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Lactobacillus plantarum AJ2 isolated from naturally fermented sausage and its effects on the technological properties of Milano-type salami

Lactobacillus plantarum AJ2 isolado de salame naturalmente fermentado e seus efeitos nas propriedades tecnológicas do salame tipo Milano

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Abstract

L. plantarum, strain AJ2, was isolated from naturally fermented sausage manufactured in the Southern region of Brazil and inoculated in Milano-type salami. The lactic culture exhibited the ability of growing in the product, decreasing pH in the first seven days of fermentation/maturation and the residual levels of nitrite and nitrate, as well as peroxide and TBARS values. The inoculated sausage had the highest intensity for brightness and red color, but did not present a difference in chemical composition and fatty acid composition, compared to the control.

Keywords: *L. plantarum; technological properties; Milano-type salami.*

Resumo

L. plantarum, cepa AJ2, foi isolado de salame naturalmente fermentado, fabricado na região Sul do Brasil e inoculado em salame tipo Milano. A cultura láctica apresentou habilidade para crescer no produto, promover a redução do pH nos primeiros sete dias de fermentação/maturação e dos níveis residuais de nitrito e nitrato, assim como menores valores de peróxidos e TBARS. O salame inoculado teve maior intensidade de brilho e da cor vermelha, mas não apresentou diferenças significativas na composição química e na composição de ácidos graxos, em relação ao controle.

Palavras-chave: *L. plantarum; propriedades tecnológicas; salame tipo Milano.*

1 Introduction

Salami is a typical fermented meat product, manufactured in large-scale by several industries in the Southern region of Brazil. Starter cultures are used in the manufacturing process and fermentation/ripening is controlled. However, in the same region, artisan fermented sausages are manufactured in several small-scale units by many rural families. In the artisan production of sausage, fat and pork meat are mixed with salt, curing agents, sugar and spices, stuffed into casings, and undergo the fermentation/ripening process in environmental conditions (25-30 °C) for a few weeks. Sausages are spontaneously fermented by the natural microbiota without the addition of a starter culture. In this case, the fermentative process is not controlled.

Spontaneous fermentation of sausages is characterized by the participation of lactic acid bacteria, Gram-positive, catalase-positive cocci, yeasts and moulds (BUCKENHÜSKES, 1993). *L. curvatus, L. plantarum* and *L. sakei* have been described as the dominant lactobacilli species in fermented sausages (HUGAS et al., 1993; PAPAMANOLI et al., 2003; SAMELIS et al., 1994; SCHILLINGER; LUCKE, 1987). Ammor et al. (2005) investigated the microbiota of a small-scale sausage production unit, and showed that, among the lactic acid bacteria isolated, *L. sakei* was the dominant species. According to Drosinos et al. (2007), *L. plantarum* was found to be the dominant species (45.6%) among 300 strains of lactic acid bacteria isolated during the fermentation and ripening period of traditionally fermented sausages in Southern Greece.

Small manufacturers continue using the traditional method, without adding starter cultures. Lactic acid bacteria present in these products come from natural contamination of meat or environment, i.e., the so-called "house flora". However, meat fermentation by natural lactic acid bacteria can sometimes fail, leading to products of poor quality, with possible presence of spoilage/pathogenic microorganisms. For this reason, addition of starter cultures has been recommended (ANDRIGUETTO; ZAMPESE; LOMBARDI, 2001; HOLZAPFEL, 2002; SANTOS et al., 1998).

The use of starter cultures (selected lactic acid bacteria, i.e., homofermentative lactobacilli and/or pediococci and Gram-positive, catalase-positive cocci (*Staphylococci* and/or

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Kokuriae)) for sausage production is becoming increasingly necessary for improving quality and guaranteeing safety and standardization of product properties, including flavor and color, and for shortening ripening time (LEROY; VERLUYTEN; VUYST, 2006; RANTSIOU et al., 2005). Lactic acid bacteria have the main role in this microbial consortium, once they affect both technological properties and microbial stability of the final product, by production of lactic and acetic acids and consequent pH decrease (DROSINOS et al., 2007). Coagulase-negative staphylococci such as *Staphylococcus xylosus* and *Staphylococcus carnosus* may be used to improve flavor of fermented meat (BERDAGUÉ et al., 1993).

Starter cultures contain lactic acid bacteria from meat, which are able to adapt to the ecology of meat fermentation and to the specific production technology (ANDRIGUETTO; ZAMPESE; LOMBARDI, 2001; HUGAS et al., 1993). The fitness of commercial meat starter cultures when applied to a particular type of salami is questionable, since a culture that performs well in one type of fermented sausage is not necessarily efficient in another type (LEROY; VERLUYTEN; VUYST, 2006).

Considering the importance of lactic acid bacteria in the fermentation of meat products and the species *L. plantarum* commonly present in the natural microbiota of fermented sausages, the aim of the present work was to determine the effects of *L. plantarum,* strain AJ2, on the technological properties of Milano-type salami. The investigation on Milano-type salami is because this product is commonly manufactured in the Southern region of Brazil and has a high lipid concentration (thus, it is important to monitor changes in lipids).

2 Materials and methods

2.1 Preparation of the lactic culture for use in the sausage

The *L. plantarum*, AJ2 strain, used in this study was selected among *L. plantarum* strains (AJ2, AL2, R2, AF5, AD3, N3 and AM2), which showed effective technological properties as starter cultures, according to Buckenhüskes (1993); Holzapfel (2002); Lee, Kim and Kunz (2006) and Saarela et al. (2006). Seven strains were selected, based on a previous study of morphological, phenotypic and molecular characterization of ten strains, isolated and characterized by Sawitzki et al. (2007). Ten characterized strains were randomly selected from 127 strains of homofermentative lactic acid bacteria, Gram-positive and catalase-negative, obtained from a total of 168 strains. These strains were isolated on the first 7 days of fermentation from 42 samples of artisan sausages, manufactured without starter cultures, from 21 manufacturers (household scale) in the Northwestern region of the state of Rio Grande do Sul, Brazil.

After fermentation, concentration and freeze-drying processes, *L. plantarum*, strain AJ2, was stored at –20 °C. For application in the salami, the freeze-dried culture was re-suspended in sterile distilled water and added to the meat batter to obtain the initial concentration of 9.0 log cfu.g^{-1} meat batter, according to Smith and Palumbo (1983) and Geisen, Lücke and Kröckel (1992).

2.2 Salami manufacturing procedure

Salami was manufactured under traditional conditions for Milano-type salami in the pilot plant of a local industry. Salami formulation was as follows: 53.5% pork meat, 17.8% beef meat, 17.8% pork back fat, 2.8% NaCl, 0.45% curing salt (containing sodium nitrate and sodium nitrite), sodium erythorbate, sugar, red wine and mixed spices. Meat and fat (both frozen) were ground (in a cutter) and mixed with the remaining ingredients. Then, meat batter was divided into two batches. *L. plantarum* AJ2 was added only to one batch (9.0 log cfu.g^{-1} meat batter). The other batch (not inoculated) was used as the control.

The salami mixture (ca. 400 g) was stuffed into synthetic casings (40 mm diameter) and left to ripen in a controlled chamber at 25 °C and 95% relative humidity (RH) for 24 hours. Then, the temperature and RH were slowly decreased, according to the following conditions: 24 °C/93% (2nd day), 23 °C/90% (3rd day), 22 °C/85% (4th day), 21 °C/80% (5th day), 20 °C/75% (6th day) and 18 °C/75% (7th day). After the 7th day, conditions were maintained for 42 days, after which the ripening period was ended.

At 0, 7, 14, 21, 28 and 42 days, samples from the inoculated salami and from the control were randomly selected and sampled in duplicate for microbiological and physicochemical analyses.

Microbiological analyses

Lactic acid bacteria, *Micrococci/Staphylococci* and spoilage/pathogenic microorganisms were analyzed in the samples according to standard methods (APHA, 1992). Twenty-five grams of each sample were homogenized in 225 mL of sterile peptone water 0.1% (w.v^{-1}) (Oxoid) for 2 minutes using a Stomacher Homogenizer (ITR, model MK 1204). Serial decimal dilutions were prepared in the same diluent and samples of appropriate dilutions were spread in duplicate on an appropriate selective medium and incubated under specific conditions: De Man, Rogosa and Sharp Agar – MRS (Oxoid) for lactic acid bacteria counting (30 °C/72 hours) in an anaerobic jar (Anaerojar Assembly – CODE AG25 – Oxoid) with a CO_2 enriched atmosphere (Anaerogen AN 2.5 L – Oxoid); Brain Heart Infusion Agar - BHI (Oxoid) for *Micrococci/Staphylococci* (35 °C/48 hours); Plate Count Agar – PCA (Oxoid) for total aerobic mesophyllic microorganisms (30 °C/72 hours); Glucose Potato Agar (Oxoid) supplemented with tetracycline (1 mg.mL^{-1}, Sigma) for yeasts and molds (25 °C/7 days); Brilliant Green Bile (2%) Broth – BGBB (Oxoid) for coliforms at 35 °C (MPN method at 35 °C/48 hours); E.C. broth (Oxoid) for thermotolerant coliforms (MPN method at 42 °C/24 hours); Baird-Parker medium (Oxoid) added of egg yolk tellurite emulsion (Oxoid) for coagulase-positive *Staphylococci* (37 °C/48 hours); sulfite-reducing *Clostridium* on Perfringens Agar Base - TSC (Oxoid) (anaerobically, at 37 °C/48 hours).

For detection of *Salmonella* ssp, pre-enrichment was done by suspending 25 g of the sample in 225 mL buffered peptone water 1.0% (w.v^{-1}) (Merck) (incubated at 37 °C/16 hours). Selective enrichment was done by transferring 0.1 mL of the pre-enriched culture to 10 mL Rappaport-Vassiliadis broth

(RVS broth, Merck) and to 10 mL Selenite Cystine Broth Base (Oxoid), followed by incubation of all tubes (42 °C/24 hours). After incubation, each selective enrichment was streaked on modified BPLS agar (Merck) and XLD agar (Merck) and all plates were incubated at 37 °C for 24 hours.

For *Listeria* ssp detection, selective enrichment was done by suspending 25 g of sample in 225 mL Fraser broth (Merck), followed by incubation at 30 °C for 24 hours. Then, 0.1 mL of the enriched culture was streaked on PALCAM agar (Merck) and OXFORD agar (Merck) and all plates were incubated at 30 °C for 48 hours.

Results of microbial population were expressed in cfu.g^{-1} of sample or MPN.g^{-1} of sample.

Physicochemical analyses

Each sample (inoculated and control) was ground and blended in a grinder (Tecnal Turratec TE-102,), frozen at –20 °C and freeze-dried (Virtis Unitop/1000 L) prior to analyses. All analyses were performed in duplicate and means and standard deviations were calculated.

After 42 days of ripening, chemical composition of the samples was determined according to standard AOAC procedures (2002), including moisture (oven air-drying method), proteins (Kjeldahl nitrogen), fat (ether extract), ash (muffle furnace) and salt (chlorine as sodium chloride).

Iron and sodium analyses were carried out according to Granadillo et al. (1995). Approximately 500 mg of samples were weighed into a digestion vessel (22 x 250 mm), added of 6.0 mL of HNO_3:$HClO_4$ 8:1 (v/v) and heated according to the following conditions: 90 °C/30 minutes, raised to 160 °C/40 minutes (10 °C/minute), 180 °C/20 minutes and held at this temperature until disappearance of a white steam. After digestion, 2.5 mL HCl 6 M were added and the volume was adjusted to 25 mL with deionized water. Iron (Fe) was determined by flame atomic spectrometry: diluted samples were directly aspirated into a spectrometer (Varian SpectrAA 220) equipped with a hollow cathode lamp, using an air/acetylene flame. Sodium (Na) was determined by flame photometry (Micronal B262). Both Fe and Na were quantified according to operating parameters provided by the manufacturer of the respective equipments.

Color measurements were carried out using a reflectance colorimeter (Chroma Meter CR-300, Minolta) and the CIE L*, a*, b* color values were measured (L*: lightness; a*: redness; b*: yellowness). Color readings were taken at three points of the surface of two slices (10 mm). Six measurements were made for each sample.

In order to monitor the dynamic chemical changes, during and at the end of the fermentation/ripening process, the following analyses were carried out every 7 days: moisture, water activity, pH value, acid value, nitrate and nitrite contents, peroxide value, TBARS and fatty acid composition. Water activity (Aw) was measured in a Testo 400 CE equipment (Testo GMBH & CO.). The pH was measured by determining the electric potential using a digital pH Meter (Digimed®). The electrode was submerged in a suspension consisting of 10 g sample added of 90 mL demineralized water. Acid value was estimated as % lactic acid (1 mL 0.1 N NaOH = 0.0090 g lactic acid) (AOAC, 2002). Nitrate and nitrite contents were determined according to the colorimetric method, with readings at 538 nm (AOAC, 2002) and expressed in mg.kg^{-1} of meat batter. The content of hydroperoxides was estimated by determining the peroxide value of lipids, expressed as mEq O_2.kg^{-1} of lipids, according to AOAC standard method (AOAC, 2002). Determination of thiobarbituric acid reactive substances (TBARS) was carried out using the distillation method, according to Tarladgis et al. (1964), modified by Crackel et al. (1988), following the recommendations of Shahidi et al. (1985) and Torres et al. (1989).

Lipids were extracted according to Folch, Less and Stanley (1957). From this extract, an aliquot of 10 mL was taken and the total lipid content was determined gravimetrically. A further aliquot, containing approximately 100 mg of lipids, was used for esterification and subsequent determination of the fatty acid composition by gas chromatography. Dried lipid extract was esterified with a solution of ammonium chloride and sulfuric acid in methanol, according to Hartman and Lago (1973). Fatty acid methyl esters were separated on a gas chromatographer (Model GC-2010, Shimadzu) coupled to a mass spectrometer (Model QP2010, Shimadzu), using a MS-5 capillary column (30 m x 0.25 mm I.D. x 0.25 µm thickness) (Restec Rtx®). The GC oven temperature was programmed from 150 to 220 °C at 2 °C.min^{-1} and held for 15 minutes. Samples of 1 µL were injected in split less mode (100:1) and the injector temperature was 250 °C. Flow rate of the carrier gas (helium) was 1.0 mL.min^{-1}. The spectrometer was operated in electron impact mode, with electron energy at 70 eV, at an ion source temperature of 220 °C. The mass spectrometer scanned m/z ratios from 40 to 400 u.

Fatty acids were identified by comparing the retention times of the sample components with those of known compounds from library databases (NIST05). Authentic standards of several detected compounds were analyzed in order to confirm the reliability of the retention times, considering a similarity higher than 90%. Results were expressed as % of normalized area. Approximate quantities of fatty acids were estimated by normalization and transformation of the area percentage into g.100 g^{-1} of sample, using the lipid conversion factor (F). An F-value of 0.956 was used for processed meat products, according to Holland et al. (1994).

Sensory evaluation

Sensory analyses were carried out, for inoculated and control salamis, by a panel of 70 untrained panelists. To evaluate the acceptance of salamis, panelists were asked to order samples from lowest to highest preference, through the Hedonic Scale method (ABNT/NBR12994/1993 and ABNT/NBR13170/1994). All panelists signed a TFSC - Term of Free and Spontaneous Consent, in accordance with Project 189/05: desenvolvimento de cultivos iniciadores para a produção de embutidos cárneos, approved on June 27, 2005, by the Committee of Ethics in Research with Human Beings of the Federal University of Santa Catarina.

Statistical analysis

The statistical analysis was performed using the t-test from the STATISTICA program, version 6.0.

3 Results and discussion

3.1 Microbiological analyses

Population of lactic acid bacteria increased to 6.52 (±0.12) log cfu.g^{-1} in the control salami and to 8.54 (±0.23) log cfu.g^{-1} in the inoculated salami during fermentation/ripening (42 days) (Figures 1 and 2). Counts of lactic acid bacteria were significantly higher ($p < 0.05$) in the inoculated salami when compared to counts of lactic acid bacteria in the control salami. These results indicate that *L. plantarum*, strain AJ2, is competitive and able to grow in the traditional formulation of Milano-type salami. Lactic acid bacteria originally isolated from traditional sausages are probably the best for improving microbiological safety of such foods, once they are well adapted to sausage conditions and should therefore be more competitive than lactic acid bacteria from other sources (AMMOR et al., 2006; PAPA et al., 1993).

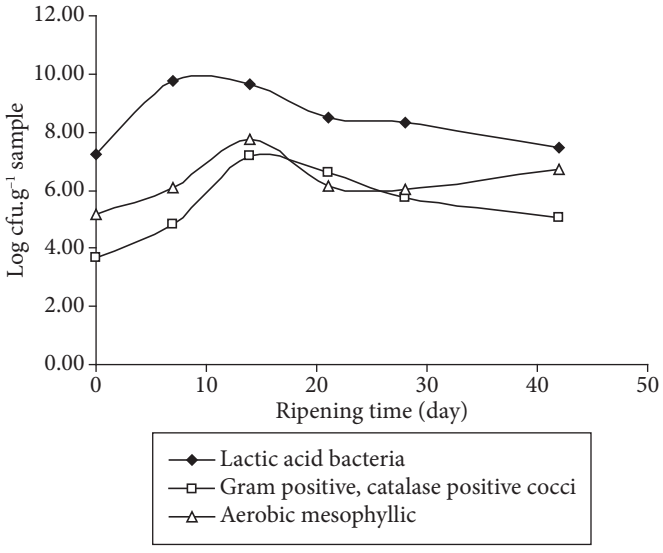

Figure 1. Microbial changes during fermentation and ripening of Milano-type salami inoculated with *L. plantarum,* strain AJ2.

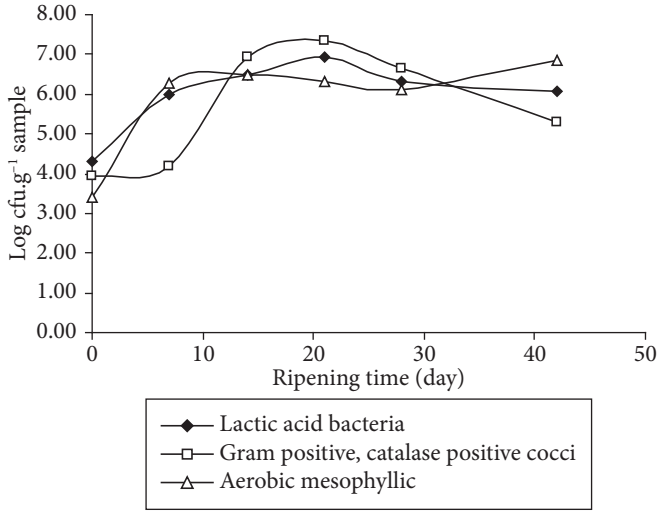

Figure 2. Microbial changes during fermentation and ripening of Milano-type salami (control).

According to Chevalier et al. (2006), Drosinos et al. (2007), Montel et al. (1993), Mauriello et al. (2004), Papamanolli et al. (2003) and Sawitzki and Terra (2000), microbial population of lactic acid bacteria in naturally fermented sausages (produced without starter cultures) are equal to or lower than 4.5 log cfu.g^{-1} at the beginning of fermentation. A similar result was observed in the present study. According to Smith and Palumbo (1983) and Geisen, Lücke and Kröckel (1992), addition of high concentrations (7.0-9.0 log cfu.g^{-1}) of desirable microorganisms will inhibit the growth of undesirable species, thereby preventing or reducing fermentation defects, once meat fermentation carried out by natural lactic acid bacteria can sometimes fail or be non competitive. Another aspect to be considered is that intentionally added starter cultures are selected and the microbiota present, as well as their effects, is predictable.

Initial counts of *Micrococci/Staphylococci* increased to 6.60 and to 7.34 log cfu.g^{-1} in control and inoculated salamis, respectively, in the first 21 days of fermentation/ripening, decreasing to 5.08 and 5.30 log cfu.g^{-1}, respectively, at the end of the ripening process (42 days). These results indicate that *L. plantarum* AJ2 and other lactic acid bacteria do not inhibit the growth of *Micrococci/Staphylococci*. For the selection of lactic acid bacteria as a meat starter culture, it is also important to guarantee no inhibition of Gram-positive, catalase-positive cocci (i.e. *S. xylosus*). According to Geisen, Lücke and Kröckel (1992), these microorganisms have a desirable influence on meat products, once they have nitrite and nitrate reductase activity, lipolytic activity, oxygen consumption and catalase activity, with effects on color and flavor, as well as decreasing rancidity of the product. To ensure sensory quality of fermented sausages, the contribution of Gram-positive, catalase-positive cocci is desirable (HUGAS; MONFORT, 1997).

In both samples (final product) no spoilage or pathogenic/toxigenic microorganisms were detected (data not shown). These results are in agreement with the Brazilian legislation – sanitary microbiological standards for ripened meat products (BRASIL/ANVISA, 2001) and suggest that, under good hygienic conditions and good manufacturing practices, it is possible to reduce contamination with undesirable microorganisms in the production of meat products.

3.2 Physicochemical analyses

Results of the chemical composition of the samples after 42 days of ripening did not present significant differences ($p > 0.05$) between salami inoculated with *L. plantarum* AJ2 and control. In both samples, average values were: moisture 28.3%; water activity 0.826; proteins 32.8%; fat 29.4%, ash

6.3%; salt (NaCl) 4.95%; sodium 2.88% and iron 1.6 mg.kg^{-1}. These results are in agreement with the Brazilian legislation – Regulamento técnico de identidade e qualidade do salame tipo Milano (BRASIL, 2000). Dynamic chemical changes (moisture, water activity, pH value, acid value, nitrate and nitrite contents, peroxide value and TBBARS) from the beginning to the end of the fermentation/ripening process are shown in Table 1.

Results for pH (first 7 days) and acid value (after 42 days of ripening) were significantly different between inoculated and control salamis ($p < 0.05$). During the first 7 days of fermentation, *L. plantarum* AJ2 was able to accelerate the production of acid. The pH values decreased from 5.60 to 4.97 in the inoculated salami, while in the control, pH values decreased from 5.68 to 5.34 (Table 1, Figure 3). Acidification is a very important aspect in sausages, once it results in denaturation of meat proteins and induction of all the reactions necessary for color formation and improvement of the stability of the product (BUCKENHÜSKES, 1993). As a result of denaturation, water-binding capacity of meat proteins is reduced, accelerating drying and shortening the processing time (JESSEN, 1995). Acidification of ground meat during the production of dry sausage, for instance, can be obtained by adding glucone-delta-lactone or by fermentation. In both cases, shelf life safety and sliceability of the sausage will be achieved, but the original taste and aroma can only be obtained by fermentation (BUCKENHÜSKES, 1993).

The ability of lactic acid bacteria, in particular lactobacilli, to decrease pH, prevents the growth of pathogenic and spoilage microorganisms, improving safety and shelf life of meat products (LUCKE, 1985; SAMELIS et al., 1994). A rapid pH drop to below 5.3 proved to be important for the inhibition of salmonellae and *Staphylococcus aureus*, if products are fermented at temperatures above 18 °C (SCHILLINGER; LÜCKE, 1989).

In both salamis, the concentrations of nitrite and nitrate in the final product (Table 1) were in agreement with the Brazilian legislation (BRASIL, 2000), which demands the addition

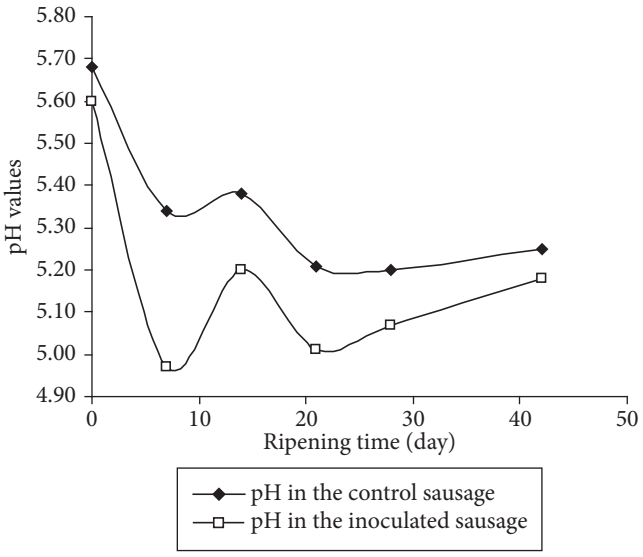

Figure 3. pH values during fermentation and ripening of Milano-type salami (inoculated with *L. plantarum* AJ2 and control salami).

Table 1. Physicochemical changes during fermentation and ripening of Milano-type salami inoculated with *L. plantarum*, strain AJ2.

Properties	Fermentation/ripening period (day)					
	0	7	14	21	28	42
Moisture (%)						
Inoculated	53.6 ± 0.27	42.0 ± 0.30	40.1 ± 0.33	39.0 ± 0.48	36.2 ± 1.02	28.2 ± 0.98
Control	52.4 ± 0.30	44.0 ± 0.28	41.7 ± 0.45	40.01 ± 0.53	35.4 ± 0.08	28.5 ± 0.10
Water activity						
Inoculated	0.954 ± 0.08	0.920 ± 0.17	0.903 ± 0.09	0.895 ± 0.11	0.858 ± 0.21	0.821 ± 0.09
Control	0.63 ± 0.07	0.932 ± 0.35	0.906 ± 0.27	0.903 ± 0.13	0.87 ± 0.28	0.832 ± 0.31
pH value						
Inoculated	5.60 ± 0.01	4.97 ± 0.03	5.20 ± 0.01	5.01 ± 0.04	5.07 ± 0.02	5.18 ± 0.06
Control	5.68 ± 0.03	5.34 ± 0.07	5.38 ± 0.05	5.21 ± 0.08	5.20 ± 0.03	5.25 ± 0.06
Acid value (mL NaOH.g^{-1})						
Inoculated	0.26 ± 0.03	0.57 ± 0.04	0.64 ± 0.02	0.79 ± 0.05	0.86 ± 0.01	1.00 ± 0.06
Control	0.25 ± 0.01	0.51 ± 0.06	0.63 ± 0.08	0.66 ± 0.03	0.86 ± 0.03	0.86 ± 0.05
Nitrate (mg.kg^{-1})						
Inoculated	276.7 ± 0.97	156.8 ± 1.07	65.4 ± 0.80	33.9 ± 0.98	21.58 ± 0.86	11.32 ± 0.95
Control	275.8 ± 1.10	178.4 ± 1.23	95.3 ± 0.97	67.4 ± 1.01	56.7 ± 0.76	48.9 ± 1.09
Nitrite (mg.kg^{-1})						
Inoculated	117.8 ± 1.09	59.7 ± 0.56	26.28 ± 0.78	17.35 ± 1.12	8.29 ± 0.93	5.12 ± 0.86
Control	118.5 ± 1.23	73.1 ± 1.10	44.7 ± 1.03	36.9 ± 0.89	32.5 ± 0.09	29.7 ± 1.10
Peroxides (mEq O_2.kg^{-1})						
Inoculated	0.65 ± 0.04	0.59 ± 0.02	0.95 ± 0.05	1.44 ± 0.07	1.77 ± 0.06	2.03 ± 0.03
Control	0.66 ± 0.02	0.95 ± 0.05	1.52 ± 0.03	2.23 ± 0.00	3.59 ± 0.06	3.89 ± 0.02
TBARS (mg MDA.kg^{-1})						
Inoculated	0.43 ± 0.03	1.11 ± 0.05	1.98 ± 0.04	1.58 ± 0.08	1.45 ± 0.06	1.32 ± 0.04
Control	0.39 ± 0.02	1.13 ± 0.03	2.04 ± 0.07	1.55 ± 0.05	1.47 ± 0.08	2.28 ± 0.06

of nitrite and/or nitrate as an ingredient in the formulation of fermented salamis, with a maximum limit of 30 $mg.kg^{-1}$ of nitrite in the final product. Nitrate and nitrite occur in the human diet from numerous different sources and the potential risk of these salts is due to their toxicity to humans when in high concentrations (fatal risk of methemoglobinaemia, particularly in newborn infants) or possible risk of diseases such as hypertension and carcinogenesis (BRADBERRY; GAZZARD; VALE, 1994; CAMMACK et al., 1999; PAIK et al., 2005). However, nitrate and nitrite have several important functions in meat products: 1) contributing to the characteristic flavor and red color of cured meat; 2) inhibiting the growth of a number of food poisoning and spoilage bacteria, especially of *Clostridium botulinum*; 3) stabilizing color; and 4) retarding the development of rancidity (CORNFORTH, 1996; GRAY; PEARSON, 1984; KILLDAY et al., 1988; OSADA et al., 2000; SANZ et al., 1997; WOODS; WOODS; GIBBS, 1990). Nitrate is a source of nitrite and the use of nitrate is interesting because it improves flavor generation compared to nitrite (OLESEN; MEYER; STAHNKE, 2004; WIRTH, 1991).

After 42 days of ripening, nitrate and nitrite values, as well as color parameters, were statistically different ($p < 0.05$) in the inoculated salami compared to the control. *L. plantarum*, strain AJ2, showed a potential effect to reduce nitrate/nitrite levels and a greater intensity of lightness and red coloration in the inoculated sausage. pH conditions in the inoculated salami might have contributed to this fact. Nitrite levels decrease during sausage fermentation due to pH conditions, which allow nitrite to be transformed into nitric oxide that reacts with myoglobin producing mononitrosylhemochrome, the characteristic pigment of cured meat (CHASCO; LIZASO; BERIAIN, 1996; MÖLLER et al., 2003; WIRTH, 1987). Results of color parameters, lightness (L values), redness (a* values) and yellowness (b* values) were the following: $L^* = 49.79 \pm 1.17$; $a^* = 16.30 \pm 1.19$ and $b^* = 6.81 \pm 0.29$ for the inoculated salami and $L^* = 42.27 \pm 1.21$; $a^* = 13.59 \pm 0.81$ and $b^* = 8.06 \pm 0.61$ for the control. Similar values for L*, a* and b* in fermented sausage (with approximately 30% fat and 40 days of ripening) were observed by Chasco, Lizaso and Beriain (1996); Dellaglio, Casiraghi and Pompei (1996); Papadima and Bloukas (1999) and Soyer, Ertas and Üzümcüoglu (2005).

Similar to color, flavor and aroma are part of the sensory properties of the product and can determine its degree of acceptability. In the sensory evaluation, inoculated salami presented 60.71% preference in relation to the control. This result suggests that *L. plantarum*, strain AJ2, promoted desirable organoleptic properties in the salami.

Another quality parameter of fermented sausages is lipid composition. According to Gandemer (1999), lipids play a key role in many quality traits of meat products, including nutritional value and sensory properties, mainly flavor. During processing of fermented sausage, lipids are progressively changed through both lipolysis and oxidation. Lipid composition and the extent of lipolysis and oxidation during processing/ripening contribute to the production of free fatty acids and flavor compounds in meat products (DEMEYER; HOOZEE; MESDOM, 1974, HERNÁNDEZ; NAVARRO; TOLDRÁ, 1999; MOLLY et al., 1996; MOTTRAM, 1998).

In the present study, after 42 days of ripening, the total fatty acid composition (% of total methyl esters) in the inoculated salami and in the control was, respectively, the following: saturated fatty acids, 39.92 and 39.43%; monounsaturated fatty acids, 46.99 and 46.82% and polyunsaturated fatty acids, 9.27 and 7.16%. Similar results for total fatty acid composition were observed by Zanardi et al. (2002) in ripened Milano-type salami (except for polyunsaturated fatty acids, which were 13.50%). Campos et al. (2006) observed values for saturated fatty acids of 39.0%; monounsaturated fatty acids of 49.3% and polyunsaturated fatty acids of 11.2% in Milano-type salami manufactured with pork meat and in salami treated with 0.5% of "mate" tea ethanol extract. Respective individual fatty acid compositions ($g.100\ g^{-1}$ of sample) in inoculated and control salamis are shown in Table 2. Results indicate that *L. plantarum* AJ2 did not have a significant action on the fatty acid composition ($p > 0.05$).

According to Molly et al. (1996), the contribution of bacteria in lipolysis in dry fermented sausage is poor because medium conditions are far from the optimal conditions for bacterial lipases. In the fermented sausages, inoculated with lipolytic and non lipolytic starter cultures and a control containing glucone-δ-lactone (GDL) and an antibiotic mixture, there was an increase in total and individual free fatty acids, but the results showed no difference ($p > 0.05$) in the level of the free fatty acids between treatments, indicating that lipolysis was most likely due to endogenous meat enzymes (KENNEALLY et al., 1998). Starter cultures, additives and spices do not seem to have an effect on lipolysis in fermented sausages (BALEV et al., 2005; GALGANO et al., 2003; MOLLY et al., 1996; ZANARDI et al., 2004). Bacterial contribution to lipolysis is considered limited in fermented sausages, but varies under different production conditions (HIERRO; HOZ; ORDÓÑES, 1997; JOHANSSON et al., 1994; MOLLY et al., 1996).

Changes which occur in lipids, mainly due to oxidation of unsaturated fatty acids, are an important phenomenon for aroma and flavor in traditional ripened meat products (CHIZZOLINI; NOVELLI; ZANARDI, 1998; MOLLY et al., 1996). However, an excess of oxidation could reach the level of rancidity, and foodstuff would no longer be acceptable for human consumption. Nevertheless, before such a condition is reached, lipid oxidation could also generate toxic molecules with possible hazards for human health (GRAY, 1978; ZANARDI et al., 2004).

Lipid oxidation is a complex chemical phenomenon where peroxidation is one of the primary mechanisms that changes lipids (GANDEMER, 2002). When cells are injured, such as in muscle foods after slaughtering, lipid peroxidation is favored, and traces of superoxide anion radical (O_2^-) and hydroperoxide (H_2O_2) are formed (KANNER, 1994). Determination of peroxide value is an estimative of hydroperoxide content in the product.

In the present study, during fermentation/ripening, estimated values of peroxides and TBARS increased in both salamis (Table 1). Similar peroxide and TBARS values were observed

Table 2. Fatty acid composition (g.100 g^{-1} of sample) during fermentation and ripening of Milano-type salami inoculated with *L. plantarum*, strain AJ2.

Fatty acid	Fermentation/ripening period (day)					
	0	7	14	21	28	42
C14:0						
Inoculated	0.33 ± 0.22	0.38 ± 0.04	0.34 ± 0.01	0.38 ± 0.02	0.44 ± 0.09	0.42± 0.04
Control	0.31 ± 0.06	0.35 ± 0.08	0.36 ± 0.00	0.40 ± 0.25	0.43 ± 0.03	0.42 ± 0.08
C16:0						
Inoculated	5.02 ± 0.37	6.00 ± 0.27	5.70 ± 0.19	6.14 ± 0.24	6.90 ± 0.85	6.76 ± 0.08
Control	5.07 ± 0.16	5.69 ± 1.03	5.78 ± 0.25	6.16 ± 0.51	6.74 ± 0.06	7.13 ± 0.38
C17:0						
Inoculated	0.09 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.04	0.10 ± 0.04
Control	0.09 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.07 ± 0.00	0.05 ± 0.00	0.07 ± 0.01
C18:0						
Inoculated	2.65 ± 1.76	3.17 ± 0.23	3.21 ± 0.18	3.33 ± 0.09	3.71 ± 0.04	3.57 ± 0.57
Control	3.08 ± 0.05	3.12 ± 0.26	3.05 ± 0.50	3.32 ± 0.15	3.54 ± 0.06	3.88 ± 0.25
C16:1 (9)						
Inoculated	0.40 ± 0.21	0.49 ± 0.04	0.46 ± 0.00	0.51 ± 0.01	0.54 ± 0.08	0.55 ± 0.06
Control	0.39 ± 0.03	0.36 ± 0.06	0.47 ± 0.02	0.50 ± 0.09	0.53 ± 0.02	0.55 ± 0.01
C18:1 (9)						
Inoculated	8.11 ± 1.72	10.01 ± 0.51	10.02 ± 0.16	10.34 ± 0.30	11.34 ± 0.06	11.50 ± 0.02
Control	9.77 ± 0.01	9.82 ± 0.11	9.93 ± 0.34	10.32 ± 0.49	11.22 ± 0.04	12.12 ± 0.42
C18:1 (11)						
Inoculated	0.54 ± 0.07	0.68 ± 0.01	0.72 ± 0.01	0.72 ± 0.24	0.81 ± 0.01	0.84 ± 0.01
Control	0.69 ± 0.04	0.67 ± 0.08	0.68 ± 0.02	0.72 ± 0.50	0.76 ± 0.01	0.87 ± 0.01
C18:2 (9, 12)						
Inoculated	1.82 ± 0.63	2.76 ± 0.07	2.69 ± 0.22	2.81 ± 0.1	2.68 ± 0.42	1.97 ± 0.83
Control	2.57 ± 0.09	2.76 ± 0.06	2.66 ± 0.21	2.50 ± 0.4	2.92 ± 0.40	2.70 ± 0.36

by Guiretti et al. (1997), Novelli et al. (1998) and Campos et al. (2007) in samples of Milano-type salami with a maturation period between 30-40 days. According to the authors, these values correspond to low lipid oxidation. After 42 days of maturation, inoculated salami showed lower values for peroxides and TBARS compared to the control salami. These results suggest that *L. plantarum*, strain AJ2, produced a good effect ($p < 0.05$) on the oxidative stability of lipids, but studies are necessary to evaluate lipid oxidation during the storage of salami.

4 Conclusion

L. plantarum, strain AJ2, presented good technological properties as a starter culture in salami, but additional studies of such properties are important, mainly when considering oxidative stability of lipids during the storage period.

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