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Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage

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ABSTRACT

In this study, a selected starter culture of *Lactobacillus plantarum* and *Staphylococcus xylosus* was used for the manufacture of a traditional Tunisian dry fermented sausage. Changes of microbiological, biochemical, sensorial and textural characteristics during the ripening of sausages were investigated.

Counts of lactic acid bacteria and staphylococci were significantly higher in inoculated sausages than in control ones. Moreover, the acidifying activity of the selective *L. plantarum* strains improved the hygienic quality of fermented sausages by reducing counts of *Enterobacteriaceae*. Both a significant decrease in pH and a significant increase in nitrite contents were observed in inoculated sausages compared to control ones. The loss of moisture during the ripening of sausages was not affected by the use of starters. Proteolysis and lipolysis were observed both in control and in inoculated sausages; however, these activities during ripening could be due to the activity of starter culture enzymes or the activity of meat endogenous enzymes. The Factorial Discriminative Analyze (FDA) of sensorial parameters (aroma, color, taste and firmness) discriminate clearly inoculated sausages from non-inoculated ones. In fact, inoculated sausages showed a more intensive red color, related to the production of nitrosomyoglobin pigment, and an acid flavor. Finally, textural parameters of sausages were not affected by the use of the selective starters.

1. Introduction

Fermented sausages are the result of microbiological, biochemical, physical and sensorial changes occurring in the meat mixture during ripening under defined conditions of temperature and relative humidity (Casaburi et al., 2007). The quality of the final product is closely related to the ripening that takes place during drying. This process, which confers to the product its particular slice ability, firmness, color and flavor, is characterized by a complex interaction of chemical and physical reactions associated with the microbiological development of the batter flora (Ordonez, Hierro, Bruna, & De la Hoz, 1999). Results of this interaction are decrease in pH, changes in the initial microflora, reduction of nitrate to nitrite and the latter to nitric oxide, formation of nitrosomyoglobin, solubilisation and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena and dehydratation (Casaburi et al., 2007). Nowadays, the need for safe products with standard and desirable technological properties has resulted in the use of starter cultures for the production of the dry fermented sausages to control the fermentation and ripening process, inhibiting the growth of other undesirable microorganisms, such as heterofermentative bacteria, biogenic amine and pathogens producer (Aro Aro et al., 2010; Drosinos et al., 2005; Tabanelli, Coloretti, Chavari, Grazia, & Lanciotti, 2012; Zaho et al., 2011). Lactic acid bacteria (LAB) and Coagulase Negative Staphylococci (CNS) are the most active indigenous microorganisms; first in the acidification process, second in the denitrification, the lipolysis and the proteolysis (Hammes & Hertel, 1998). In addition, depending on the product, other groups may play a role, such as molds, enterococci and yeasts (Casaburi et al., 2007).

LAB, in dry fermented meat products, are responsible for a rapid fermentation of carbohydrates added to the mixture, leading to a decrease in the pH. The acidification below the isoelectric point of muscle protein affects proteins coagulation responsible for sliceability, firmness and cohesiveness of the final product (Drosinos, Paramithiotis, Kolovos, Tsikouras, & Metaxopoulos, 2007). The

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protective effect of LAB starter cultures is in relation to pathogenic and spoilage bacteria through the antimicrobial properties of their metabolites, such as organic acids, hydrogen peroxide and bacteriocins (Ammor & Mayo, 2007).

CNS participate in the development and stability of good red color by the nitrate reductase activity that leads to the formation of nitrosomyoglobin. In addition, nitrate reduction produces nitrites that limit the lipid oxidation (Talon, Walter, Chartier, Barrière & Montel, 1999). Also, catalase and superoxide dismutase activities on CNS are important to decompose the hydrogen peroxide and to prevent lipid oxidation (Barrière et al., 2001). Various aromatic substances and organic acids are released by protease and lipase activities of CNS which has a considerable role in the development of the flavor of fermented meat due to the formation of low molecular weight compounds, including peptides, amino acids, aldehydes, amines and free fatty acids (Simonová et al., 2006).

The most promising bacteria for starter cultures are those which are isolated from the indigenous microflora of traditional products. These micro-organisms are well adapted in the meat environment and are capable of dominating the microflora of products (Drosinos et al., 2005). Among LAB and CNS that can be used as starter cultures, strains of *Staphylococcus xylosus* and *Lactobacillus plantarum* showed interesting technological properties leading them to be used as starter cultures for the manufacture of dry meat products (Essid, Ben Ismail, Bel Hadj Ahmed, Ghedamsi & Hassouna, 2007; Essid, Medini, & Hassouna, 2009).

In Tunisia, dry fermented sausages are generally produced without use of selected starter. The natural fermentation of meat, carried out spontaneously by the microflora of the raw meat and environment, may produce products which are either inferior or even unsafe for consumption. Thus the use of starter cultures for the production of fermented sausages is becoming necessary to guarantee safety and standardize properties (Essid, Ben Ismail, Ben Hadj Ahmed, Ghedamsi, & Mnasser, 2007).

The aim of this work was to study the effect of autochthonous strains of *S. xylosus* and *L. plantarum,* previously isolated from a traditional salted meat and selected for their technological properties, on the microbiological, biochemical, textural and sensory characteristics during the ripening of a Tunisian dry fermented beef sausage.

2. Material and methods

2.1. Bacterial strains and culture condition

The *S. xylosus* and the *L. plantarum* strains used in this study were both isolated from a Tunisian traditional salted meat "kadid" as previously reported by Essid et al. (2007) and Essid et al. (2009). These strains were selected for having the best attributes for use as starter meat cultures (Essid et al., 2007, 2009). Strains of *S. xylosus* were cultivated in Tryptone Soy Broth (Pronadisa, Spain) at 37 °C, whereas strains of *L. plantarum* were cultivated in Man Rogosa and Sharpe Broth (MRS broth) (Pronadisa, Spain) at 30 °C. After 24 h of incubation, the bacterial suspensions were centrifuged at 12,000 *g* for 15 min (Universal, 320) and the pellets were washed with sterile distilled water and resuspended in 10 ml of the nutrient broth (Pronadisa, Spain). Cells were harvested by centrifugation (12,000 *g* for 15 min) and resuspended in 5 ml of distilled water. Finally, the number of bacterial cells in each suspension was adjusted to reach the range of 7 log CFU/ml by using a spectrophotometer (Jenway 6305).

2.2. Sausage manufacture and sampling

Ten kilograms of sausages batter was prepared from fresh boneless beef meat (75%) (w/w) and beef fat (25%) (w/w) purchased from a local market in Tunis one day before the sausage preparation and stored overnight at 4 °C. The meat and the beef fat were minced and mixed in a rotating bowl meat cutter (Rowenta, Universo, Germany), with 400 g of salt, 20 g of black pepper (Kamy, Tunisia), 20 g of paprika (Kamy, Tunisia), 100 g of glucose and 1 g of KNO_3. After mixing them, the sausage mixture was divided into two batches: one batch was inoculated with strains of *S. xylosus* and *L. plantarum* with 7 log CFU/g for each strain and the other one was non-inoculated, to act as a control. The mixture was manually stuffed into a natural casing (20–25 cm of length and about 4 cm of diameter) at approximately 330 g each and placed in a drying-ripening chamber (BCR, CF 1B, France). Sausages were subjected to the following conditions of temperature and relative humidity: fermentation for 5 days at 23 °C and 85–90% RH followed by drying at 14 °C and 75–80% RH for 23 days. For sampling, three sausages of each batch at 0 day (mix before stuffing) and after 7, 14, 21 and 28 days of ripening were taken for microbiological, physicochemical and textural analyzes and each analysis was carried out in triplicate. Sensory analysis was carried out at the end of process.

2.3. Microbiological analysis

For microbiological analysis, 10 g of each sample of sausage batch were collected aseptically, transferred into a sterile plastic bag and were homogenized with 90 ml of peptone water (Pronadisa, Spain) using a Stomacher 80 Biomaster. Serial 10-fold dilutions were prepared in sterile peptone water. Appropriate dilution samples (1 or 0.1 ml) were poured or spread in duplicate on different growth media. Total viable counts were enumerated on Plat Count Agar (PCA) (Pronadisa, Spain) after 48 h of incubation at 30 °C; LAB on MRS agar (Pronadisa, Spain) after 48 h of incubation at 30 °C; staphylococci on Mannitol Salt Agar (Pronadisa, Spain) after 37 h of incubation at 37 °C; yeasts and molds on Sabouraud Agar (Pronadisa, Spain) after 5 days of incubation at 25 °C and *Enterobacteriacea* on Violet Red Bile Glucose Agar (VRBG) (Pronadisa, Spain) after 24 h at 37 °C (Guiraud, 1998).

2.4. Moisture, pH, nitrite and color determination

For the pH measurement, 10 g of tested samples were diluted in 90 ml of distilled water. After the homogenization, pH values were determined with a digital pH-meter (WTW pH 315i/SET.Wissenschaftlich). The moisture percentage was calculated by weight loss experimented by the sample (5 g) maintained in an oven (Memmert, UL 60) at 105 °C, until constant weight according to the ISO recommended method (ISO, 1973). Nitrite content was determined following the ISO Standards (1975). Color measurements were carried out using a colorimeter (Minolta Chroma Meter CR-300). Samples were cut into slices before color determination. L^*, a^* and b^* were determined which indicate respectively lightness, redness and yellowness.

2.5. Determination of free amino acids

Amino acids were extracted from the samples with HCl acid: 5 g of each sample were chopped and mixed with 4 ml of HCl (6 M). After incubation at 105 °C for 24 h, hydrolyze was stopped by addition of 7 ml of NaOH (6 M). The solution was filtered (0.45 μm) and the filtrate was stored at 4 °C. The injection solution was composed of 0.5 μl of filtrate, 2.5 μl of tris-borate, 0.5 μl of H_2O and 0.5 μl of o-phthaldialdehyde solution. The analysis of amino acids was performed in a reverse phase HPLC (Agilent L1100) equipped with FLD injector and detector, in a Hypersil ODS C_{18} column (250 × 4.6 mm). Excitation and emission absorbance were respectively 340 and 450 nm. Amino acids were identified by