strain was not found in the control group, which indicates that no native strain of that species was present or could proliferate during fermentation. In other words, *L. plantarum* was able to adapt and survive in an environment in which it is not usually found. The adaptation of a species not usually found in that environment facilitates the tracking and quality control of probiotic viability in the finished product since there will be no autochthonous *L plantarum* to interfere with the growth of the added probiotic strain.

Lactiplantibacillus plantarum viability during 43 d at room storage and cold storage is shown in Fig. 2a and Fig. 2b, respectively. Due to the fact that *L. plantarum* viability was affected by storage time interaction, *L. plantarum* counts decreased during all storage days (*P*< 0.001). The number of viable probiotics in the meat product is affected by several factors, such as fat content, food ingredients, temperature, processing conditions, moisture, % NaCl, a_w, etc.

(Ranadheera, Baines & Adams, 2010). However, higher *L. plantarum* counts at the final of storage were reached due to the addition of chestnut flour (P< 0.001). As is already known, the chestnut fruit has a remarkable content of fibre and polyphenols, including Gallic and Ferulic acids (Barreira, Ferreira, Oliveira, & Pereira, 2008). Therefore, this improvement in viability could be attributed to the buffering capacity of the fibres present in chestnut flour and to the considerable amount of nutrients that promote bacterial survival (Espírito-Santo *et al*., 2012; Frumento *et al*., 2013). Final *L. plantarum* counts indicate that the presence of chestnut flour supports *L. plantarum* probiotic viability (P< 0.001). Other authors have also demonstrated that the use of fruit by-products to enrich fermented products is a successful strategy to increase probiotic viability during storage (Espírito-Santo *et al*., 2012; Frumento *et al*., 2013; Casarotti *et al.,* 2018). In addition, phenolic compounds can exert a prebiotic function and increase the probiotic population, suggesting a mutual relationship between phenolic compounds and probiotics while improving the general quality characteristics of foods and potentiating the healthy beneficial effects of both components (De Llano *et al*. 2017; De Souza *et al*., 2019). Phenolic compounds can selectively inhibit the growth of pathogenic bacteria without affecting probiotic viability (Pacheco-Ordaz *et al.,* 2018). In the same line, *L. plantarum* has been described to hold several enzymatic activities such as phenolic acid decarboxylase (PAD), benzyl alcohol dehydrogenase and the ability to degrade some phenolic compounds (Rodríguez *et al*., 2009). In this work, probiotic strain viability was affected by temperatures during storage (P= 0.002). As has been described by Vermeulen *et al*. (2015), this type of dry-cured meat product does not traditionally need refrigeration; nevertheless, when the probiotic strain was added to the formulation, viability was improved under refrigeration (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011).

Lactic acid bacteria viability during 43 d at room and cold storage are shown in Fig. 3a and Fig. 3b, respectively. LAB populations were affected by storage temperature and storage time interaction (P< 0.001), by probiotic strain and chestnut flour interaction (P= 0.001) and by probiotic strain and storage time interaction (P< 0.001). At 43 d of storage, LAB count improvements by cooling were observed (P= 0.001). In addition, regardless of the presence of the probiotic, chestnut flour addition kept the LAB count higher than treatments with no chestnut flour during storage (P= 0.001). This could be explained by the same reasons why chestnut flour improves probiotic viability. Final LAB counts for the control case were 5.08

and 5.13 log CFU/g at 4 and 20 °C, respectively, which is in accordance with reports by Pavli *et al*. (2020).

3.3 Lipid oxidation (TBARS)

Lipid oxidation, expressed as TBARS value, is shown in Table 1. No significant effects were observed due to the probiotic strain in TBARS values (*P*= 0.294). As was expected, room storage caused higher TBARS values with respect to cold storage during 43 d (*P*= 0.001) (Aksu & Kaya 2005). TBARS values increments throughout storage at 20 °C and 4°C for all treatments, except for CPL, were observed (Table 1). Chestnut flour in the Longaniza de Pascua formulation at room storage caused a higher increase in TBARS values with respect to treatments without chestnut flour (*P*= 0.022). As explained by Sirini *et al*. (2020), since phenolic antioxidants can initiate an auto oxidation process, chestnut flour could finally behave like pro-oxidants. In the same line, the addition of chestnut flour and its subsequent room storage led to the highest TBARS values (*P*= 0.020). It is worth noting that the amount of malonaldehyde formed during storage at both temperatures was in the range of 0.23–0.70, in accordance with other authors (Slima *et al*., 2017; Bis-Souza *et al*., 2020), and resulted in TBARS values much lower than 2.0 mg MDA/kg, which is accepted as the deterioration and sensory detection level (Campo *et al.,* 2006). The results of this work suggest that vacuum packaged Longaniza de Pascua with *L. plantarum* and chestnut flour could be stored at room storage or cold storage up to 43 d without causing any serious rancidity problems.

3.4 Colour

Pérez-Alvarez (1996) has studied a dry-cured behaviour in dry-cured sausages. This author mentions that Lightness (L*) was decreased by dehydration process while a* (red-green colour co-ordinate) increased. This increase is caused by a nitrosated-heme pigments formation (main nitrosomyoglobin) and pigment concentration by a dehydration process. The yellow-blue (b*) colour co-ordinate was decreased by meat pigments interconversion. The results of this work indicate that control L* values ranged between 38 and 45, which is in accordance with other authors (Dong *et al*., 2020; Aksu & Kaya 2005). Storage temperature and storage time interaction affected lightness values (*P*< 0.001). L* values decreased at both temperatures in the first days of storage. Then, L* values remained for all treatments except for CPL, where there were no significant changes throughout storage. In addition, Pérez-Alvarez & Fernández-López, (2012) mentioned that L* can be influenced by “water

free" movements on the meat product surface. Thus, L* decreased due to a decrease in moisture content, less free-water on the sausage surface and pigments concentration. Due to the fact that chestnut flour had high L* values (L* 87.56; a* 1.15; b* 12.60; C*12.66; h*1.48) and retained water due to the chestnut starch (due to its water holding capacity), the addition of chestnut flour causes a lower decrease in L* when compared to those treatments that do not have chestnut flour (*P*= 0.040).

Redness (a*) is used as an indicator of colour stability in meat and meat products. Although there is an interaction between storage temperature and storage time, it did not affect redness behaviour (*P* = 0.007). A significant increase in redness values between days 0 and 15 at both temperatures in all treatments was registered (Table 2). This redness increase might be explained by the formation of nitrosyl-myoglobin as a reaction product between myoglobin and nitrites under mildly acidic conditions (Turantaş & Kemahlıoğlu, 2010). From the final of storage, a decrease in redness values at both temperatures for all treatments, except for CPL, was observed. The addition of a probiotic strain to the Longaniza de Pascua formulation (PL) at 4 °C led to a faster redness decrease compared to non-probiotic strain treatments (*P*= 0.005). Both redness decreases may be attributed to the oxidation of nitrosyl-myoglobin (Wenjiao *et al*., 2013). As has been reported by other authors, the application of probiotic microorganisms in fermented meat products might interfere significantly by increasing rancidity and discoloration in the final product. This is due to the fact that most lactobacilli form hydrogen peroxide, which could have an influence over lipid oxidation and colour losses in meat products (Caplice *et al*., 1999; Kołożyn-Krajewska & Dolatowski, 2012). This could be the reason for an earlier a* decrease in probiotic strain treatments (PL). However, the combined use of chestnut and probiotic strain (CPL) caused higher a* values than each factor used separately at 20 °C (*P*< 0.001). Therefore, this combination could mitigate the oxidizing effect of chestnut flour and probiotic strain added alone.

Yellowness is the colour parameter which could be mainly related to lipid oxidation. Under the same behaviour pattern, the addition of a probiotic strain caused higher b* values earlier than in those treatments with no probiotic addition (*P* = 0.004). This could be explained for the same reason argued in the redness discussion. Yellowness was affected by storage temperature and probiotic strain interaction (*P*= 0.020). The pigments interconversion generated by the metabolic action of the probiotic would affect b*, which is associated with storage temperature. The higher the temperature, the greater the change observed, which

would indicate that it is preferable to keep Longaniza de Pascua at 4 °C than at 20 °C. In the same line, the combined use of chestnut flour and *L. plantarum* generates higher b* values than the ingredients alone (P< 0.001). This is due to the direct effect of the yellow components of the colour coming from the chestnut flour.

Chroma (C*) and hue (h*) are better correlated with human visual colour perception. Chroma (C*) values describe brightness or vividness of colour. An increase in C* values at the beginning of storage and a subsequent decline were observed. In the same line, lower C* values were found at room storage compared to cold storage (P= 0.001). In addition, the combined use of *L. plantarum* and chestnut flour in Longaniza de Pascua at room storage caused higher C* values compared to each factor separately (P< 0.001). These results could be related to the fact that oxidation reduces colour vividness (Salueña, Gamasa, Rubial, & Odriozola, 2019), as shown in Table 1. Although a storage time and probiotic strain interaction existed (P= 0.001), it did not affect C* values behaviour.

Regarding hue values, no effect by storage temperature was observed (P= 0.742). Independently of the probiotic strain addition, the presence of chestnut flour in the Longaniza de Pascua formulation (CL) led to approximately constant hue values (P= 0.002). This could be due to the fact that the addition of chestnut flour to Longaniza de Pascua increases TBA values and oxidation reduces colour vividness at an approximately constant hue (Salueña *et al.,* 2019). Although a significant interaction between the probiotic strain and storage time existed, the probiotic strain did not cause variety in h* value behaviour (P= 0.032). Hue values were in the red hue range (30-60) during all storage for all treatments, which is typical of Longaniza de Pascua colour.

In accordance with Prandl, Fischer, Schmidhofer & Sinell (1994), in this work colour differences (ΔE*) were less evident throughout storage at both temperatures owing to the addition of the probiotic strain, whereas the opposite occurs at 4 ° C due to the addition of chestnut flour in the Longaniza de Pascua formulation. In the case of a combined use of chestnut flour and *L. plantarum* at both temperatures, very evident differences in colour during the first days of storage were observed, while differences were not very evident towards the end (Table 3).

3.5 PFGE genotyping

As shown in Figure 4, the eight bacterial isolates analysed had identical PFGE profiles. According to the number and size of the fragments, it was possible to distinguish only one chromosomal restriction pattern or "Fingerprint" for the eight isolates, ensuring the presence of one strain of *L. plantarum* throughout the whole study.

4. Conclusion

In this research work, an *L. plantarum* counts improvement at the final of storage was reached due to the addition of chestnut flour. Probiotic strain viability was affected by storage temperature, so the addition of a probiotic strain requires refrigeration for Longaniza de Pascua during its shelf-life. Vacuum packaged Longaniza de Pascua with *L. plantarum* and chestnut flour could be stored at room storage (20 °C) or cold storage (4 °C) until the end of its shelf life without causing any serious rancidity problems. For all treatments, the h* values remained within the red zone, which is typical of Longaniza de Pascua. The incorporation of chestnut flour and *L. plantarum* did not modify the shelf life of dry-cured sausages compared to the control during all their shelf life at both temperatures analysed. Chestnut flour and probiotic strain addition to the Longaniza de Pascua formulation could represent a good alternative to improve and give added value to this kind of fermented meat products. Longaniza de Pascua is able to act as a good carrier of probiotic bacteria by maintaining probiotic bacteria viability.

Acknowledgments

Noelí Sirini, Laureano Frizzo, Raquel Lucas-González, Manuel Viuda- Martos, Juana Fernández-López, José Ángel Pérez-Álvarez and Marcelo Rosmini are members of HealthyMeat-Network, founded by CYTED (ref. 119RT0568). This study was funded by the National Agency for Scientific and Technological Promotion (Proyecto PICT 2016-3495) and Universidad Nacional del Litoral (Proyecto CAI+D 50120150100152LI, CAI+D 50120150100151LI, CAI+D Orientado 2016 2-14, CAI+D 50620190100152LI and CAI+D 50620190100062LI).

Author Statement

Noelí Sirini, Raquel Lucas González and Manuel Viuda Martos carried out the preparation of Longanizas de Pascua and performed experiments (pH, TABARS and color measurement; BAL and

L.plantarum counts). María Virginia Zbrun collaborated in conducting the pulsed field analyzes and interpreting these results

Noelí Sirini processed the experimental data, drafted the manuscript, and designed the tables and figures. Laureano Frizzo and María Virginia Zbrun aided in interpreting the results and worked on the manuscript

Marcelo Signorini contributed to the design of the research, analysis of the results, and manuscript writing.

Juana López-Fernández, José Ángel Pérez-Álvarez, Manuel Viuda Martos and Marcelo Rosmini developed the project and main conceptual ideas and were in charge of overall direction and planning.

All authors provided critical feedback and helped organized the research, analysis, and manuscript.

Conflict of Interest

There is not any conflict or competitive interest.

References

Aksu, M. I., KAYA, M., & Ockerman, H. W. (2005). Effect of modified atmosphere packaging and temperature on the shelf life of sliced pastirma produced from frozen/thawed meat. *Journal of Muscle foods, 16(3),* 192-206.

Agüero, N., Frizzo, L. S., Ouwehand, A. C., Aleu, G., & Rosmini, M. R. (2020). Technological Characterisation of Probiotic Lactic Acid Bacteria as Starter Cultures for Dry Fermented Sausages. *Foods, 9(5),* 596.

Alvarez, J. Á. P. (1996) *Contribucion al estudio objetivo del color en productos carnicos crudo-curados* (Doctoral dissertation, Universitat Politècnica de València).

Aquilanti, L., Garofalo, C., Osimani, A., & Clementi, F. (2016). Ecology of lactic acid bacteria and coagulase negative cocci in fermented dry sausages manufactured in Italy and other Mediterranean countries: an overview. *International Food Research Journal*, *23*(2).

Aymerich, T., Martin, B., Garriga, M., & Hugas, M. (2003). Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages. *Applied and environmental microbiology, 69(8),* 4583-4594.

Barone, A. M., Banovic, M., Asioli, D., Wallace, E., Ruiz-Capillas, C., & Grasso, S. (2021). The Usual Suspect: How to Co-Create Healthier Meat Products. *Food Research International*, 110304.

Barreira, J. C., Ferreira, I. C., Oliveira, M. B. P., & Pereira, J. A. (2008). Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food chemistry, 107(3),* 1106-1113.

Bis-Souza, C. V., Penna, A. L. B., & da Silva Barretto, A. C. (2020). Applicability of potentially probiotic Lactobacillus casei in low-fat Italian type salami with added fructooligosaccharides: in vitro screening and technological evaluation. *Meat Science*, 108186.

Blajman, J., Gaziano, C., Zbrun, M. V., Soto, L., Astesana, D., Berisvil, A., ... & Frizzo, L. (2015). In vitro and in vivo screening of native lactic acid bacteria toward their selection as a probiotic in broiler chickens. *Research in veterinary science*, *101*, 50-56.

Bujalance, C., Jiménez-Valera, M., Moreno, E., & Ruiz-Bravo, A. (2006). A selective differential medium for Lactobacillus plantarum. *Journal of Microbiological Methods, 66(3),* 572-575.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of food engineering, 104(4),* 467-483.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72(2),* 303-311

Caplice, E., & Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International journal of food microbiology*, *50*(1-2), 131-149.

Casaburi, A., Aristoy, M. C., Cavella, S., Di Monaco, R., Ercolini, D., Toldrá, F., & Villani, F. (2007). Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Science*, 76(2), 295-307.

Casarotti, S. N., Borgonovi, T. F., Batista, C. L., & Penna, A. L. B. (2018). Guava, orange and passion fruit by-products: Characterization and its impacts on kinetics of acidification and properties of probiotic fermented products. *LWT*, 98, 69-76.

Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. F., & Charalampopoulos, D. (2011). Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *International Journal of Food Microbiology, 149(3*), 185–193.

Cirillo, M., Rizzello, F., Badolato, L., De Angelis, D., Evangelisti, P., Coccia, M. E., & Fatini, C. (2021). The effects of COVID-19 lockdown on lifestyle and emotional state in women

undergoing assisted reproductive technology: Results of an Italian survey. *Journal of Gynecology Obstetrics and Human Reproduction*, *50*(8), 102079.

Cocolin, L., Dolci, P., & Rantsiou, K. (2011). Biodiversity and dynamics of meat fermentations: the contribution of molecular methods for a better comprehension of a complex ecosystem. *Meat Science*, *89*(3), 296-302.

Dong, C., Wang, B., Li, F., Zhong, Q., Xia, X., & Kong, B. (2020). Effects of edible chitosan coating on Harbin red sausage storage stability at room temperature. *Meat science, 159*, 107919.

Do Espírito Santo, A. P., Cartolano, N. S., Silva, T. F., Soares, F. A., Gioielli, L. A., Perego, P., ... & Oliveira, M. N. (2012). Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts. *International Journal of Food Microbiology*, 154(3), 135-144.

Drosinos, E. H., Mataragas, M., Xiraphi, N., Moschonas, G., Gaitis, F., & Metaxopoulos, J. (2005). Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science*, *69*(2), 307-317.

Fernández–López, J., Viuda-Martos, M., Lucas-González, R., & Pérez-Álvarez, J. A. (2019). In Chestnut flour as source of dietary fiber and polyphenols. 19th ICC Conference. Vienna, Austria: *International Association for Cereal Science and Technology*, 24-25 April. Book of abstract.

Frumento, D., do Espirito Santo, A. P., Aliakbarian, B., Casazza, A. A., Gallo, M., Converti, A., & Perego, P. (2013). Development of milk fermented with Lactobacillus acidophilus fortified with Vitis vinifera marc flour. *Food Technology and Biotechnology, 51(3),* 370.

Gao, X., Xie, L., Wang, Z., Li, X., Luo, H., Ma, C., & Dai, R. (2013). Effect of postmortem time on the metmyoglobin reductase activity, oxygen consumption, and colour stability of different lamb muscles. *European Food Research and Technology*, *236*(4), 579-587.

Grimoud, J., Durand, H., Courtin, C., Monsan, P., Ouarné, F., Theodorou, V., & Roques, C. (2010). In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics. *Anaerobe*, *16*(5), 493-500.

Gosiewski, T., & Brzychczy-Włoch, M. (2015). The use of PFGE method in genotyping of selected bacteria species of the Lactobacillus genus. In *Pulse Field Gel Electrophoresis* (pp. 225-240). Humana Press, New York, NY.

Gullón, B., Gagaoua, M., Barba, F. J., Gullón, P., Zhang, W., & Lorenzo, J. M. (2020). Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. *Trends in Food Science & Technology*, *100*, 1-18.

Hedberg, M., Hasslöf, P., Sjöström, I., Twetman, S., & Stecksén-Blicks, C. (2008). Sugar fermentation in probiotic bacteria–an in vitro study. *Oral microbiology and immunology, 23(6),* 482-485.

Hilbig, J., Hartlieb, K., Herrmann, K., Weiss, J., & Gibis, M. (2020). Influence of phosphates and pH value on white efflorescence formation on dry fermented sausages. *European Food Research and Technology*, *246*(3), 471-484.

Hunt, M. C., King, A., Barbut, S., Clause, J., Cornforth, D., Hanson, D., ... & Weber, M. (2012). AMSA meat color measurement guidelines. *American Meat Science Association, Champaign, Illinois USA*, *61820*, 1-135.

Jin, S.-K., Choi, J. S., Yang, H.-S., Park, T.-S., & Yim, D.-G. (2018). Natural curing agents as nitrite alternatives and their effects on the physicochemical, microbiological properties and sensory evaluation of sausages during storage. *Meat Science, 146*, 34–40.

Kim, I. S., Jo, C., Lee, K. H., Lee, E. J., Ahn, D. U., & Kang, S. N. (2012). Effects of low-level gamma irradiation on the characteristics of fermented pork sausage during storage. *Radiation Physics and Chemistry*, *81*(4), 466-472.

Kołożyn-Krajewska, D., & Dolatowski, Z. J. (2012). Probiotic meat products and human nutrition. *Process Biochemistry, 47(12*), 1761-1772.Kuo, C. C., & Chu, C. Y. (2003). Quality characteristics of Chinese sausages made from PSE pork. *Meat science, 64(4),* 441-449.

Leistner, L., & Rodel, W. (2012, December). The Significance of Water Activity for Micro-organisms in Meats. In *Water Relations of Foods: Proceedings of an International Symposium held in Glasgow, September 1974* (p. 309). Elsevier.

de Llano, D. G., Gil-Sánchez, I., Esteban-Fernández, A., Ramos, A. M., Fernández-Díaz, M., Cueva, C., ... & Bartolomé, B. (2017). Reciprocal beneficial effects between wine polyphenols

and probiotics: an exploratory study. *European Food Research and Technology, 243(3),* 531-538.

Martín-Sánchez, A. M., Cherif, S., Vilella-Esplá, J., Ben-Abda, J., Kuri, V., Pérez-Álvarez, J. Á., & Sayas-Barberá, E. (2014). Characterization of novel intermediate food products from Spanish date palm (Phoenix dactylifera L., cv. Confitera) co-products for industrial use. *Food chemistry*, *154*, 269-275.

Nediani, M. T., García, L., Saavedra, L., Martínez, S., Lopez Alzogaray, S., & Fadda, S. (2017). Adding value to goat meat: biochemical and technological characterization of autochthonous lactic acid bacteria to achieve high-quality fermented sausages. *Microorganisms*, *5*(2), 26.

Ordóñez, J. A., Hierro, E. M., Bruna, J. M., & Hoz, L. D. L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical reviews in food science and nutrition*, *39*(4), 329-367.

Pavli, F. G., Argyri, A. A., Chorianopoulos, N. G., Nychas, G. J. E., & Tassou, C. C. (2020). Effect of Lactobacillus plantarum L125 strain with probiotic potential on physicochemical, microbiological and sensorial characteristics of dry-fermented sausages. *LWT*, 118, 108810.

Pacheco-Ordaz, R., Wall-Medrano, A., Goñi, M. G., Ramos-Clamont-Montfort, G., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2018). Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. *Letters in applied microbiology, 66(1),* 25-31.

Papadima, S. N., & Bloukas, J. G. (1999). Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science*, *51*(2), 103-113.

Pérez-Alvarez, J.A., Fernández-López, J. (2012). Chemical and Biochemical aspects of color in Muscle-Based Foods. In Food Biochemistry and Food Processing (2nd Edition). Ed. B.K. Simpson, L.M.L. Nollet, F. Toldrá, S. Benjakul, G. Paliyath, Hui, Y.H. Wiley-Blackwell. Sussex, UK. 317-330. ISBN-13:978-0-8138-0874-1/2012

Reinoso, B. D., Couto, D., Moure, A., Fernandes, E., Domínguez, H., & Parajó, J. C. (2012). Optimization of antioxidants–Extraction from Castanea sativa leaves. *Chemical Engineering Journal, 203, 101-109*.

Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. *Food research international, 43(1),* 1-7.

Rebucci, R., Sangalli, L., Fava, M., Bersani, C., Cantoni, C., & Baldi, A. (2007). Evaluation of functional aspects in Lactobacillus strains isolated from dry fermented sausages. *Journal of food quality*, *30*(2), 187-201.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., ... & Muñoz, R. (2009). Food phenolics and lactic acid bacteria. *International journal of food microbiology*, 132(2-3), 79-90.

Rosmini, M. R., Perlo, F., Pérez-Alvarez, J. A., Pagán-Moreno, M. J., Gago-Gago, A., López-Santoveña, F., et al. (1996). TBA test by an extractive method applied to 'paté'. *Meat Science, 42(1),* 103–110.

Rubio, R., Jofré, A., Aymerich, T., Guàrdia, M. D., & Garriga, M. (2014). Nutritionally enhanced fermented sausages as a vehicle for potential probiotic lactobacilli delivery. *Meat Science, 96(2),* 937-942.

Ruiz-Moyano, S., Martín, A., Benito, M. J., Hernández, A., Casquete, R., & de Guia Córdoba, M. (2011). Application of Lactobacillus fermentum HL57 and Pediococcus acidilactici SP979 as potential probiotics in the manufacture of traditional Iberian dry-fermented sausages. *Food Microbiology, 28(5),* 839-847.

Salueña, B. H., Gamasa, C. S., Rubial, I. M. D., & Odriozola, C. A. (2019). CIELAB color paths during meat shelf life. *Meat science*, *151*, 107889.

Sánchez-Zapata, E., Fuentes-Zaragoza, E., De Vera, C. N. R., Sayas, E., Sendra, E., Fernández-López, J., & Pérez-Alvarez, J. A. (2011). Effects of tuna pâté thickness and background on CIEL∗ a∗ b∗ color parameters and reflectance spectra. *Food Control*, *22*(8), 1226-1232.

Sánchez-Zapata, E., Díaz-Vela, J., Pérez-Chabela, M. L., Pérez-Alvarez, J. A., & Fernández-López, J. (2013). Evaluation of the effect of tiger nut fibre as a carrier of unsaturated fatty acids rich oil on the quality of dry-cured sausages. *Food and bioprocess technology*, *6*(5), 1181-1190.

Sayas-Barberá, E., Viuda-Martos, M., Fernández-López, F., Pérez-Alvarez, J. A., & Sendra, E. (2012). Combined use of a probiotic culture and citrus fiber in a traditional sausage 'Longaniza de Pascua'. *Food Control*, *27*(2), 343-350.

Prandl, O., Fischer, A., Schmidhofer, T., & Sinell, H. J. (1994). *Tecnología e higiene de la carne* (No. TS1975. T42 1994.). Zaragoza: Acribia.

Shori, A. B. (2015). The potential applications of probiotics on dairy and non-dairy foods focusing on viability during storage. *Biocatalysis and Agricultural Biotechnology, 4(4)*, 423-431.

Slima, S. B., Ktari, N., Trabelsi, I., Triki, M., Feki-Tounsi, M., Moussa, H., ... & Salah, R. B. (2017). Effect of partial replacement of nitrite with a novel probiotic Lactobacillus plantarum TN8 on color, 20hysic-chemical, texture and microbiological properties of beef sausages. *LWT*, 86, 219-226.

Sirini, N., Roldán, A., Lucas-González, R., Fernández-López, J., Viuda-Martos, M., Pérez-Álvarez, J. A., ... & Rosmini, M. R. (2020). Effect of chestnut flour and probiotic microorganism on the functionality of dry-cured meat sausages. *LWT*, 134, 110197.

Sirini, N., Frizzo, L. S., Aleu, G., Soto, L. P., & Rosmini, M. R. (2021). Use of probiotic microorganisms in the formulation of healthy meat products. *Current Opinion in Food Science,* 38, 141-146.

de Souza, E. L., de Albuquerque, T. M. R., Dos Santos, A. S., Massa, N. M. L., & de Brito Alves, J. L. (2019). Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities–a review. *Critical reviews in food science and nutrition,* 59(10), 1645-1659.

Turantaş, F., & Kemahlıoğlu, K. (2012). Fate of some pathogenic bacteria and molds in Turkish Tarhana during fermentation and storage period. *Journal of food science and technology, 49(5*), 601-607.

Vermeulen, A., Marvig, C. L., Daelman, J., Xhaferi, R., Nielsen, D. S., & Devlieghere, F. (2015). Strategies to increase the stability of intermediate moisture foods towards Zygosaccharomyces rouxii: the effect of temperature, ethanol, pH and water activity, with or without the influence of organic acids. *Food microbiology*, 45, 119-125.

Wenjiao, F., Yunchuan, C., Junxiu, S., & Yongkui, Z. (2014). Effects of tea polyphenol on quality and shelf life of pork sausages. *Journal of Food science and Technology*, *51*(1), 191-195.

Zhang, W., Xiao, S., Samaraweera, H., Lee, E. J., & Ahn, D. U. (2010). Improving functional value of meat products. *Meat science,* 86(1), 15-31.

Table 1. TBARS values (mean ± standard deviation) during 43 d of storage of Longaniza de Pascua at room and cold storage temperature.

		TBA (mg malonaldhyde/kg)		
Type of sausage	**Time (d)**	**Room storage (20 °C)**	**Cold storage (4 °C)**	**SEM**
CL	0	0.27^{b}	0.27^{a}	0.02
	15	0.26^{b}	0.32^{a}	0.01
	22	0.52^{a}	0.33^{a}	0.04
	43	0.70^{a}	0.33^{a}	0.03
	F.E	0.32^{A}	$0.24^{A,B}$	0.05
CPL	0	0.25^{a}	$0.2[illegible]^{a}$	0.04
	15	0.23^{a}	$0.2[illegible]^{a}$	0.03
	22	0.58^{a}	0.22^{a}	0.19
	43	0.43^{a}	0.34^{a}	0.05
	F.E	0.37^{A}	0.28^{A}	0.05
PL	0	0.13^{b}	0.13^{b}	0.01
	15	0.14^{b}	$0.16^{a,b}$	0.01
	22	0.21^{a}	0.12^{b}	0.02
	43	0.29^{a}	0.23^{a}	0.01
	F.E	0.21^{A}	0.18^{B}	0.07
L	0	0.14^{b}	0.14^{b}	0.01
	15	0.13	0.13^{b}	0.00
	22	0.21^{b}	0.08^{b}	0.01
	43	0.36^{a}	0.31^{a}	0.01
	F.E	0.43^{A}	0.28^{A}	0.17

***F.E**: Fixed Effect Average. Capital letters represent treatment effects. Lowercase letters represent storage time effects. **CL**: 3% chestnut flour; **CPL**: 3% chestnut flour and 8.5 log CFU/g L. plantarum; **PL**: 8.5 log CFU/g L. plantarum; **L**: control.*

Table 2. Colour parameters (mean ± standard deviation) of Longaniza de Pascua at room (20 °C) and cold (4 °C) storage temperature.

Storage temperature	**Type of sausage**	**Time (d)**	**L***	**a***	**b***	**C***	**h***
	CL	0	45.5 ± 3.6^{a}	4.4 ± 1.9^{b}	3.5 ± 1.5^{b}	5.7 ± 2.2^{b}	34.7 ± 6.8^{a}
		15	41.0 ± 2.5^{b}	$5.0\pm2.0^{a,b}$	$4.3\pm3.0^{a,b}$	$6.7\pm3.3^{a,b}$	46.0 ± 4.5^{a}
		22	$42.4\pm3.1^{a,b}$	7.4 ± 1.8^{a}	6.6 ± 1.8^{a}	10.0 ± 2.2^{a}	42.2 ± 5.7^{a}
		43	$42.2\pm3.6^{a,b}$	4.3 ± 1.8^{b}	$4.\pm1.4^{b}$	5.9 ± 2.0^{b}	55.8 ± 7.2^{a}
		F.E	42.8 ±	5.3 ±	4.6 ± 2.2^{C}	7.0 ± 2.9^{C}	40.9 ±

(20°C)			3.5	2.2			11.6
	CPL	0	42.2±2.0^{a}	4.5±1.0^{b}	3.2±1.0^{c}	5.4±1.4^{c}	39.2±11.0^{c}
		15	43.0±2.3^{a}	8.4±1.2^{a}	8.6±0.8^{b}	12.1±1.4^{b,a}	36.6±11.5^{b}
		22	40.8±3.0^{a}	8.1±2.0^{a}	7.3±1.4^{b}	11.0±1.9^{b}	41.4±7.0^{c,b}
		43	42.5±2.8^{a}	7.7±2.0^{a}	11.2±0.9^{a}	13.7±4.6^{a}	46.3±15.3^{a}
		F.E	42.2 ± 2.6^{A}	7.2± 2.2^{A}	7.6 ±3.1^{A}	10.6 ±3.4^{A}	44.7 ±9.7^{A}
	PL	0	42.2±1.6^{a}	4.4±1.3^{b}	2.7±0.8^{c}	5.2±1.5^{b}	31.3±4.2^{b}
		15	37.4±2.1^{b}	7.6±1.0^{a}	7.2±1.1^{a}	10.5±1.2^{a}	43.5±5.3^{a}
		22	39.4±2.2^{b}	7.2±0.9^{a}	5.6±1.0^{b}	9.1±1.0^{a}	37.6±5.5^{a}
		43	42.2±1.3^{a}	4.4±1.3^{b}	7.0±0.3^{a}	5.2±1.5^{b}	31.3±4.2^{b}
		F.E	40.2± 2.9^{B}	6.0 ±1.8^{B}	5.7± 2.4^{B}	8.4 ±2.5^{B}	41.8 ±11.0^{A,B}
	L	0	45.0±3.5^{a}	5.5±1.7^{b}	4.1±1.4^{b}	6.9±2.2^{b}	37.0±4.6^{b}
		15	38.0±3.4^{b}	9.7±2.1^{a}	8.7±1.6^{a}	13.0±2.3^{a}	42.1±6.3^{b}
		22	41.7±4.1^{a,b}	8.6±1.3^{a}	8.0±1.9^{a}	11.9±1.5^{a}	43.0±7.7^{b}
		43	41.7±2.8^{a,b}	4.9±1.4	8.9±2.9^{a}	8.9±2.3^{b}	54.6±10.8^{a}
		F.E	41.8± 4.0^{A}	7.0± 2.7	6.0 ±2.9^{B}	9.2 ±3.8^{B}	38.3 ±7.4^{B}
(4°C)	**CL**	0	45.6±3.6^{a}	4.4±1.9^{b}	3.5±1.5^{c}	5.7±2.2^{c}	37.0±4.6^{a}
		15	45.5±3.8^{a}	8.0±1.7^{a}	8.6±2.7^{a,b}	11.9±2.7^{a}	46.5±11.1^{a}
		22	44.3±1.9^{b}	9.4±2.0^{a}	9.4±1.8^{a}	13.3±2.5^{a}	44.3±4.8^{a}
		43	45.5±4.5^{a}	6.6±3.0^{a,b}	6.7±2.0^{b}	9.5±3.4^{b}	45.4±6.8^{a}
		F.E	42.7 ±4.0^{A}	7.3±2.6^{A}	7.0± 2.9^{A}	10.2± 3.7^{A}	42.8 ±7.9^{A,B}
	CPL	0	42.2±2.1^{a}	4.5±1.0^{b}	3.2±1.0^{b}	5.3±1.4^{b}	39.2±11.0^{b}
		15	40.8±3.5^{a}	9.4±1.2^{a}	8.1±1.5^{a}	11.9±1.7^{a}	46.4±8.0^{a,b}
		22	40.6±3.7^{a}	7.3±2.6^{a}	7.3±2.46^{a}	10.4±2.7^{a}	45.2±4.2^{a}
		43	39.5±2.0^{a}	7.6±1.6^{a}	6.3±1.1^{a}	10.3±1.8^{a}	48.0±11.3^{a,b}
		F.E	40.6± 3.5^{B}	7.3 ±2.5^{A}	6.4± 2.5^{A}	9.8 ±3.3^{A}	40.5 ±7.9^{A,B}
	PL	0	42.2±1.6^{a}	4.4±1.3^{b}	2.7±0.8^{c}	5.2±1.5^{b}	34.7±6.8^{c}
		15	40.8±1.7^{a,b}	7.9±1.5^{a}	8.1±1.5^{a}	11.4±1.8^{a}	40.6±5.5^{a}
		22	40.0±3.0^{a,b}	8.4±1.7^{a}	6.2±1.8^{b}	10.5±2.3^{a}	45.6±7.7^{b,c}
		43	38.6±3.9^{b}	8.1±1.9^{a}	7.0±1.3^{a,b}	10.4±1.7^{a}	40.2±4.8^{a,b}
		F.E	40.3± 2.7^{B}	7.0± 2.2^{A}	6.0 ±2.8^{A}	9.4 ±3.2^{A}	39.6 ±8.2^{B}
	L	0	45.0±3.5^{a}	5.4±1.7^{b}	4.1±1.4^{b}	6.9±2.1^{b}	31.3±4.3^{b}
		15	43.0±2.0^{a,b}	7.2±1.9^{a,b}	8.1±2.7^{a}	11.0±2.9^{a}	46.0±5.4^{a}
		22	40.2±3.0^{b,c}	8.5±2.6^{a}	8.2±2.5^{a}	11.8±3.4^{a}	35.9±5.0^{a,b}

43	38.0±2.8	7.3±2.2 [b]	7.3±1.9	10.4±2.0 [b]	41.3±8.5
F.E	43.4 ±3.9[A]	7.0± 2.9[A]	6.8 ±2.9[A]	9.8 ±3.5[A]	44.0 ±9.3[A]

F.E: *Fixed Effect Average. Capital letters represent treatment effects. Lowercase letters represent storage time effects.* ***CL****: 3% chestnut flour;* ***CPL****: 3% chestnut flour and 8.5 log CFU/g L. plantarum;* ***PL****: 8.5 log CFU/g L. plantarum;* ***L****: control.*

Table 3: Colour Differences (ΔE*) during storage at room (20 °C) and cold (4 °C) storage temperature.

Storage temperature	Day	ΔE CL	ΔE CPL	ΔE PL
20 °C	0	1.33	3.11	3.31
	15	7.06	5.15	2.64
	22	1.97	1.20	3.62
	43	4.91	3.76	1.93
4 °C	0	1.33	3.11	3.31
	15	2.62	3.08	2.30
	22	1.50	1.49	2.00
	43	7.57	1.86	1.07

CL*: 3% chestnut flour;* ***CPL****: 3% chestnut flour and 8.5 log CFU/g L. plantarum;* ***PL****: 8.5 log CFU/g L. plantarum;* ***L****: control.*

Fig. 1a. Changes in pH during 43 d at room storage (20 °C). Letters represent significant differences between treatments per day.

Fig. 1b. Changes in pH during 43 d at cold storage (4 °C). Letters represent significant differences between treatments per day.

Fig. 2a. *L. plantarum* viability at room storage (20 °C). Letters represent significant differences between treatments per day.

Fig. 2b. *L. plantarum* viability at cold storage (4 °C). Letters represent significant differences between treatments per day.

Fig. 3a. LAB viability at room storage (20 °C). Letters represent significant differences between treatments per day.

Fig. 3b. LAB viability at cold storage (4 °C). Letters represent significant differences between treatments per day.

Fig. 4. PFGE patterns of digested genomic DNA of *L. plantarum* isolated from pharmaceutical product (1), *L. plantarum* spray dried powder (2), *L. plantarum* inocula used in CPL (3), *L. plantarum* at day zero in CPL treatment (4), *L. plantarum* at day 43 in CPL treatment (5), *L. plantarum* inocula used in PL (6), L. plantarum at day zero in PL treatment (7) and *L.*

plantarum at day 43 in PL treatment (8). Letters a and b represent Ladder PTG Marker (New England Biolabs, Ipswich, USA).

Highlights

- The addition of chestnut flour in Longaniza de Pascua caused a higher *L. plantarum* counts at final of storage.
- Vacuum packaged Longaniza de Pascua with *L. plantarum* and chestnut flour could be stored at 20 °C or 4 °C up to 43 d without causing any rancidity problem.
- The *L. plantarum* addition to Longaniza de Pascua requires cold storage temperature to keep the probiotic viability.
- The hue always remained around red, which is typical of Longaniza de Pascua.

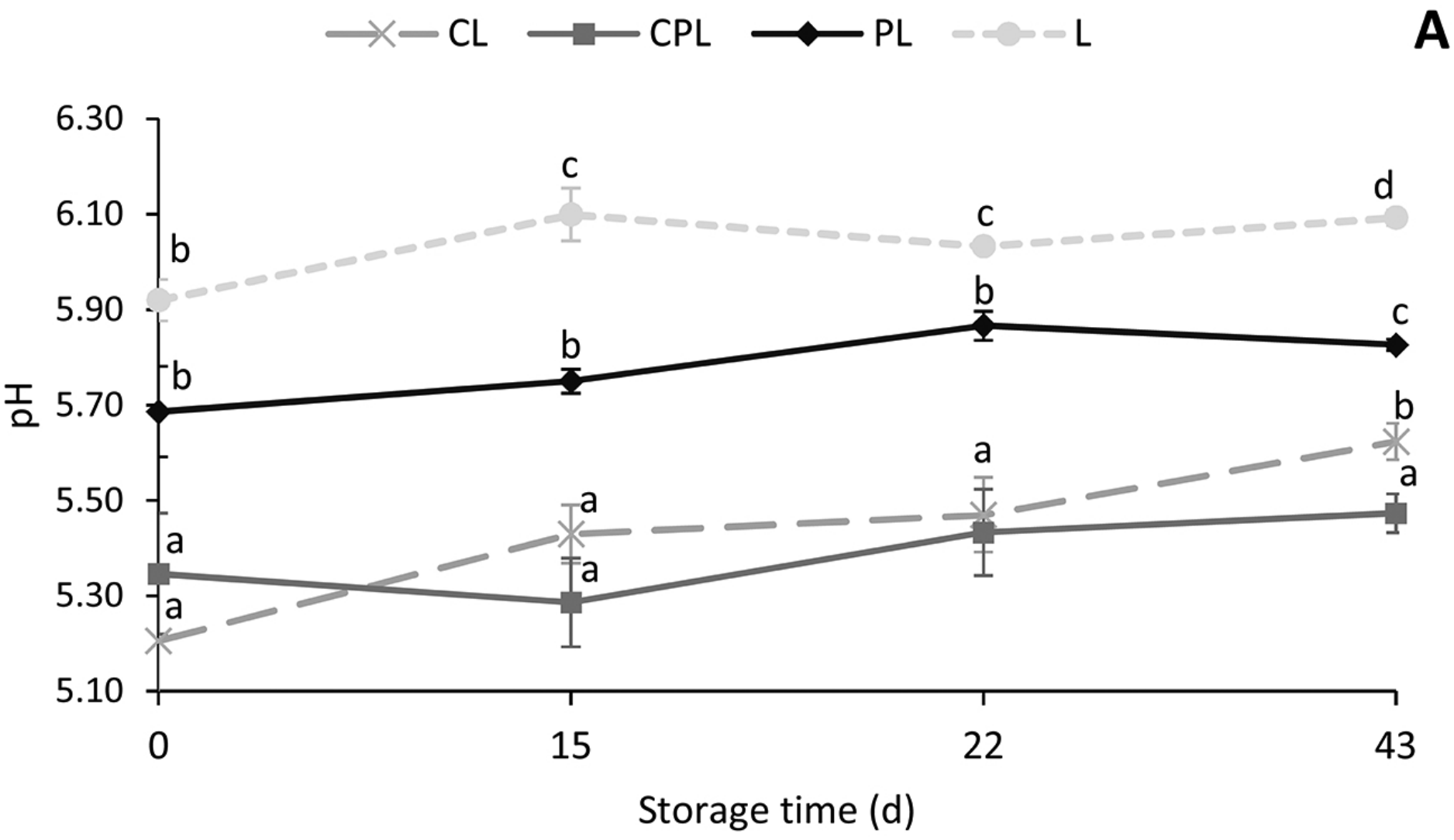
CL
CPL
PL
L
A
6.30
6.10
5.90
5.70
5.50
5.30
5.10
pH
0
15
22
43
Storage time (d)
a
a
a
a
a
a
b
b
b
b
c
c
c
d

Figure 1a

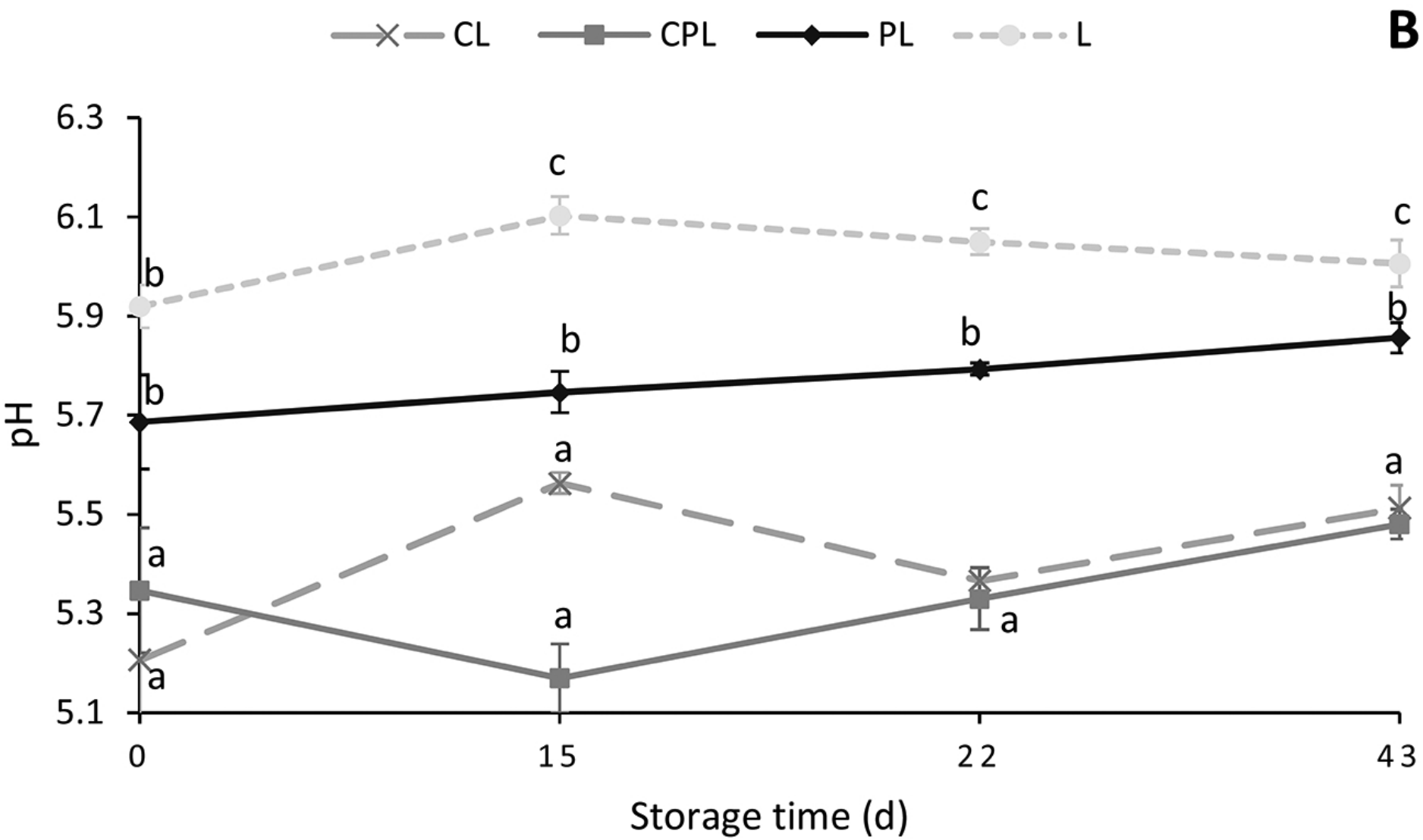

Figure 1b

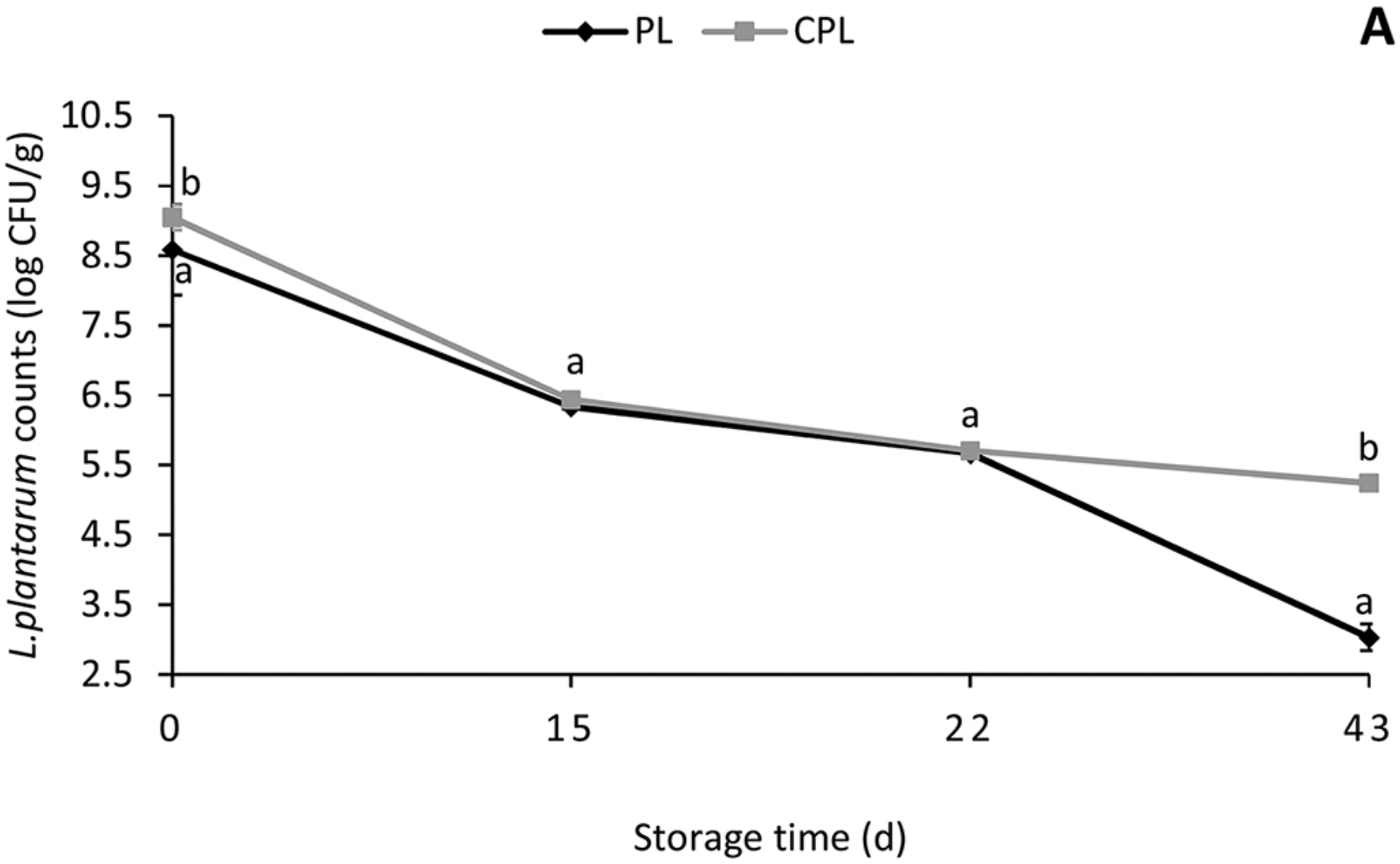
PL
CPL
A
L.plantarum counts (log CFU/g)
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
b
a
a
a
b
a
0
15
22
43
Storage time (d)

Figure 2a

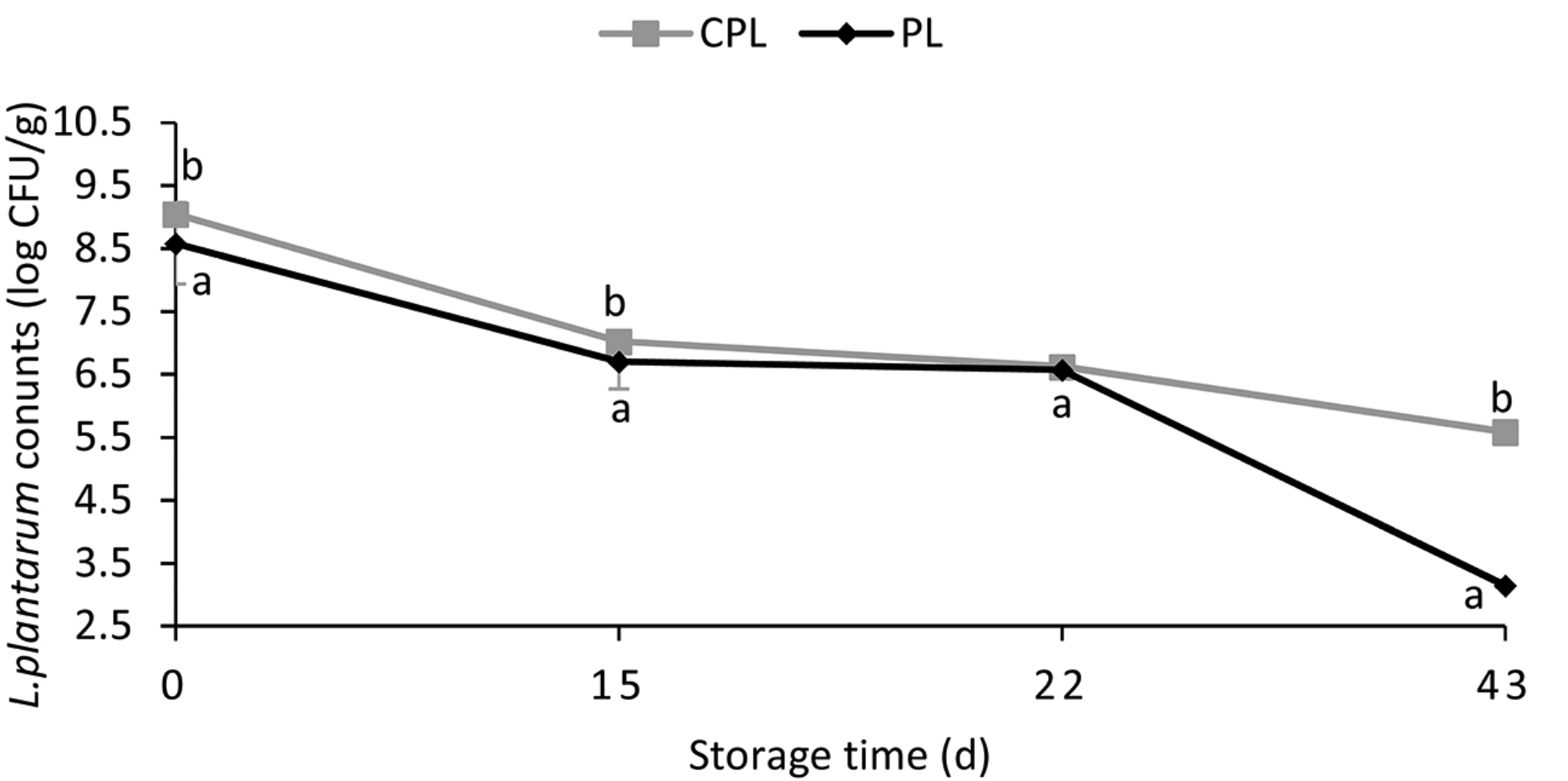
B
CPL
PL
L.plantarum counts (log CFU/g)
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
b
a
b
a
a
b
a
0
15
22
43
Storage time (d)

Figure 2b

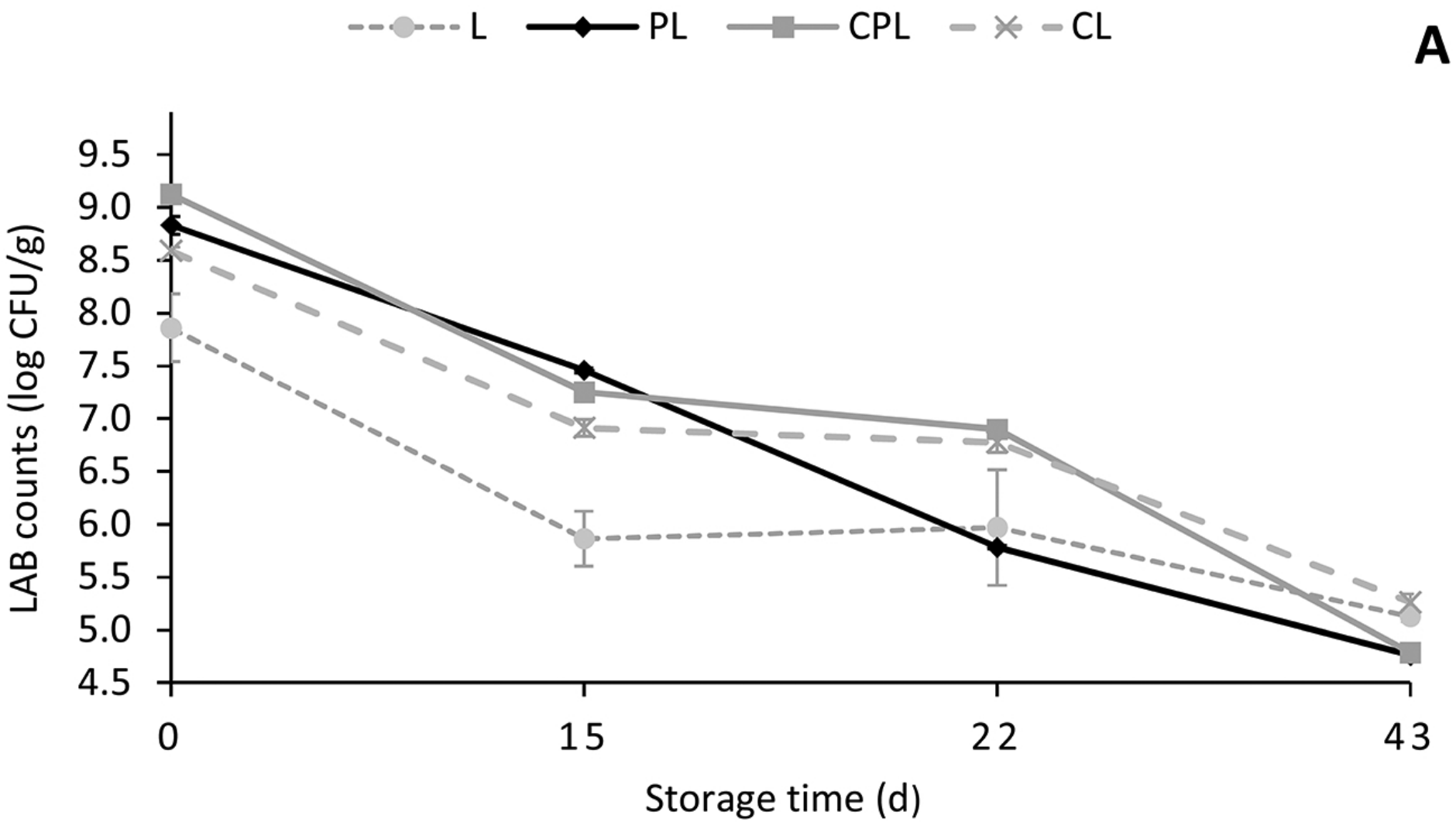
L
PL
CPL
CL
A
LAB counts (log CFU/g)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
0
15
22
43
Storage time (d)

Figure 3a

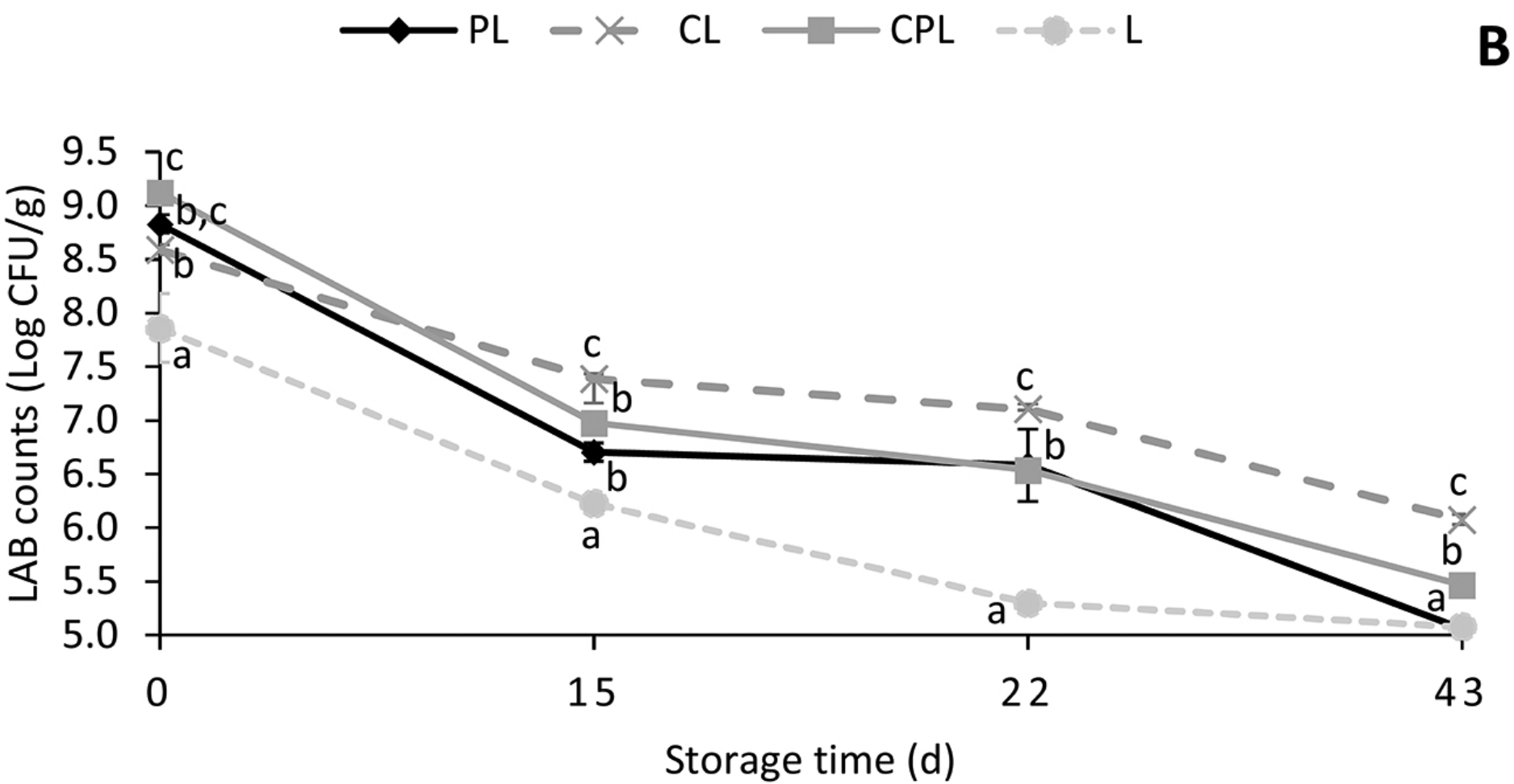
PL
CL
CPL
L
B
LAB counts (Log CFU/g)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
0
15
22
43
Storage time (d)
c
b,c
b
a
c
b
b
a
c
b
a
c
b
a

Figure 3b

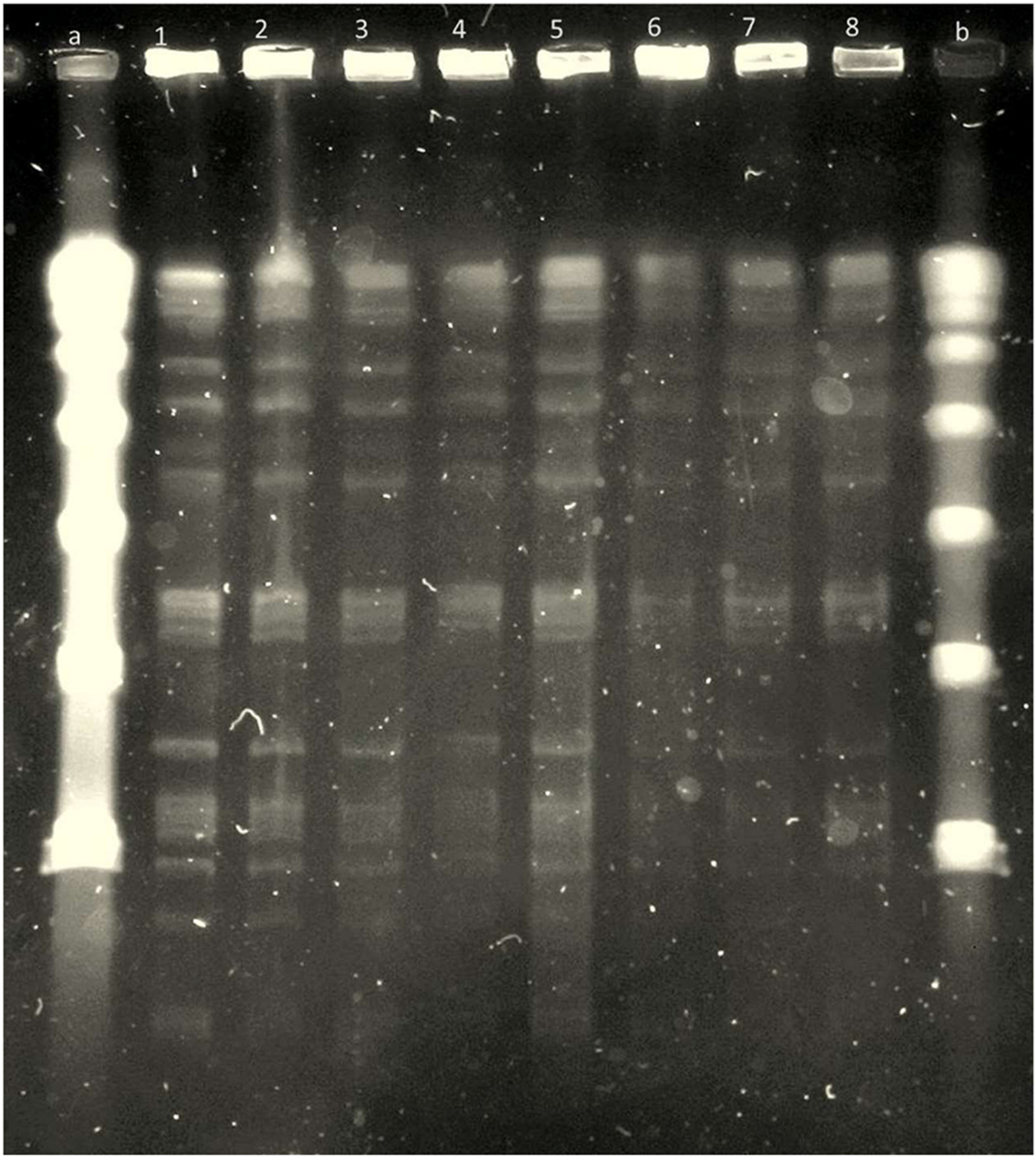

Figure 4