PE4.109 Safety of a Traditional Low Acid Sausage with Eco-efficiently Obtained Orange Fiber and Lactobacillus casei CECT 475 399.00

Ana María Martín-Sánchez (1), Esther Sendra(1) esther.sendra@umh.es, Estrella Sayas (1), Juana Fernández-López (1) José Ángel Pérez-Álvarez (1)
(1)All authors are in the IPOA Research Group, Food Technology Department, Universidad Miguel Hernández, Grupo REVIV. Ctra. de Beniel km 3.2, 03312, Orihuela (Alicante) SPAIN. (corresponding author phone: 0034 966749735; fax: 0034 966749677; e-mail: esthe

Abstract—Microbial populations of a low acid sausage 'Longaniza de Pascua' including 1% orange fiber and Lactobacillus casei CECT 475 have been studied during 8 days of drying time. At the end of ripening pH of all types of sausage was higher than 5.3 and water activity was below 0.91. L. casei CECT 475 acted as a starter culture accelerating the decline in pH. The incorporation of 1% orange fiber favored the growth of lactic acid bacteria, Lactobacilli and Micrococci. Sausages made with orange fiber and L. casei CECT 475 had better guarantees of food safety, due to its low pH and water activity, and decreased counts of Enterobacteria.

I. INTRODUCTION

'LONGANIZA de Pascua' is a traditional low acid Spanish sausage, whose final pH is above 5.3. Such sausages are produced by spontaneous fermentation, and consumers reject an excessive acid taste [14]. Sausage drying takes place at low temperature, enhanced by their thin diameter (about 15 mm) which allows a rapid decrease of water activity and constitutes their first defense against the proliferation of pathogens. Sausages made with starter cultures usually contain a high number of Lactobacilli. The most common species of Lactobacilli in starter cultures are: L. plantarum, L. sake, L. curvatus. The most common meat starter cultures used in Spain contain, other than Lactobacilli, are Pediococcus pentosaceus, Staphylococcus xylosus and Micrococci, while in the rest of Europe there is a greater predominance of other lactic acid bacteria [11]. Fadda et al., [5,6] studied the effect of Lactibacillus casei (along with conventional starter cultures) in the process of proteolysis during fermentation of sausages. The interest of the addition of these cultures was their ability to acidify and release bacteriocins which could accelerate ripening and enhance food safety. Initial counts of L. casei were 7 log CFU / g, and increased to 8 log CFU / g, to stay until the end of fermentation. Orange fiber addition to fermented milks has been reported to enhance the growth and metabolism of L. casei [13]. The addition of citrus fiber to dry-cured sausages has been reported to reduce residual nitrite [1,2], and enhance the growth of Microccoci [7]. In the present study, we aim to evaluate microbial populations of 'Longaniza de Pascua' manufactured with the addition of orange fiber (eco-efficiently obtained from orange juice by products) and a potentially probiotic strain that has been successfully added to dairy products [13] and for which the sausage can be an excellent carrier: as it is not subject to heat treatment and have a pH higher than that of fermented milks.

II. MATERIALS AND METHODS

A. Sausage manufacture

Eco-efficiently extracted fiber from orange juice by products was prepared [8].Four types of 'Longaniza de Pascua' were manufactured following a traditional formula (60% lean meat, 40% pancetta. Other ingredients were calculated on meat basis: 5% water, 2% salt, 0.2% dextrose, 0.02% pepper, 0.005% ascorbic acid, 10 mg/kg nitrite, 0.001% anis). Type A: control, Type B including 1% orange fiber, Type C inoculated with Lactobacillus casei CECT 475; Type D: with 1% orange fiber and inoculated with Lactobacillus casei CECT 475. The procedure is described in Figure 1

B. Analytical determinations

Samples were taken at days 0, 1, 3, 6 and 8 of drying for analysis. pH and water activity were determined. The following microbial analysis were run: aerobic mesophilic bacteria (AMB) using PetrifilmTM incubated at 35ºC for 48 h.; Micrococci in Manitol Salt agar incubated at 35ºC for 48 h.; lactic acid bacteria (LAB) in Petrifilm TM plates, using Man Rogosa Sharpe broth as suspension media, incubation took place under anaerobic conditions at 37ºC for 48 h.; counts on Man Rogosa Sharpe agar with vancomycine were determine as estimates of counts of L. casei, although L. plantarum, L. sakei and other meat Lactobacilli may grow in this media. Incubation took place under anaerobiosis, at 37ºC for 48 h. Counts of moulds and yeasts were obteined in Bengala Rose Agar, incubated at 28ºC for 5 days. Counts of Enterobacteria were obtained in Enterobacteria plates PetrifilmTM incubated at 37ºC for 24 h.

C. *Statistical Analysis*

Three independent replicates of each experiment were run. All determinations were run in duplicate. General Lineal Model procedure together with Multivariate ANOVA was used to analyze results to study the effect of 2 fixed effects: drying time and sausage type. Nine levels for drying time (0, 1, 2, 3, 4, 5, 6, 7 and 8 day) and four levels for sausage type (A, B, C, D). Tukey test was applied for comparisons among means (confidence level 0.05%). SPSS 16.0 for Windows was used (SPSS Inc., Chicago, Illinois, USA, 2008).

III. RESULTS AND DISCUSSION

The pH was between 5.42 and 5.59 (Figure 2), coinciding with the findings in Italian low acid sausages [4]. As already mentioned the Longaniza de Pascua is a low-acid sausage, due to its final pH above 5.3 [14]. All independent variables that significantly affect the pH studied (sausage type and time) (Table 1). Longaniza with fiber and L. casei showed the lowest pH values in line with its large population of lactic bacteria, and the highest pH was for Longaniza control. The presence of fiber helps in lowering pH. Samples with starter cultures, have higher population of lactic bacteria that consume the sugars and release a greater proportion of acid batch control and hence a lower pH. Furthermore, the fiber served as a substrate for lactic acid bacteria and thus enhanced their metabolism and the consequent decrease in pH. Meat products of pH above 5.2 require a water activity below 0.91 to be self-stable, these products require no refrigeration and their shelf life is not only limited by microbial growth, but by physical and chemical spoilage [9]. Longaniza the Pascua meets these characteristics (Figure 2).

It can be seen that no significant differences in terms of sausage type ($P > 0.05$) were observed, whereas highly significant differences ($P < 0.01$) were due to drying time (Table 2). Water activity of low acid Italian sausages is between 0.80 and 0.84 [4]. Sausage type and time significantly affected AMB counts (Table 3), which increased with drying time and were lowest in sausages containing L. casei CECT 475 (Figure 3). LAB and L. casei, due to their anaerobic nature may have not been fully recovered in the medium and incubation conditions of AMB. LAB counts were significantly affected by sausage type and drying time (Table 4, Figure 4), which increased with drying time and the presence of L. casei. Spaziani et al., [14] detected LAB counts in the order of 8-9 log CFU / g Italian sausages with low acidity. LAB has been reported to be the predominant microbiota in fermented Italian sausages with counts of 8 log CFU / g [4]. Counts of 7-8-9 log CFU / g of LAB have been reported by other authors [3,10]. In orange fiber enriched sausages, similar AMB and LAB counts to those of the present study have been reported [7]. Counts of moulds and yeasts were high (Figure 5) and both sausage type and drying time significantly affected such counts (Table 5). Counts increased with time and were much higher in sausages with orange fiber. The reported high counts of these sausages may have been due to their small diameter

which a high relation surface/volume. Longaniza de Pascua is usually consumed with the casing, and so the analyzed aliquots included the casings. Reported counts of moulds and yeast are much lower but are referred to sausages of much higher diameter: 2 log units CFU / g at the beginning of fermentation, increasing in control sausages but stable in sausages with starter cultures [4]; about 3 log CFU / g in salchichón stable from day 9 [7]; above 4 log CFU / g in sobrasada [12]. No references have been found with counts as high as the reported in our study. Counts of Microccoci were significantly affected by sausage type and drying time (Table 6), which increased with fiber presence and drying time (Figure 6).

Increased populations of microccoci in salchichón due to the addition of 1% orange fiber had been previously reported [7]. Other reported counts of Microccoci in dry-cured sausages are: 6 log UFC/100g in low acid Italian sausages [14]; 4-5 log CFU / g in Italian sausages, even when Microccoci were added as starter culture and the initial population of 7 log units, it was subsequently decreased to 5, probably by the injurious effects of the acid liberated during curing [4]; 3-4.5 log CFU / g at 8 days of ripening [3]; about 4 log CFU / g [10]. In our case the observed Micrococci counts were higher than those observed by other authors, probably due to the high pH of this type of sausage that allows a greater survival and multiplication of this group of microorganisms, together with the beneficial effect of the presence of fiber. Estimate counts of L. casei obtained in MRS Vancomycine were affected by sausage type and drying time (Table 7).

It is not surprising that sausages without addition of L. casei presented microbial growth in this medium since L. plantarum, L. sakei or L. curvatus can grow well in this environment and are common in the microbiota of fermented meat [11]. All counts increased (Figure 7) with drying time and the addition of L. casei. The presence of fiber would have favored the development of this group of lactobacilli. Counts of Enterobacteria were significantly affected by drying time and sausage type (Table 8), which increased with time and were lowest in sausages containing L. casei (Figure 8).

The presence of fiber also contributed to decreased counts of enterobacteria. Other authors reported decreased counts of enterobacteria when starter cultures were added [4], propably due the decrease in pH caused by bacterial metabolism. In dry-cured sausages counts of Enterobacteriaceae tend to decrease with curing time: from 4 log CFU / g at 15 days and decreasing to undetectable after 35 days [10]. In orange fiber enriched salchichón initial counts of Enterobacteria of 2 log CFU / g decreased to undetectable at 23 days of curing [7]. During curing of sobrasada, non detectable Enterobacteria have been reported from 30 days of curing [12]. In our case there is no cure for such time and therefore Enterobacteria are still present at 8 days of curing.

IV. CONCLUSION

The addition of Lactobacillus casei CECT 475 to a low acid sausage 'Longaniza de Pascua' accelerates the curing process by accelerating the decline in pH, acting as a starter culture. The incorporation of 1% orange fiber promotes the growth and survival of Lactobacilli and Micrococci. Sausages made with orange fiber and L. casei CECT 475 have better guarantees of food safety, due to its low pH and water activity, and this effect is reflected in decreased counts of Enterobacteria. Low acid sausages can be a good vehicle for probiotic bacteria, as they allow high viability of the bacteria added, the addition of orange fiber further enhances the microbial quality of the sausages. Further studies on probiotic verified bacteria addition to meat products would be of great interest, especially if combined with proper fibers.

ACKNOWLEDGEMENT

Authors are grateful to the Caja de Ahorros del Mediterráneo (CAM).

REFERENCES

[1] Alesón-Carbonell, L.; Fernández- López, J.; Sayas-Barberá, E.; Sendra, E.; Pérez-Álvarez, J.A. (2003). Utilization of lemon albedo in dry-cured sausages. Journal of Food Science, vol.68: 1826-1830.

[2] Alesón-Carbonell, L.; Fernández- López, J.; Sendra, E.; Sayas-Barberá, E.; Pérez-Álvarez, J.A. (2004). Quality charasteristics of a non-fermented dry-cured sausages formulated with lemon albedo. Journal of the Sicence of Food and Agriculture, 84: 2077-2084.

[3] Bruna, J.M.; Ordóñez, J.A.; Fernández, M.; Herranz, B.; de la Hoz, L. (2001): Microbial and physico-chemical change during the ripening of dry fermented sausages superficially inoculated with or having added an intracellular cell-free extract of Penicillinum aurantiogriseum. Meat Science, 59: 87-96.

[4] 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 startes cultures. Meat Science 76: 295-307.

[5] Fadda, S.; Oliver, G.; Vignolo, G. (2002): Protein degradation by Lactobacillus plantarum and Lactobacillus casei in a sausage model system. Journal of Food Science Vol.67, Nr 3.

[6] Fadda, S.; Vignolo, G.; Aristoy, M.-C.; Toldrá, F. (2001): Effect of curing conditions and Lactobacillus casei CRL705 on the hydrolysis of meat proteins. Journal of Applied Microbiology 91: 478-487.

[7] Fernández-López, J.; Sendra, E.; Sayas-Barberá, E.; Navarro, C.; Pérez-Álvarez, J.A. (2008): Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. Meat Science, 80: 410-417.

[8] García Pérez, F.J.; Sendra, E.; Fernández-Ginés, J.M.; Fernández-López, J.; Sayas, E.; Pérez-Álvarez, J.A. (2003). Características de fibra de subproductos de la industria de zumo de naranja. Alimentaria, 345: 71-74.

[9] Leistner L., Roedel W. (1975). The significance of water activity for microorganisms in meat. En: R.B. Duckworht (Ed.), Water relations of foods. Academic Press, Londres pp: 309-323.

[10] Lizaso, G.; Chasco, J.; Beriain, M.J. (1999): Microbiological and biochemical changes during ripening of salchichón, a Spanish dry cured sausage. Food Microbiology 16: 219-228.

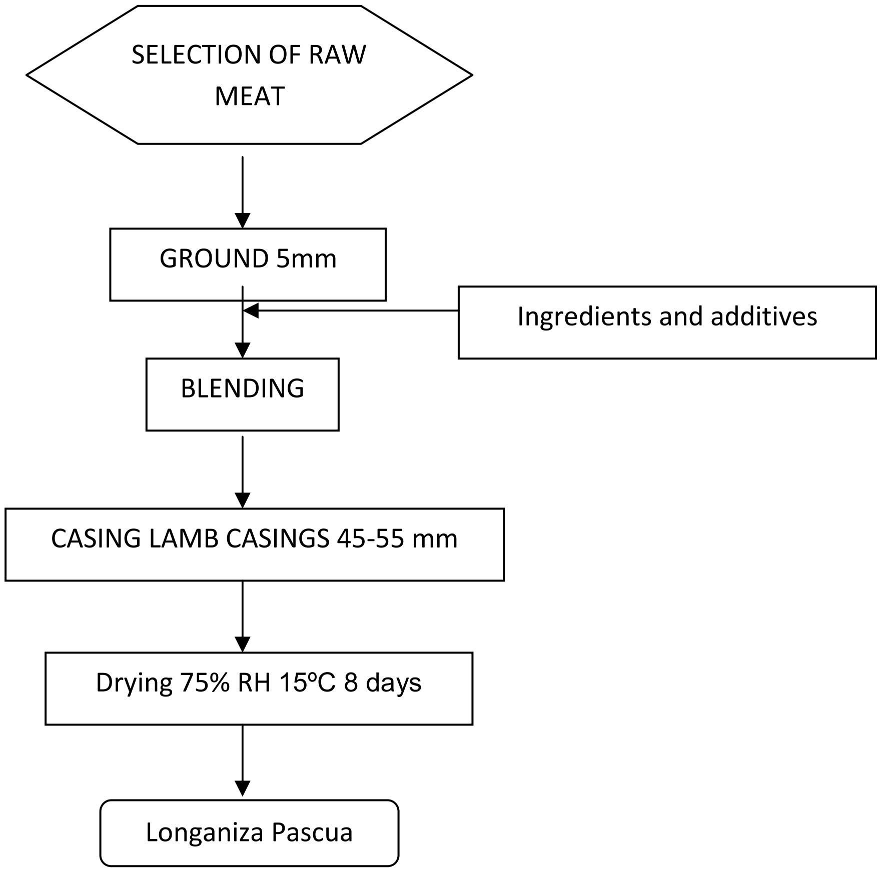
[11] López, M.C. (1999).Utilización de cultivos iniciadores en la elaboración de embutidos. Eurocarne, 80: 35-45.

[12] Roselló, C.; Barbas, J.I.; Berna, A.; López, N. (1994). Microbial and chemical changes in "sobrasada" during ripening. Meat Science, 40: 379-385.

[13] Sendra, E.; Fayos, P.; Lario, Y.; Fernández-López, J.; Sayas-Barberá E.; Pérez-Álvarez, J.A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiology, 25: 13-21.

[14] Spaziani, M.; Del Torre, M.; Stecchini, M.L. (2009). Changes of physicochemical, microbiological and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. Meat Science, 81(1): 77-85.

Figure 1: Flow diagram of 'Longaniza de Pascua' manufacturing.



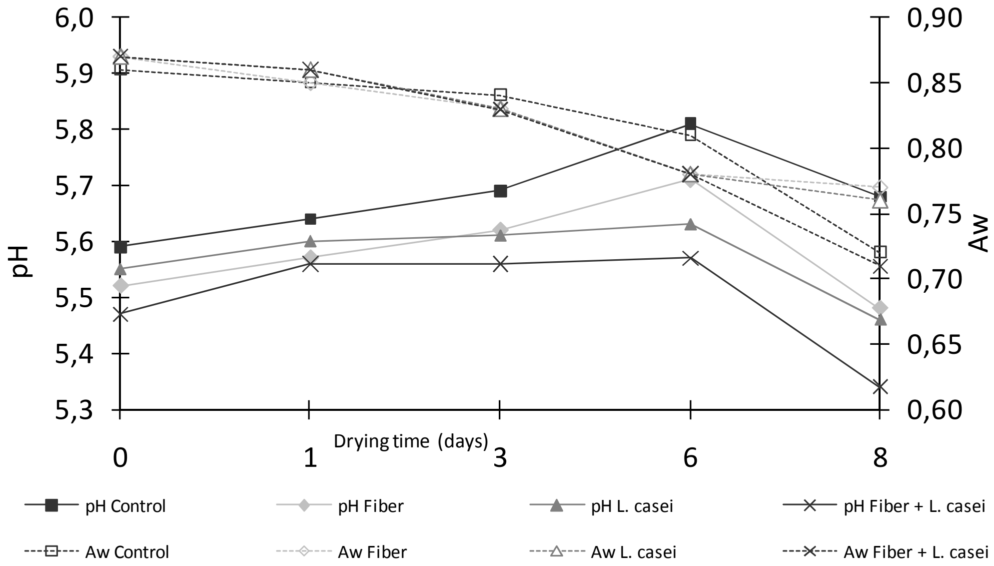


Figure 2. pH and water activity of dry cured sausages enriched with orange fiber and *L. casei* CECT 475 during drying time.

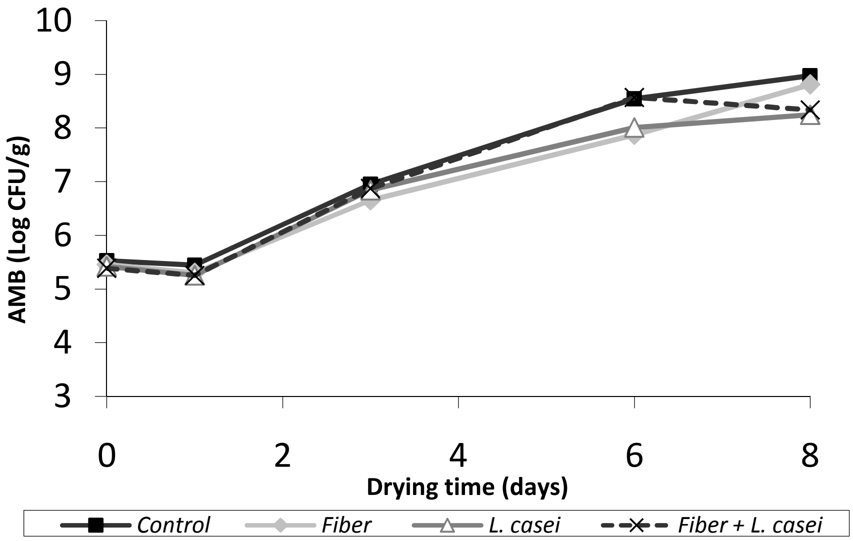


Figure 3. Aerobic Mesophilic Bacteria (AMB) counts in *Longaniza de Pascua*: evolution during dry curing.

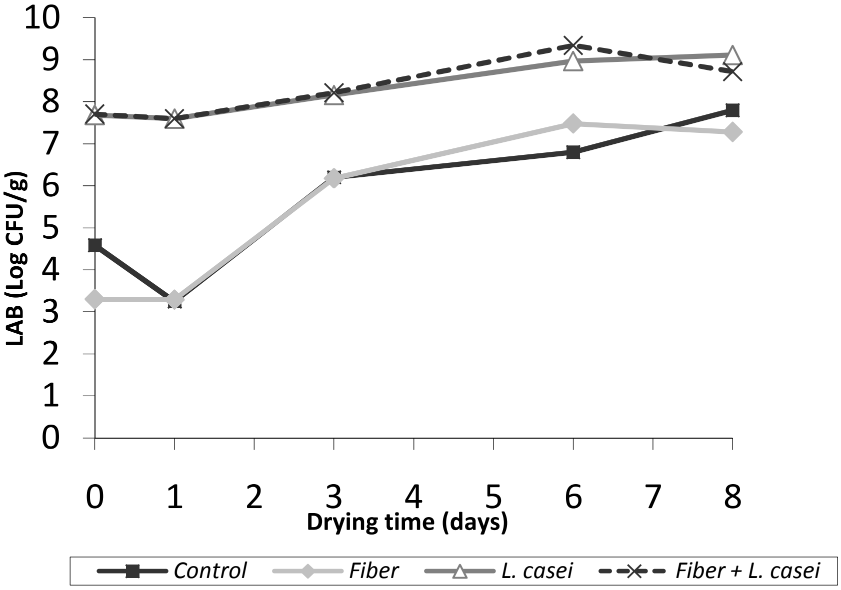


Figure 4. Lactic Acid Bacteria (LAB) counts in *Longaniza de Pascua*: evolution during dry curing.

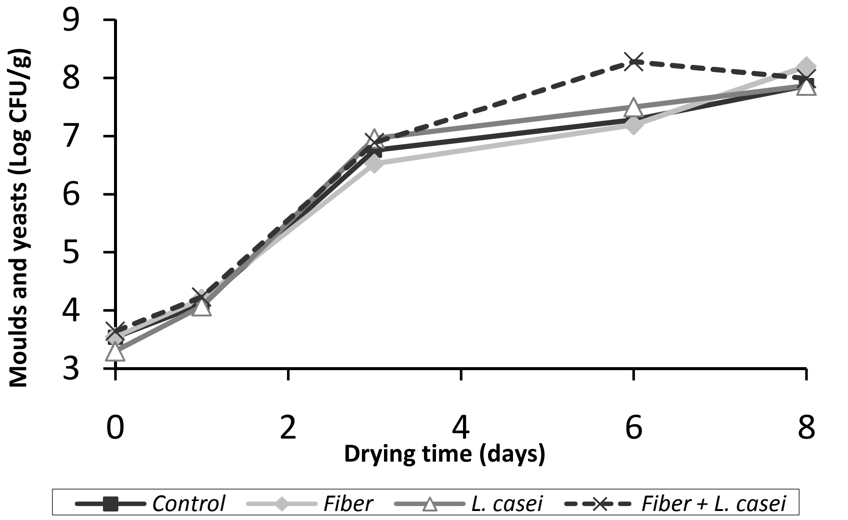


Figure 5. Moulds and yeasts counts in *Longaniza de Pascua*: evolution during dry curing.

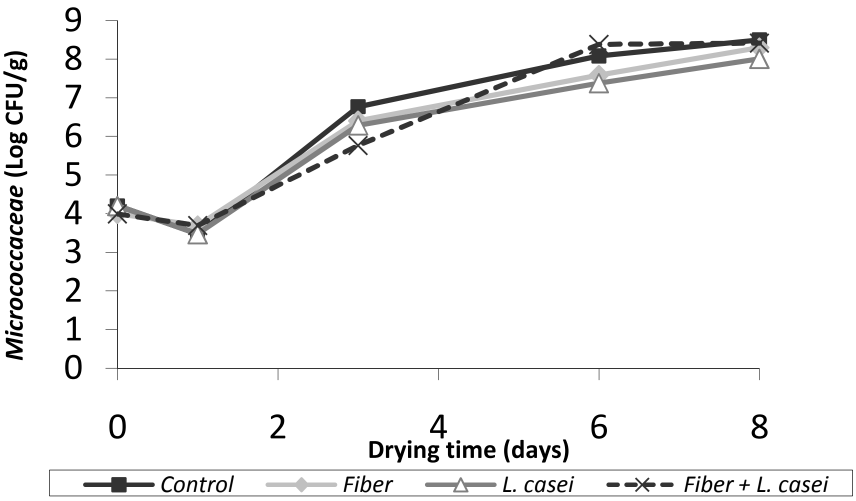


Figure 6. Micrococci counts in *Longaniza de Pascua*: evolution during dry curing.

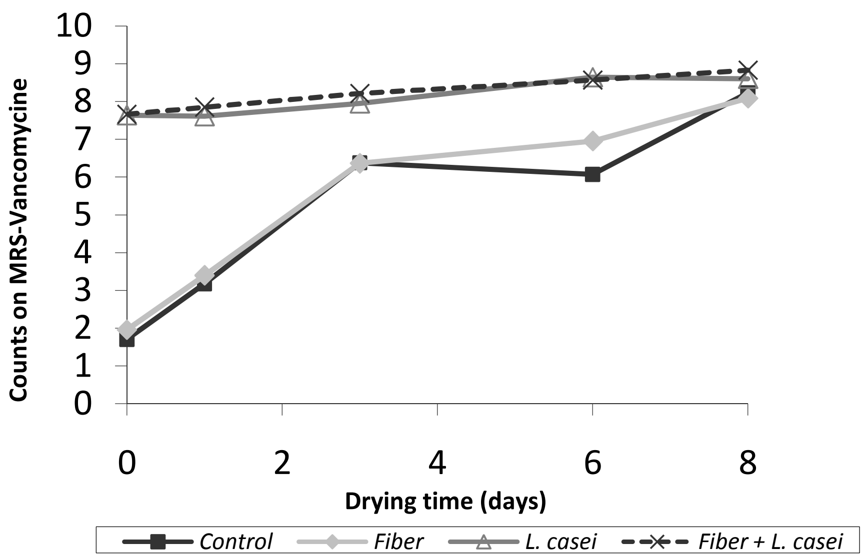


Figure 7. Estimated counts of meat Lactobacilli on *Longaniza de Pascua* during dry curing

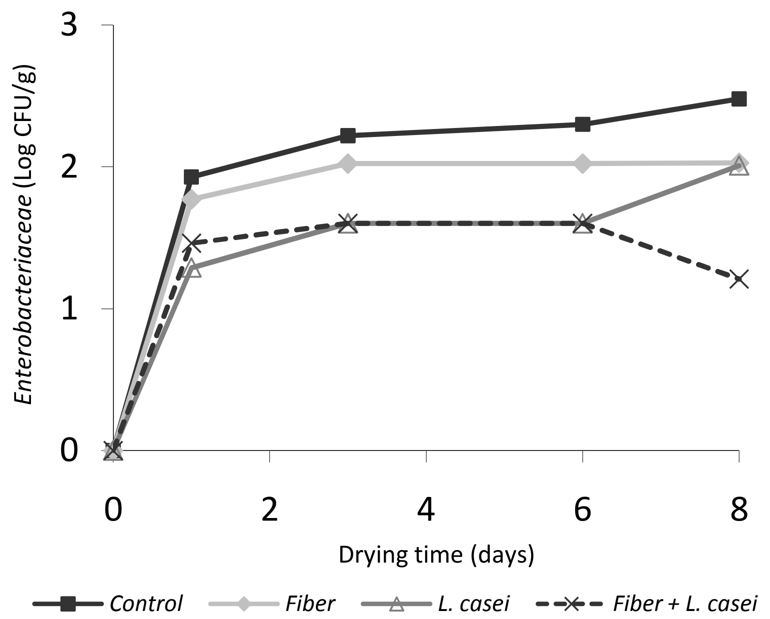


Figure 8. Evolution of Enterobacteria population in *Longaniza de Pascua* during drying

Table 1: Homogeneous groups obtained by multiple comparison Tukey test for pH for factors sausage type and drying time.

	Sausage type				Drying time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
pH	b	ab	ab	a	ab	ab	ab	b	a

Table 2: Homogeneous groups obtained by multiple comparison Tukey test for water activity for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
a_w	a	a	a	a	c	c	bc	b	a

Table 3: Homogeneous groups obtained by multiple comparison Tukey test for aerobic mesophilic bacteria for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
AMB	c	b	a	b	a	a	a	b	c

Table 4: Homogeneous groups obtained by multiple comparison Tukey test for lactic acid bacteria for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
LAB	a	a	b	c	a	a	a	b	b

Table 5: Homogeneous groups obtained by multiple comparison Tukey test for moulds and yeasts for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
MY	a	b	a	b	a	a	a	b	c

Table 6: Homogeneous groups obtained by multiple comparison Tukey test for Micrococci for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
Micrococci	c	b	a	b	a	a	a	b	c

Table 7: Homogeneous groups obtained by multiple comparison Tukey test for counts on MRS Vancomycine for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
Counts MRS-V	a	a	b	c	a	a	a	b	c

Table 8: Homogeneous groups obtained by multiple comparison Tukey test for enterobacteria for factors sausage type and drying time.

	Sausage type				Time				
	Control	Fiber	*L. casei*	Fiber+*L.casei*	0	1	3	6	8
Enterobacteria	c	b	a	a	a	b	c	c	d