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MICROBIOLOGICAL, BIOCHEMICAL AND TEXTURAL CHARACTERISTICS OF A TUNISIAN DRY FERMENTED POULTRY MEAT SAUSAGE INOCULATED WITH SELECTED STARTER CULTURES

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ABSTRACT

The effect of selected starter cultures (*Lactobacillus sakei* + *Staphylococcus carnosus* or *L. sakei* + *S. carnosus* + *Staphylococcus xylosus*) on microbiological, biochemical and textural characteristics of a traditional Tunisian dry fermented poultry meat sausage was investigated. The microbial results showed that lactic acid bacteria and coagulase negative staphylococci represent the two predominant microflora in all stages of ripening. Moreover, the acidifying activity of *L. sakei* improved the hygienic quality of fermented sausages by reducing the Enterobacteriaceae counts. The moisture content and water activity of control and inoculated sausages decreased in all stages of ripening. Proteolysis and lipolysis were observed both in control and in inoculated sausages. Finally, textural parameters of sausages were not affected by the use of selective starters.

PRACTICAL APPLICATIONS

Poultry meat products provide a suitable environment for proliferation of meat spoilage microorganisms. The increase in resistance of food spoilage microorganisms to current preservatives, the consumer's demand for safe and minimally processed foods and the hazards associated with the use of high doses of chemical preservatives have led to the need for finding safer alternatives in food preservation. Consumers have also become more critical about the use of synthetic additives to preserve food or enhance characteristics such as color and flavor. Hence, there is a growing trend toward minimally processed foods. As a consequence, bacterial antagonism has received considerable attention in food preservation. 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, inhibit the growth of undesirable microorganisms and extend the shelf life of the product.

INTRODUCTION

Poultry meat products are among the fastest growing food commodities on the market in many parts of the world (Chouliara *et al.* 2007) due to their low cost of production as compared to meat products such as beef, lamb or pork; low fat content; high nutritional value; and distinct flavor (Barbut 2002). An added benefit is that poultry meat is not restricted by most cultural and religious laws, and it is consumed by both Jews and Muslims (Cavani *et al.* 2009). Poultry meat products present an ideal substrate supporting the growth of several spoilage and pathogenic bacteria (Mataragas *et al.* 2008). Thus, application of suitable agents possessing antimicrobial activities may be useful for

maintaining meat quality and preventing economic loss (Sallam *et al.* 2004; Gao *et al.* 2014). Nowadays, the use of selected starter cultures for sausage production with appropriate metabolic performances and good ability for implantation in sausages is becoming increasingly necessary to guarantee food safety, reduce the fermentation time, extend the shelf life and enhance the organoleptic characteristics of the final product (Parente *et al.* 2001; Drosinos *et al.* 2005; Leroy *et al.* 2006; Casaburi *et al.* 2008; Tabanelli *et al.* 2012).

Most of the dry fermented sausages are produced with starter culture combination of lactic acid bacteria (LAB) and coagulase negative staphylococci (CNS) (Ammor and Mayo 2007).

LAB play an important role in meat preservation and fermentation processes because they affect both the technological properties and the microbial stability of the final product (Drosinos *et al.* 2007). In fact, the main role of LAB is to acidify the sausage, although they may also slow down proteolytic and lipolytic activity (Ammor and Mayo 2007). The positive technological aspects of this acidification are several such as the inhibition of spoilage and pathogenic microorganisms, faster drying and improved texture profile through denaturation and coagulation of proteins and the activation of muscle proteases and reddening through the formation of nitric oxide and nitrosyl myoglobin (Bedia *et al.* 2011).

The main LAB species used are *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus casei*, *Lactobacillus pentosus*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* (Hugas and Monfort 1997).

CNS participate in the development of characteristic sausage flavor and odor as various aromatic substances and organic acids such as free fatty acids (FFAs), aldehydes, amines, ketones and alcohols are released from their protease and lipase activity (Montel *et al.* 1996; Leroy *et al.* 2006; Fonseca *et al.* 2013). CNS are gram-positive, catalase-positive cocci with antioxidant activities, preventing the formation of off-flavors and rancidity; their nitrate reductase activity is important for color formation (Ravyts *et al.* 2010). Unfortunately, CNS are hardly competitive in the presence of acidifying LAB, as the sausage fermentation conditions are not favorable to their growth (Cocolin *et al.* 2006; Ravyts *et al.* 2010).

The species mostly used for the production of dry fermented sausages are *Staphylococcus carnosus* and *Staphylococcus xylosus* (Laukova *et al.* 2010).

In Tunisia, sausages are mainly produced from red meat. The aims of this work are to produce a skinless poultry meat sausage previously inoculated with selected starters of *L. sakei*, *S. carnosus* and *S. xylosus*, and to study the microbiological, biochemical and textural quality during ripening.

MATERIAL AND METHODS

Preparation of Dry Fermented Sausages

The sausage formulation included 6.750 kg of poultry meat (75%), 2.250 kg of poultry fat (25%), 360 g of salt, 18 g of black pepper, 18 g of paprika, 90 g glucose and 0.9 g of potassium nitrate. After chopping and mixing the ingredients, the mixture was divided into three batches (3 kg for each batch): batch 1, inoculated with a commercial starter culture starter A (20 g/200 kg): *L. sakei* + *S. carnosus* + *S. xylosus* (TEXEL SA-201, DANISCO, Paris, France); batch 2, inoculated with a commercial starter culture starter B (25 g/100 kg): *L. sakei* + *S. carnosus* (BFL-F06, CHR HANSEN, Nienburg, Germany); and batch 3, control without inoculation. Starter A and starter B were added to sausages according to manufacturer's recommendations.

The mixture of each batch was stuffed into artificial casings, giving approximately 500 g as the final mass of each sausage and then placed in a fermentation chamber (BCR, CF 1 B, Antony, France). The sausages were fermented for 5 days at 24C and 80% relative humidity (RH). After 5 days of processing, the temperature was decreased to 14C for 23 days and the RH value was 80%. 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 analyses, and each analysis was carried out in triplicate.

Microbiological Analysis

Sausage samples (10 g) of each batch were homogenized with 90 mL of sterile peptone water (Biolife, Milan, Italy) and decimal dilutions were prepared. Mesophilic LAB were enumerated on MRS (de Man, Rogosa and Sharpe) agar (Biolife) after 48 h of incubation at 30C. The number of staphylococci was determined on mannitol salt agar (Biolife) after incubation at 37C for 48 h. Yeasts and molds were enumerated on Sabouraud Dextrose Agar (Biokar, Beauvais, France) at 28C for 4 days.

Total viable counts were determined on standard plate count agar (Biolife) at 30C for 48 h. Enterobacteriaceae were determined on Violet Red Bile Glucose (VRBG) (Biokar) at 37C for 24 h.

Physicochemical Analysis

pH, a_w, Moisture and Weight Loss. The pH values were measured in homogenates prepared by blending 10 g of sausage (Moulinex DPA141, Lyon, France) with 50 mL of distilled water for 2 min. Measurements were taken with a pH meter (Microprocessor pH meter BT-500, Boeco, Hamburg, Germany). Water activity (a_w) was measured

with water activity meter (HygroLab 3, Rotronic, Croissy-Beaubourg, France). The moisture percentage was calculated by weight loss experimented by the sample (5 g) maintained in an oven (Memmert, UL 60, Germany) at 105C, until constant weight according to the ISO recommended method (ISO 1973). Weight loss was expressed as percentage of the initial weight (Liaros *et al.* 2009).

Color Measurement. Color measurements were carried out using a CR-300 colorimeter (Minolta Chroma Meter CR-300, Tokyo, Japan). Each sausage was cut and the color of the slices was measured three times for each analytical point; L^*, a^* and b^* scale coordinates were obtained: L^* (lightness), a^* (redness) and b^* (yellowness). Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Free Amino Acid (FAA) Content. The FAA content was determined by reversed phase high-performance liquid chromatography (HPLC). The amino acids were extracted after hydrolysis of meat proteins in the presence of concentrated hydrochloric acid. Thus, 5 g of dry fermented sausages was chopped and added to 4 mL of HCl 37% (6 M). The mixture was homogenized and then placed in an oven at 105C for 24 h. Hydrolysis was stopped by adding approximately 6 mL of NaOH (6 N). Then, the mixture was filtered through a syringe filter and the filtrate was stored at 4C until injection. The separation of the protein fraction of sausages was performed using HPLC Agilent (Province, Canada) L 100 system, on a column C18 (250 mm × 4.6 mm dimension of the column, 5-μm porosity). This system is composed of high pressure pump, an automatic injector fluorescence detector (FLD), FLD, and control software and acquisition of data (ChemStation). The separation was carried out for 30 min; the flow rate of the mobile phase is 1 mL/min. The excitation is at 340 nm and the emission is at 440 nm. The injection solution is composed of 2.5 μL of borate, buffer supplemented with 0.5 μL of the sample, 0.5 μL of H_2O and 0.5 μL of *o*-phthaldialdehyde solution. The whole is mixed with 3.5 μL of air. Then, everything is injected. The injection of reference amino acids allowed determining their retention times. To determine the concentration of amino acids in different samples, straight standards have been established relating the concentration of each reference amino acid to the area of the peak obtained.

Determination of FFAs. Lipids were extracted according to Soxhlet method (ISO 1996). The FFA compositions were determined by means of gas chromatography coupled with mass spectrometry (GC-MS) after methyl esterification. The analysis was performed using an Agilent chromatograph (Agilent 5975 B) equipped with split injector and flame ionization detector in an Agilent 19091S-933 column (1% phenylmethylsiloxane, 30 m × 250 μm × 0.25 μm). Helium was used as the carrier gas. The oven temperature increased from 80 to 300C at a rate of 3C/min and 10 min at 300C. The individual FFAs were identified in comparison with retention times of chemical standards by mass spectrometry. FFAs were expressed as percentage of total fatty acids.

Instrumental Texture Measurement. Instrumental texture analysis was performed with a texture analyzer (TA-XT2, Stable Micro Systems, Haslemere, U.K.). Each sample of sausage was cut in a cylinder (3 cm × 3 cm). Each cylinder was compressed with a cylindrical probe at 5 mm/s speed and the level of compression was 60% of the thickness of the sample. The parameter determined from the force–time curves was hardness. Hardness was defined by peak force during compression and expressed in newtons (N). The sample elasticity was estimated by using the apparently Young's modulus, which was calculated as the rate of strain (σ) (N/m^2) as a function of stress (ε) (%) in the straight line of the deformation curve. The apparent Young's modulus value (N/m^2) is higher as the sausage is less elastic.

Statistical Analysis. Data were statistically analyzed using the one-way analysis of variance (ANOVA) procedure of SPSS 17.0 (SPSS, Inc., Chicago, IL). Duncan's multiple range test was used to determine any significant difference between mean values, and evaluations were based on a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Microbiological Results

Figure 1 shows the evolution of LAB, staphylococci, yeasts and molds, Enterobacteriaceae and total viable counts during fermentation of starter inoculated and control sausages.

Our results showed that the addition of selected starter cultures did not significantly affect ($P > 0.05$) the quantitative evolution of different microbial groups, with the exception of staphylococci ($P < 0.05$), whereas the growth of microorganisms has been significantly affected ($P < 0.05$) by the time parameter.

The numbers of total viable counts remain significantly higher in sausages inoculated compared with those measured on control samples at all stages of ripening due to the prior inoculation of sausages by *S. carnosus*, *S. xylosus* and *L. sakei* (Fig. 1). Our results are in agreement with those obtained by Drosinos *et al.* (2005) and Ciuciu Simion *et al.* (2014).

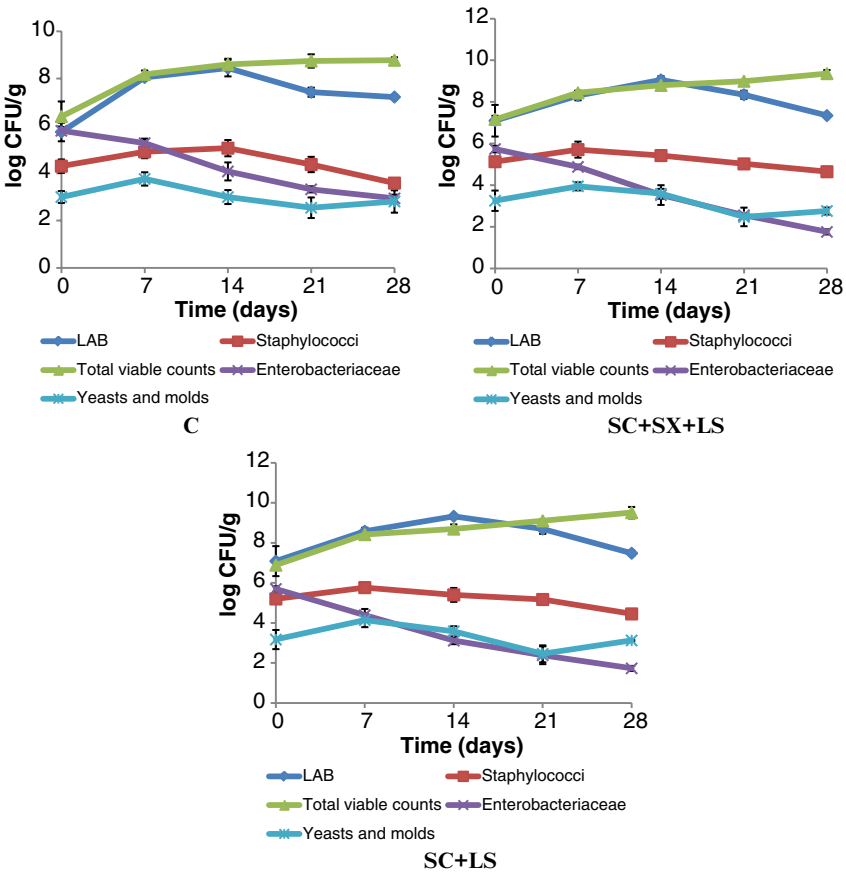

FIG. 1. EVOLUTION OF MICROBIAL POPULATION DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), SC + LS (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

LAB are the dominant microflora in both control and starter inoculated sausages. Their number is higher in sausages inoculated respectively with starter A and starter B than in control sausages (Fig. 1). This result is in agreement with many other studies reporting that LAB are the dominant microflora of dry fermented sausages (Casaburi *et al.* 2008; Albano *et al.* 2009; Bedia *et al.* 2011; Janssens *et al.* 2012; Fonseca *et al.* 2013; Ciuciu Simion *et al.* 2014). *L. sakei* is usually reported as the most dominant LAB species in fermented sausages (Papamanoli *et al.* 2003; Comi *et al.* 2005; Drosinos *et al.* 2007; Lebert *et al.* 2007). *L. sakei* is highly adapted to the fermented meat matrix. This species possesses several genes that enable it to cope with environmental stresses such as the presence of curing salt (Marceau *et al.* 2001; Chaillou *et al.* 2005), to use alternative energy sources such as arginine (Rimaux *et al.* 2011) and to produce bacteriocins (Leroy and De Vuyst 2005; Ravyts *et al.* 2008) and well adapted to low temperatures (Dossmann *et al.* 1996; Ammor and Mayo 2007).

Maximum LAB counts were reached after 2 weeks of ripening in control and inoculated sausages. Beyond the 14th day, the number of LAB decreased slightly. This slight decrease is due to the exhaustion of the sugar and the low temperature conditions (14C) (Fernandez-Lopez *et al.* 2008; Fonseca *et al.* 2013).

Staphylococci are the dominant microflora next to LAB in both control and inoculated sausages at all stages of ripening. This result is in agreement with many other studies reporting that LAB and staphylococci are the dominant microflora in dry fermented sausages (Rubio *et al.* 2007; Casaburi *et al.* 2008; Essid and Hassouna 2013).

The numbers of staphylococci remain significantly higher in sausages inoculated compared with those measured on control samples at all stages of ripening.

In control sausages, the number of staphylococci increased during the first 2 weeks of ripening to reach 5.063 log cfu/g at 14th day and later decreased throughout the process, whereas the staphylococci counts increased in the inoculated batches during the first 7 days of ripening and then decreased steadily during the process (Fig. 1). This inhibition of staphylococci is due to the decrease of pH caused by lactobacilli (Johansson *et al.* 1994; Lizaso *et al.* 1999; Olesen and Stahnke 2004; Ravyts *et al.* 2010; Zaho *et al.* 2011).

The number of Enterobacteriaceae decreased during the ripening phase of control and inoculated dry fermented sausages (Fig. 1). In fact, the number of Enterobacteriaceae is significantly lower in sausages inoculated with starter A and starter B than those measured on control samples. Our results are in agreement with other studies reporting that the sausages inoculated with starter cultures have the lowest number of Enterobacteriaceae than the control (Papamanoli *et al.* 2003; Rubio *et al.* 2007; Casaburi *et al.* 2008; Ciuciu Simion *et al.* 2014). This decrease of the number of Enterobacteriaceae is explained by the decrease in pH caused by LAB (Bronomo *et al.* 2008; Janssens *et al.* 2012; Essid and Hassouna 2013; Ciuciu Simion *et al.* 2014). Moreover, the antimicrobial compounds excreted by LAB such as bacteriocins contributed to the reduction of the number of viable cells of Enterobacteriaceae (Deumier and Collignan 2003; Leroy *et al.* 2006; Ammor and Mayo 2007; Gao *et al.* 2014). In fact, *L. sakei* often produce sakacins, a class II-a bacteriocin with a strong antimicrobial activity against a broad range of food spoilage microorganisms and foodborne pathogens. These bacteriocins are characterized by a cationic nature, a high isoelectric point and the presence of hydrophobic and hydrophilic regions, allowing them to permeabilize membrane cells (Cintas *et al.* 2001).

Finally, the number of yeasts and molds increased steadily during the first 7 days of ripening for all samples of sausages. Then, their concentrations decreased. At the end of maturation, yeasts and molds reached values of 2.81, 2.763 and 3.127 log cfu/g, respectively, for control samples and sausages inoculated with starter A and starter B, respectively (Fig. 1). Our results are in agreement with other studies (Casaburi *et al.* 2008; Janssens *et al.* 2012) reporting that yeasts and molds of both control and inoculated sausages reached values between 10^2 and 10^3 cfu/g at the end of maturation.

pH, Water Activity, Moisture and Weight Loss

The initial pH of all of sausages tested was 6.3. The pH decreased during the first 2 weeks of ripening to reach values of 5.27, 5.16 and 4.95, respectively, at the 14th day for control samples and sausages inoculated with starter A and starter B (Fig. 2). The fastest pH drop was observed in sausages inoculated with starter B. The decrease in pH was presumably caused by an accumulation of organic acids, mainly lactic, present in this type of sausages as a result of carbohydrate breakdown during fermentation (Zaho *et al.* 2011). During the last 2 weeks of ripening, the pH of control and inoculated sausages increased gradually. This increase in pH is explained, first of all, by the reduction in the number of LAB due to the exhaustion of the sugar and, second, to proteolytic activity generated by microorganisms.

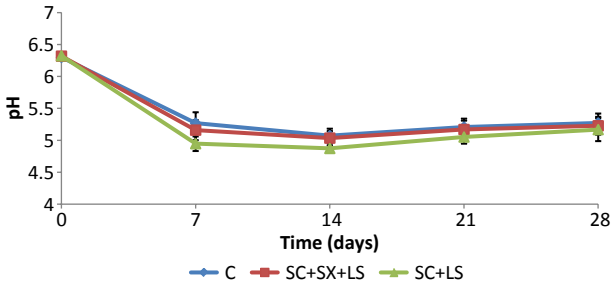

FIG. 2. EVOLUTION OF pH DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), SC + LS (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

Bacterial proteases induce proteolytic degradation, generating peptides, amino acids and amines, which have a buffering effect on the organic acids produced by LAB during fermentation (Benito *et al.* 2007; Ruiz-Moyano *et al.* 2011).

Our results showed that the sausages inoculated with starter A and starter B have a lower pH than the control sausages. These results are in agreement with many other studies (Bozkurt and Erkmen 2002; Drosinos *et al.* 2005; Kaban and Kaya 2006; Casaburi *et al.* 2008; Essid and Hassouna 2013; Ciuciu Simion *et al.* 2014) reporting that the pH of a dry fermented sausage inoculated with LAB is lower than the pH of the control sausage.

Values of water activity (a_w) decreased gradually during the manufacturing process, reaching values of 0.822, 0.743 and 0.792, respectively, for control sausages and sausages inoculated with starter A and starter B at the end of maturation (Fig. 3). The usage of starter cultures had no significant effect ($P > 0.05$) on a_w. The same occurred regarding moisture content values, which decreased from 59.37, 58.47

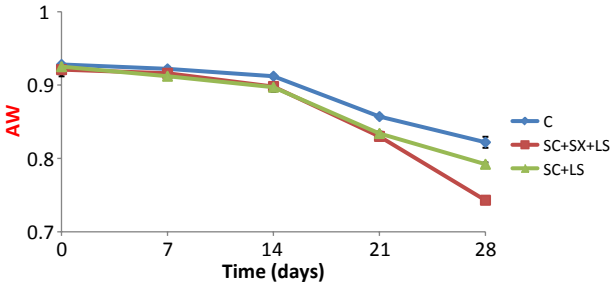

FIG. 3. EVOLUTION OF WATER ACTIVITY DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), SC + LS (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

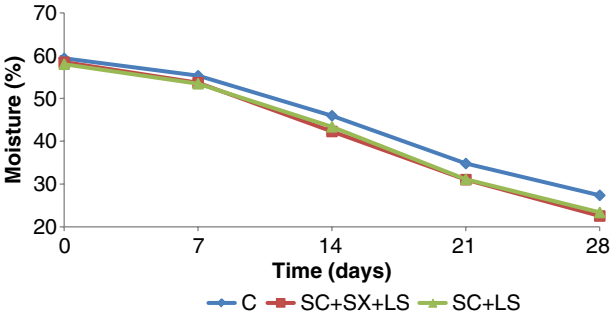

FIG. 4. EVOLUTION OF MOISTURE DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus Sakei*), SC + LS (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

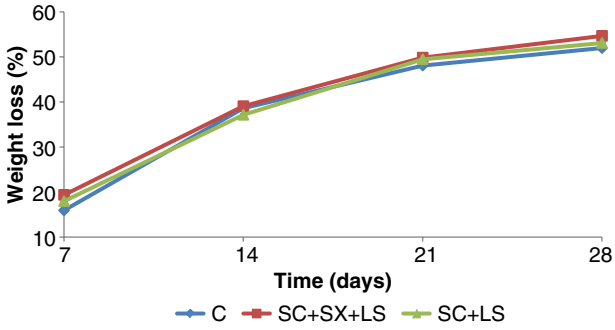

FIG. 5. EVOLUTION OF WEIGHT LOSS DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SX (sausage inoculated with *Staphylococcus xylosus*), LP (sausage inoculated with *Lactobacillus plantarum*), SX + LP (sausage inoculated with mixed starter culture *S. xylosus* and *L. plantarum*).

and 58%, respectively, for control sausages and sausages inoculated with starter A and starter B, to reach values of 27.37, 22.54 and 23.37% at day 28 (Fig. 4). This water loss especially during the first 2 weeks of ripening is due, first of all, to the elevated temperature of fermentation (24C) that accelerates the dehydration of the product and, second, to RH. RH was controlled in the fermentation chamber and it was fixed at 80%. Water has to diffuse from the inner part of the sausage to the surface and then it is evaporated to the chamber environment. Both rates, diffusion and evaporation, should proceed in a similar fashion. Hence, the maintenance of this high humidity (80%) in the fermentation chamber is necessary to prevent excessive weight loss and to provide some control of microbial spoilage. Moreover, this water loss is due to the decrease of pH which causes protein denaturation and thus a decrease in water retention capacity of myofibrillar proteins (Solignat 1999). Our results are in agreement with many other studies (González-Fernández *et al.* 2003; Ferreiria *et al.* 2007; Jin *et al.* 2010; Casquete *et al.* 2011; Fonseca *et al.* 2013; Ciuciu Simion *et al.* 2014).

The weight of control and inoculated sausages decreased during the ripening period (Fig. 5). This loss in weight is due to the temperature of fermentation (24C) that accelerates the dehydration of the product. Our results are in agreement with other studies (Liaros *et al.* 2009; Jin *et al.* 2010) reporting that the weight loss of dry fermented sausage increased during the ripening period.

Color Measurement

The color development was significantly affected by the ripening time of sausages ($P < 0.05$) and not by the addition of starters ($P > 0.05$).

The quality parameters L^*, $a^*$and b^* values underwent a decrease through the ripening period of different samples studied (Fig. 6). Our results are in agreement with those found by Casaburi *et al.* (2007) and Olivares *et al.* (2010). In relation to L^* values, a decrease was observed during ripening as sausage became darker due to weight loss (Olivares *et al.* 2010). With respect to a^* values, a decrease was observed during the first 2 weeks of maturation, followed by a slight increase. The variation of the parameter color a^* during ripening of dry fermented sausages is linked to the

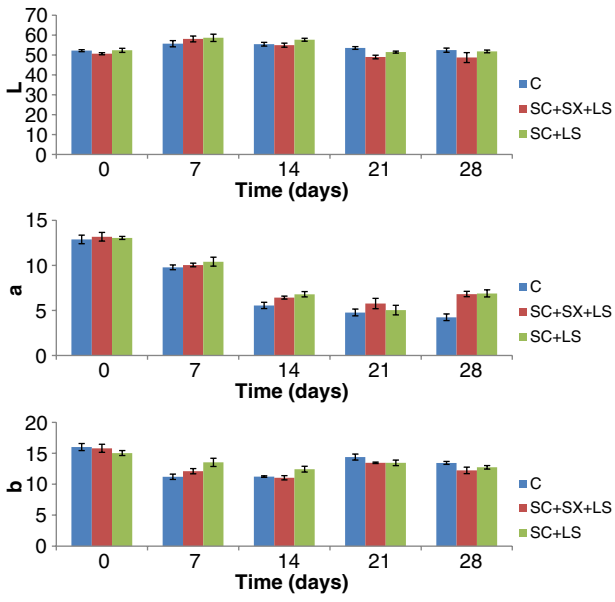

FIG. 6. EVOLUTION OF L^*, a^*AND b^* VALUES DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus Sakei*), SC + LS (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

TABLE 1. FREE AMINO ACID CONTENT (MG AMINO ACIDS/100 G OF SAUSAGE) DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES

Amino acids (mg amino acids/100 g of sausage)	Time (days)						
	0	14			28		
	C	C	SC + LS	SC + SX + LS	C	SC + LS	SC + SX + LS
Aspartic acid	3.436 ± 0.04	3.629 ± 0.06	4.032 ± 0.04	3.847 ± 0.07	3.344 ± 0.03	3.511 ± 0.01	2.97 ± 0.06
Glutamic acid	3.398 ± 0.03	3.499 ± 0.05	3.883 ± 0.05	3.766 ± 0.06	3.16 ± 0.03	3.278 ± 0.02	2.754 ± 0.05
Serine + Glutamine	3.583 ± 0.03	3.630 ± 0.09	4.173 ± 0.04	4.474 ± 0.03	2.976 ± 0.07	4.275 ± 0.23	3.877 ± 0.06
Arginine + Threonine	74.696 ± 1,25	80.256 ± 1.72	87.157 ± 0.19	88.313 ± 0.88	82.173 ± 0.38	102.593 ± 0.73	108.628 ± 1.6
Alanine	5.597 ± 0.04	5.415 ± 0.13	6.507 ± 0.04	6.850 ± 0.05	4.803 ± 0.07	4.553 ± 0.88	4.403 ± 0.24
Tyrosine	4.459 ± 0.01	4.636 ± 0.15	5.46 ± 0.18	6.005 ± 1.72	5.044 ± 0.29	5.075 ± 0.13	3.310 ± 0.06
Valine + Methionine	3.994 ± 0.02	4.116 ± 0.05	4.537 ± 0.05	4.993 ± 0.08	3.715 ± 0.01	4.503 ± 0.01	3.794 ± 0.06
Tryptophan	0.735 ± 0.16	0.531 ± 0.09	0.611 ± 0.02	0.684 ± 0.07	0.328 ± 0.08	0.569 ± 0.02	0.38 ± 0.02
Phenylalanine	3.642 ± 0.08	3.238 ± 0.03	3.534 ± 0.15	4.097 ± 0.25	3.072 ± 0.04	3.074 ± 0.01	2.852 ± 0.19
Leucine	6.610 ± 0.05	6.802 ± 0.13	8.004 ± 0.08	7.631 ± 0.11	5.934 ± 0.03	6.444 ± 0.09	5.111 ± 0.1
Isoleucine	3.372 ± 0.07	3.634 ± 0.06	4.073 ± 0.05	3.917 ± 0.11	3.281 ± 0.03	3.344 ± 0.04	2.759 ± 0.04
Lysine	1.641 ± 0.13	1.778 ± 0.06	2.218 ± 0.07	1.918 ± 0.15	1.464 ± 0.01	1.276 ± 0.07	0.871 ± 0.03
Total	**114.983**	**121.164**	**134.189**	**136.494**	**119.294**	**142.495**	**141.709**

Note: C (control sausage), SC + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus* and *Lactobacillus sakei*), SC + SX + LS (sausage inoculated with mixed starter culture *S. carnosus*, *Staphylococcus xylosus* and *L. sakei*).

formation of a small amount of nitroso myoglobin pigment (pink-red). Indeed, chicken muscle has lower myoglobin content (Mielnik *et al.* 2002; Yilmaz *et al.* 2002).

FAA Content

To evaluate the effect of starter of *S. xylosus*, *S. carnosus* and *L. sakei* on proteolysis, FAAs were determined through ripening using a chromatographic approach. The concentrations of total FAAs during ripening are shown in Table 1.

The total FAA content of sausages increased from 114.983 mg/100 g on day 0 to 119.294, 142.495 and 141.709 mg/100 g for control sausages and sausages inoculated with starter A and starter B, respectively. Our results are in agreement with many other studies (Casaburi *et al.* 2008; Candogan *et al.* 2009; AroAro *et al.* 2010; Lorenzo and Franco 2012; Nie *et al.* 2014) reporting an increase in total FAA content during ripening of sausages. The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and FAAs; this degradation can be produced by endogenous and microbial enzymes as reported by different authors (Fadda *et al.* 2002; Hughes *et al.* 2002; AroAro *et al.* 2010). The concentrations of total FAAs during ripening were higher in inoculated sausages than that in control samples. This difference in the evolution of total FAAs are related to the proteolytic activities of endogenous and microbial enzymes activated by the decrease in pH of the medium ($pH_{op} = 4.5–5.5$) and by the decrease in temperature ($T_{op} = 15–20C$) (Casaburi *et al.* 2008).

In addition, the results showed that the highest enzymatic activity took place at the beginning of the process in coincidence with the fermentation stage. This increase has been attributed to the higher temperatures applied during fermentation compared with the low temperature applied during drying (AroAro *et al.* 2010; Essid and Hassouna 2013). A decrease in amino acid content was noted during the last 2 weeks of ripening of control sausage; this decrease may indicate their metabolism by bacteria (Bover-Cid *et al.* 2000; Sekikawa *et al.* 2003; AroAro *et al.* 2010; Essid and Hassouna 2013).

FFA Content

The concentrations of saturated, monounsaturated and polyunsaturated fatty acids during ripening of the three types of sausages are reported in Table 2. The results showed that total FFAs increased through ripening; they reached 98.83, 99.1 and 99.07% in control sausages and sausages inoculated with starter A and starter B, respectively. This increase is due to endogenous and microbial lipase activity. Our results are in agreement with those reported by Casaburi *et al.* (2008), Essid and Hassouna (2013), Rubio *et al.* (2007) and Zaho *et al.* (2011). Moreover, the monounsaturated fatty acids displayed concentrations higher than polyunsaturated and saturated in all the samples analyzed during ripening. Our results are similar to those of Casaburi *et al.* (2008), Rubio *et al.* (2007) and Pereira *et al.* (2000). On the contrary, Essid and Hassouna (2013) and Yildiz-Trup and Serdaroglu (2008) found that saturated fatty acids dominate sausages. High variability between sausages is normal as the concentration of FFAs in the fat depends on many factors, such as the raw materials used to prepare the sausages, the length of the process

TABLE 2. FREE FATTY ACID COMPOSITION (%) DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES

Fatty acid composition (%)	Time (days) 0	14			28		
	C	C	SC + LS	SC + SX + LS	C	SC + LS	SC + SX + LS
C14:0	0.44 ± 0.02	0.41 ± 0.04	0.42 ± 0.03	0.4 ± 0.02	0.42 ± 0.04	0.44 ± 0.03	0.47 ± 0.02
C15:0	0	0	0	0	0	0	0
C16:2	0.4 ± 0.01	0	0	0	0	0	0
C16:1	5.42 ± 0.45	5.5 ± 0.71	5.9 ± 0.24	5.44 ± 0.38	5.41 ± 0.49	5.64 ± 0.2	5.61 ± 0.11
C16:0	28.75 ± 2.47	29.04 ± 3.62	34.01 ± 1.12	31.65 ± 0.21	29.47 ± 0.16	32.30 ± 0.12	33.13 ± 2.2
C17:0	0.08 ± 0.01	0	0	0	0	0	0
C18:3	0	0	0	0	0	0	0
C18:2	7.70 ± 0.35	8.96 ± 0.05	6.43 ± 0.29	7.51 ± 0.75	8.63 ± 0.57	6.62 ± 1,94	6.43 ± 0.11
C18:1	45.72 ± 0.4	47.02 ± 3.1	44.61 ± 0.66	46.37 ± 0.15	46.07 ± 1,04	45.69 ± 0.83	44.68 ± 0.16
C18:0	8.26 ± 0.17	7.99 ± 0.32	8.17 ± 0.03	8.24 ± 0.05	8.50 ± 0.37	8.41 ± 1.11	8.75 ± 2.5
C20:1	0	0	0	0	0.33 ± 0.01	0	0
C20:0	0.09 ± 0.03	0	0	0	0	0	0
ΣSFA	37.62	37.44	42.60	40.29	38.39	41.15	42.35
ΣMUFA	51.14	52.52	50.51	51.81	51.81	51.33	50.29
ΣPUFA	7.74	8.96	6.43	7.51	8.63	6.62	6.43
Total	**96.5**	**98.92**	**99.54**	**99.61**	**98.83**	**99.1**	**99.07**

Note: C (control sausage), SC + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus* and *Lactobacillus sakei*), SC + SX + LS (sausage inoculated with mixed starter culture *S carnosus*, *Staphylococcus xylosus* and *L. sakei*).
MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

and the starter cultures (Lorenzo and Franco 2012). Furthermore, inoculated and control sausages showed the same FFA composition, considering that the major release of these latter was probably due to endogenous lipases. Therefore, the lipolytic strains *S. xylosus* and *S. carnosus* did not prove to be effective in the lipolysis of fermented sausage during ripening. The same result was observed by Casaburi *et al.* (2008). Moreover, the acid present in greater percentage was the oleic acid followed by palmitic acid and stearic acid. The same result was observed by Pereira *et al.* (2000), who showed that oleic acid and palmitic acid were the predominant FFAs during ripening of Brazilian poultry sausages.

Texture Measurements

Results concerning the instrumental texture showed that no significant differences were observed between control and inoculated sausages; however, hardness and elasticity were affected by time ripening. Hardness (Fig. 7) and Young's modulus (Fig. 8) increased significantly through ripening time for control and inoculated sausages. The increase of hardness and elasticity through the ripening of sausages are mostly due to water loss. These results match with those found by Liaros *et al.* (2009), who reported that higher hardness in sausages is probably due to much higher weight loss. Generally, the major changes in fermented sausage structure take place during fermentation when the pH declines and the myofibrillar proteins aggregate to from a gel. After fermentation, drying is a major factor affecting rheological properties (Gonzalez-Fernandez *et al.*, 2006).

CONCLUSION

This work studies the effect of selected starter cultures on microbiological, biochemical and textural characteristics of a Tunisian dry fermented poultry meat sausage. The use of starter cultures could be useful for maintaining hygienic quality of sausages by inhibition of spoilage and pathogenic microorganisms, which allows a good preservation of sau-

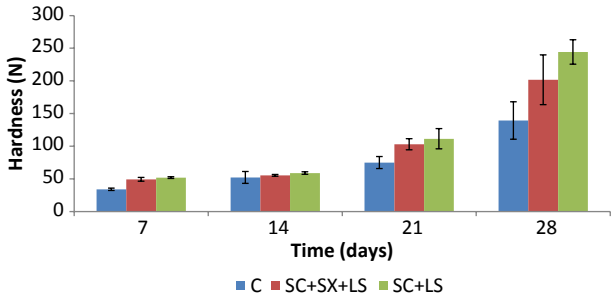

FIG. 7. EVOLUTION OF HARDNESS DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), SC + LS (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

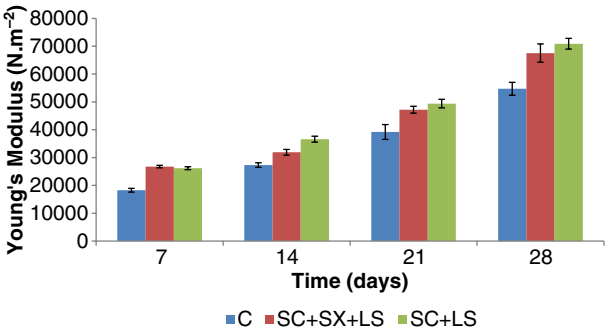

FIG. 8. EVOLUTION OF YOUNG'S MODULUS DURING THE RIPENING OF CONTROL AND INOCULATED DRY FERMENTED SAUSAGES
C (control sausage), SC + SX + LS (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), SC + LS (Sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*).

sages and consequently improved their shelf life. Textural parameters of sausages were not affected by the use of selective starters.

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