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ORIGINAL PAPER

Influence of autochthonous starter cultures on microbial dynamics and chemical-physical features of traditional fermented sausages of Basilicata region

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Abstract In this study, a combination of a *Lactobacillus sakei* strain and a *Staphylococcus equorum* strain was used as autochthonous starter for an experimental production of Basilicata fermented sausages. The influence of starter addition on the safety and quality parameters and microbiological and chemical-physical properties of the products was investigated. Microbial counts of safety indicators were lower in the samples with the addition of starter culture, and, at the end of ripening, *Enterobacteriaceae* and Gram negative bacteria were detected only in samples made without the starter addition. The addition of starter led to a final product with better microbiological and chemical-physical features, and a positive effect on flavor and aroma compounds formation by a good proteolytic and lipolytic activities. The use of autochthonous starter cultures allows to obtain products with the organoleptic characteristics expected and steady in time and to standardize the production process, improving the safety and quality, but preserving the essential character of the product.

Keywords Traditional fermented sausages · Autochthonous starter culture · Experimental processing

Introduction

Fermented sausage is defined as a mixture of comminuted fat and lean meat, and it contains sugars, salt, nitrite and nitrate, ascorbates and spices. The raw sausage mixture is stuffed into casings and hung vertically in fermentation and ripening chambers for several weeks (Hugas and Monfort 1997; Tyopponen et al. 2003). The ripening process, which confers on the product its particular slice ability, firmness, texture, nutritional qualities, colour and flavour, is characterized by a complex interaction of biochemical and physical reactions and microbiological and sensorial changes associated with the development and metabolic activities of the autochthonous microflora (Casaburi et al. 2007; Martin et al. 2007; Ammor and Mayo 2007). The spontaneous fermentation of sausages involves the participation of Lactic Acid Bacteria (LAB), Coagulase Negative Cocci (CNC; mostly *Staphylococcus* and *Kocuria* species), and, less importantly, yeasts and moulds. These microrganisms affect the quality, the safety and the formation of aroma and flavour compounds (Comi et al. 2005; Montel et al. 1998). The Basilicata region offers different kinds of fermented sausages, artisanal products mostly manufactured by traditional technologies without starter addition (Amato et al. 1997); so, the specific composition of the house flora is responsible of the distinctive qualities of artisanal products, resulting in a greater quality than controlled fermentations inoculated with industrial starters, but leading to a large product diversity (Lebert et al. 2007; Lucke 2000). As it is not possible to ensure that the number and the strains of microorganisms present in the raw material will always be the same and behave in the same way, the use of starter cultures for sausage production became necessary to guarantee food safety and to standardize product properties (Zambonelli et al. 1992; Bonomo et al. 2008a; Baruzzi et al. 2006). The commercial starter cultures commonly used in sausage production are not always able to compete well with the house flora, so that their use often results in losses of desirable sensory properties. So, appropriate starter cultures have to be selected from indigenous microorganisms, that are more competitive and well

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adapted to the particular product and to the specific production technology, and with high metabolic capacities which can beneficially affect product quality and safety, preserving their typical character (Rebecchi et al. 1998; Leroy et al. 2006). Microorganisms originating from fermented meats are particularly well adapted to the ecological niche of meat fermentation (Hugas and Monfort 1997), therefore the study of the microbial ecology of fermented sausages is important to select them as native starter cultures able to produce the many typical regional fermented sausages with their specific properties (Lucke 1985; Aymerich et al. 2003). According to the definition of Hammes (1996), meat starter cultures are "preparations which contain living or resting microorganisms that develop the desired metabolic activity in the meat" and they are used to change and improve the sensory properties of the food (Tyopponen et al. 2003). In previous studies, the authors identified, characterized and selected, from traditional fermented sausages of Basilicata region, strains of lactic acid bacteria and coagulase-negative staphylococci suitable to use as autochthonous starters, endowed with specific technological and physiological features that make the products unique (Bonomo et al. 2008a, b).

The purpose of this study was to determine the effects of starter culture addition during the ripening of traditional fermented sausages to define the chemical-physical and microbial changes occurring in these products.

Materials and methods

Starter culture formulation

Bacterial strains used as autochthonous starter cultures in this study are reported in Table 1. They were chosen in previous studies on the basis of their safety and technological characterization and the intraspecific biodiversity evaluation (Bonomo et al. 2008a, b). All strains were maintained as freeze-dried stocks in reconstituted (11% w/v) skim milk, containing 0.1% w/v ascorbic acid (Riedel-de Haën, Sigma–Aldrich, Milan, Italy) and lactic acid bacteria (LAB) were routinely cultivated in MRS broth at 30°C for 16 h, while coagulase-negative staphylococci (CNS) were routinely cultivated on TSAYE slants, and routinely cultivated in Tryptone Soya Broth with 0.6% (w/v) Yeast Extract (TSBYE) at 30°C for 16 h. These strains were tested individually and each combined with others; different associations (25 combinations) were obtained between each LAB strain and each staphylococcal strain (shown in Table 1).

Tests in a meat model dough

A meat model dough was applied in our laboratory to test the behaviour of the potential autochthonous starters selected previously (Bonomo et al. 2008a, b). A recipe for traditional Basilicata sausages was used to prepare the meat mixture containing 5 kg of pork meat, 2.5% of NaCl, 0.2% of glucose and 50 ppm of potassium nitrate. The frozen meat was minced and mixed with the ingredients, then the strains were added to the mix in different combinations at a final concentration of 10^6 c.f.u./g. After inoculation, the meat mixture was distributed into 50 mm plates and then incubated at 20°C for 24 h and 7 days in anaerobiosis. At the end of incubation, the effects of the different starter combinations on pH, proteolysis and colour of product were evaluated. Duplicate determinations were performed. The pH was measured by using an electrode (Hanna Instruments Italia, Padova, Italy) inserted directly into the mixture after 24 h and 7 days of incubation. After 7 days

Table 1 Bacterial strains used as autochthonous starter cultures in this study

Species	Strains	Origin[a]	Technological properties[b]	Reference
Lactobacillus sakei	DBPZ0062	DBDBAF	Acidifying activity	Bonomo et al. (2008a)
Lactobacillus sakei	DBPZ0098	DBDBAF	Proteolytic activity	Bonomo et al. (2008a)
			Ability to hydrolyze sarcoplasmatic proteins	
Lactobacillus sakei	DBPZ0338	DBDBAF	Acidifying activity	Bonomo et al. (2008a)
Lactobacillus sakei	DBPZ0329	DBDBAF	Proteolytic activity	Bonomo et al. (2008a)
Lactobacillus sakei	DBPZ0416	DBDBAF		Bonomo et al. (2008a)
Staphylococcus equorum	DBPZ0241	DBDBAF	Growth at low pH values	Bonomo et al. (2008b)
Staphylococcus equorum	DBPZ0248	DBDBAF	Growth in presence of salts (NaCl, KNO_2)	Bonomo et al. (2008b)
Staphylococcus succinus	DBPZ0251	DBDBAF	Proteolytic activity	Bonomo et al. (2008b)
Staphylococcus xylosus	DBPZ0224	DBDBAF	Nitrate reductase activity	Bonomo et al. (2008b)
Staphylococcus equorum	DBPZ0044	DBDBAF	Ability to hydrolyze sarcoplasmatic and myofibrillar proteins	Bonomo et al. (2008b)

[a] DBDBAF: Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata

[b] Technological properties evaluated in order to select potential starters

of incubation, the proteolytic activity of strains was evaluated according to the Cd-ninhydrin method (Folkertsma and Fox 1992) reading the absorbance at 507 nm, by UV–Visible Beckman DU-65 spectrophotometer. To analyse mixture colour, a colorimeter Minolta Chromameter-2 Reflectance was used, by evaluating parameter L^* (mix luminosity), parameter hue angle with a^* and b^* coordinates (chromatic intensity) and parameter chroma (mix colour brilliancy).

Sausage experimental manufacturing and sampling procedure

Fermented sausages were prepared in an artisanal industry of the Basilicata region using traditional techniques. The sausage formulation included 62% pork lean meat, 33% fat, skim milk powder 4%, NaCl 2.8–3%, glucose 0.2%, ascorbic acid 0.08%, spices (ground and whole black pepper, fennel seeds, sweet and hot chili pepper powder) in variable amount according to the individual sausage recipe and nitrite/nitrate 50 ppm. The meat was minced and mixed with the other ingredients and then, after mixing, the meat mixture was divided into three batches: a, b and c. Batches a and b contained NaCl and glucose, and batch c contained NaCl, glucose and nitrate. Two of these batches (b and c) were inoculated with starter culture, while the batch (a) was non-inoculated, so acted as control.

The starter culture was composed of a mixture of *Lb. sakei* (DBPZ0062) and *S. equorum* (DBPZ0241). Freeze-dried starter culture was added to each batch at a final concentration of 10^6 c.f.u./g. Subsequently, the meat mixture was manually stuffed into natural casings and placed in a ripening chamber. Each batch was subdivided into two lots that were ripened for 28–30 days. The ripening was performed as follows: The first stage consisted of 1 day of stewing with the relative humidity (RH) of 85–90% and a temperature of 24°C; the second stage consisted of 7 days of drying at 18–22°C and 80–90% RH; and the last stage consisted of 20–22 days of seasoning with 80–85% RH and a temperature of 10–14°C. The sausages were vacuum packaged and stored at 4°C for 90 days. Samplings were taken before stuffing ($t = 0$) and, during processing, at the end of drying ($t = 7$ days), at the mid-ripening ($t = 15$–20 days) and then at the end of ripening ($t = 28$–30 days). Samples were also taken at 60 and 90 days of storage. Samplings were collected in duplicate and used for the analyses.

Microbiological analyses

The fermented sausages were subjected to microbiological analysis to monitor the dynamic changes in the populations responsible for the ripening, but also to determine their hygienic quality. In particular, 10 g of each sample was transferred into a sterile stomacher bag, 10-fold diluted with sterile saline/peptone water (8.5 g/l NaCl and 0.1 g/l bacteriological peptone) and homogenized for 2 min in a stomacher (Lab-Blender, Seward, London, UK). Further decimal dilutions were made and inoculated into agar plates of appropriate and selective media. Gram-negative bacteria were enumerated on Plate Count Agar (PCA), containing 0.1% crystal violet solution in ethanol and 1% triphenyltetrazolium chloride solution, incubated at 30°C for 24–48 h; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBGA) incubated at 37°C for 24 h; LAB were determined on MRS agar in anaerobic conditions incubated at 30°C for 48 h; staphylococci on Baird Parker Medium Agar supplemented with egg yolk tellurite emulsion incubated at 37°C for 24–48 h; and yeasts and moulds on Rose Bengal Chloramphenicol Agar (RBCA) incubated at 25°C for 5 days. Tests were carried out in duplicate, then, after counting, means and standard deviations were calculated and the results were expressed as log (c.f.u./g).

Chemical-physical analyses

Moisture, pH, water activity and colour determination

Chemical-physical analyses during ripening included measurement of moisture, pH and water activity. Moisture content (%) was determined by drying at thermostatic 105°C until constant weight (after about 3 days) (Johansson et al. 1994; Dalmis and Soyer 2008). Percentage of moisture of each sample was calculated by the difference between the sample initial weight and the sample weight after drying. Means of two measurements were calculated. The pH measurement was performed by using an electrode for liquids of the Hanna Instrument 8417 pH-meter. The pH was determined after mixing and homogenizing 10 g of test samples with 90 ml of distilled water and equilibrating for 5 min. Means were calculated from three measurements. Water activity (a_w) was measured by Hygrometer Decagon (Rotronic Hygroscop BT) at 24°C. Calibration was performed at 80% of humidity using 40% saturated solutions of lithium chloride (Papadima and Bloukas 1999). Means of two measurements for each sample were obtained. In order to evaluate the effect of starter addition on sample colour, a colorimeter Minolta Chromameter-2 Reflectance was used. The a^* and b^* coordinates, L^*, hue angle and chroma parameters were determined. The a^* and b^* are coordinates of chromaticity: negative values of a^* represented a green colour, while positive values a red colour; then, negative values of b^* stood for a blue colour, while positive values a yellow colour. Moreover, parameter L^* represented the sample luminosity (expressed in percentage), parameter hue angle the chromatic intensity, with

low values that indicate the red colour and values near at 90° the yellow colour, and parameter chroma the sample colour brilliancy. The colorimeter was calibrated using a white standard plate ($L = 100$). Three readings were carried out on each slice, only the lean colour was measured and care was taken to avoid any fat.

Free amino acids (FAA) and free fatty acids (FFA)

The free amino acid release in each sample was evaluated according to the Cd-ninhydrin method (Folkertsma and Fox 1992) reading the absorbance at 507 nm respectively, by UV–Visible Beckman DU-65 spectrophotometer. The free fatty acid release was determined evaluating the enzymatic activity by determination of the free fatty acid concentration by spectrophotometric measurements at 715 nm according to Lowry and Tinsley (1976). All enzymatic activity assays were performed in duplicate.

Statistical analysis

Statistical analysis was performed using Systat 10.0 for Windows (SPSS, Chicago, IL, USA). In the meat model dough and in the experimental processing, differences between the control sausage and sausages with autochthonous starter, with regard to microbiological and physic-chemical parameters during ripening phases, were tested by ANOVA analysis ($P < 0.05$) to evaluate the effect of starter culture addition on the quality attributes.

Reagents and media

Unless otherwise specified, all reagents were obtained from Sigma–Aldrich (Milan, Italy), while bacteriological media and ingredients were obtained from Oxoid (Basingstoke, Hampshire, England).

Results and discussion

Meat model dough

The primary purpose of this study was to test the effect of specific strains, previously chosen as potential autochthonous starters, in a meat model system in order to select the best starter combination to use in an experimental processing of traditional sausages. The meat dough was inoculated with selected strains (Table 1) individually and each combined in different associations with others. The initial pH values in control and starter-inoculated samples were 5.8 and 6.2, respectively; then, after 24 h (at the beginning of ripening) in all samples, including control, a fair pH drop was recorded, ranging between 4.9 and 5.2, with significant differences between control and starter-inoculated samples. Samples inoculated with the DBPZ0062 strain combined with each staphylococcal strain presented the lower values at this time. After 7 days of incubation, all samples showed a pH increase, and that inoculated with DBPZ0416–DBPZ0044 produced the highest value, very probably linked to the strong proteolytic activity of the two strains (Table 2). As to proteolytic activity, the effect of starter combinations on the degradation of proteins during ripening (after 7 days) was analysed, detecting concentration of amino acids with Cd-ninhydrin. Most of the inoculated samples showed an amino acid release higher than the control, ranged between 0.51 and 1.28 (Table 2). Moreover, all culture combinations, except some including DBPZ0098 strain, produced a red colour, with high value of a^* chromaticity coordinate and low values of hue angle parameter. In addition, this red colour was luminous, with high values of L^* parameter, and brilliant, with high values of chroma parameter (Table 2). In particular, *S. equorum* strains (DBPZ0044 and DBPZ0241) combined with *Lb. sakei* strains (DBPZ0416 and DBPZ0062) caused it. On the basis of the results of tests in this model meat dough, we selected the starter combination endowed with better features tested and composed of DBPZ0062 *Lb. sakei* strain and DBPZ0241 *S. equorum* strain to use in an experimental processing of artisanal fermented sausages.

Microbiological analyses

Enterobacteriaceae and Gram negative bacteria

The microbial changes in traditional fermented sausages during ripening and storage are shown in Table 3. Initial Enterobacteriaceae numbers were similar in all batches, ranged between 2.5 and 2.8 log c.f.u./g, range usually reported in other studies on this type of sausage (Casaburi et al. 2007; Dominguez et al. 1989; Sanz et al. 1997). However, the growth of these microorganisms during ripening was clearly different between the formulations with and without starter addition. The Enterobacteriaceae level was significantly ($P < 0.05$) lower in starter-inoculated samples than control during all ripening phases. At the end of drying (after 7 days), Enterobacteriaceae count was still elevated, due to the high values of a_w; then, in the mid-ripening phase, the addition of starter in batch c caused a significant decrease of approximately 1.60 log c.f.u/g, while the control and the batch b showed a slight reduction over the same period of time, even if the difference between control and formulations with starter was of 1 log c.f.u/g. At the end of ripening, the Enterobacteriaceae count was 1.58 log c.f.u/g in control batch and non-detectable in batches with starter addition. During the

Table 2 Effects of potential autochthonous starters selected on pH, proteolysis and mixture colour in a meat model dough

Strain combinations	Proteolysis ($OD_{507\ nm}$)[a]	pH[b]			Mixture colour analysis[c]			
		24 h	7 days		L^* (%)	Hue angle	a^*	b^*
Control	0.49	5.12	5.26	47.71	48.89	9.11	10.11	13.51
DBPZ0062	0.80	5.06	5.44	42.49	39.93	10.12	8.32	13.02
DBPZ0098	0.60	5.07	5.27	46.50	44.21	9.44	8.82	12.54
DBPZ0338	0.71	4.97	5.31	43.11	39.98	10.53	8.62	13.51
DBPZ0329	0.86	5.00	5.25	45.59	40.18	8.38	7.09	10.59
DBPZ0416	1.28	4.95	5.31	42.55	39.99	10.41	8.56	13.49
DBPZ0241	0.60	4.96	5.33	47.51	41.49	9.58	7.09	10.51
DBPZ0248	0.78	5.07	5.30	46.09	42.23	8.61	8.57	12.19
DBPZ0044	0.93	4.96	5.28	42.59	40.03	8.97	7.45	11.49
DBPZ0251	0.49	5.06	5.37	45.01	43.97	7.58	8.92	13.10
DBPZ0224	0.61	4.90	5.32	47.55	46.87	8.39	8.71	12.01
DBPZ0062 + DBPZ0241	0.72	5.05	5.33	43.50	40.01	10.27	7.79	13.32
DBPZ0062 + DBPZ0248	0.51	5.06	5.34	42.45	41.23	9.89	8.02	12.97
DBPZ0062 + DBPZ0044	0.75	5.04	5.35	42.47	40.02	10.10	7.97	13.51
DBPZ0062 + DBPZ0251	0.63	5.03	5.33	41.98	42.34	10.11	9.01	13.02
DBPZ0062 + DBPZ0224	0.60	5.00	5.33	42.11	40.04	10.48	8.82	13.62
DBPZ0098 + DBPZ0241	0.88	5.00	5.33	41.51	58.76	5.98	9.54	11.31
DBPZ0098 + DBPZ0248	0.77	5.15	5.33	40.88	53.65	8.34	9.56	12.33
DBPZ0098 + DBPZ0044	0.79	5.17	5.35	41.48	40.03	7.98	8.21	12.98
DBPZ0098 + DBPZ0251	0.64	5.10	5.33	39.04	46.74	7.89	8.87	11.88
DBPZ0098 + DBPZ0224	0.75	5.07	5.33	37.32	52.23	6.57	8.71	10.87
DBPZ0338 + DBPZ0241	0.78	5.11	5.31	43.23	57.96	9.48	8.74	12.45
DBPZ0338 + DBPZ0248	0.79	5.13	5.30	43.67	55.32	8.73	8.67	11.73
DBPZ0338 + DBPZ0044	0.77	5.12	5.33	40.57	46.23	9.21	8.91	11.98
DBPZ0338 + DBPZ0251	0.67	5.12	5.34	40.04	46.74	8.09	9.83	11.87
DBPZ0338 + DBPZ0224	0.77	5.10	5.32	39.92	52.38	8.87	9.71	10.77
DBPZ0329 + DBPZ0241	0.74	5.12	5.32	41.50	40.11	10.17	8.79	12.37
DBPZ0329 + DBPZ0248	0.68	5.14	5.33	40.97	41.26	10.04	9.12	12.77
DBPZ0329 + DBPZ0044	0.72	5.13	5.29	41.34	42.00	10.11	8.99	13.01
DBPZ0329 + DBPZ0251	0.53	5.15	5.34	40.98	41.77	10.09	9.56	13.02
DBPZ0329 + DBPZ0224	0.63	5.11	5.32	41.15	41.04	10.11	9.32	13.66
DBPZ0416 + DBPZ0241	0.91	5.12	5.40	42.52	39.78	9.47	7.59	12.11
DBPZ0416 + DBPZ0248	0.84	5.14	5.43	40.31	41.47	8.54	8.78	10.68
DBPZ0416 + DBPZ0044	0.79	5.13	5.82	42.52	42.23	11.10	9.19	13.42
DBPZ0416 + DBPZ0251	0.63	5.12	5.56	41.78	43.56	9.97	9.32	13.22
DBPZ0416 + DBPZ0224	0.61	5.10	5.44	43.55	44.63	8.78	8.41	12.21

All values represent the mean of two measurements

[a] Proteolysis ($OD_{507\ nm}$): proteolytic activity according to Cd-ninhydrin absorbance values at 507 nm

[b] pH: measurements of mixture pH after 24 h and 7 days of incubation

[c] Mixture colour analysis: evaluation of parameter L^* (mix luminosity), parameter hue angle with a^* and b^* coordinates (chromatic intensity) and parameter chroma (mix colour brilliancy)

whole storage period (90 days at 4°C), starter-inoculated samples continued to show a total inhibition of Enterobacteriaceae, while the control, with a initial presence over 1 log c.f.u/g, proved an intensive decrease, until a total inhibition reached at the end of storage. A similar trend was observed in evolution of Gram-negative bacteria during ripening. The initial count (about 4.3 log c.f.u./g) dropped to 2.5 log c.f.u./g at mid-ripening phase and to a total inhibition at the end of ripening in the batches inoculated with starter; while the control showed a high level at

Table 3 Microbial counts (log c.f.u./g) of traditional fermented sausages at four different ripening times: before stuffing ($t = 0$) and, during processing, at the end of drying ($t = 7$ days), at the mid-ripening ($t = 15$–20 days), at the end of ripening ($t = 28$–30 days) and then during storage

Microrganisms	$t = 0$	$t = 7$ days	$t = 15$–20 days	$t = 28$–30 days	$t = 60$ days	$t = 90$ days
Lactic acid bacteria						
Batch a	3.52 ± 0.04	8.51 ± 0.07	8.63 ± 0.14	7.73 ± 0.03	7.76 ± 0.08	7.80 ± 0.23
Batch b	3.59 ± 0.05	9.02 ± 0.08	9.15 ± 0.07	8.84 ± 0.15	8.80 ± 0.20	8.76 ± 0.17
Batch c	4.03 ± 0.07	9.03 ± 0.08	9.18 ± 0.08	8.87 ± 0.11	8.83 ± 0.20	8.79 ± 0.15
Coagulase-negative staphylococci						
Batch a	3.31 ± 0.07	5.96 ± 0.11	8.53 ± 0.23	6.03 ± 0.07	6.72 ± 0.22	6.72 ± 0.11
Batch b	3.49 ± 0.15	6.12 ± 0.09	9.06 ± 0.07	6.81 ± 0.11	6.81 ± 0.14	6.81 ± 0.14
Batch c	3.50 ± 0.08	6.10 ± 0.03	9.05 ± 0.12	6.69 ± 0.09	6.69 ± 0.08	6.69 ± 0.08
Enterobacteriaceae						
Batch a	2.79 ± 0.24	3.44 ± 0.03	3.26 ± 0.12	1.58 ± 0.21	0.61 ± 0.07	ND
Batch b	2.51 ± 0.15	2.83 ± 0.06	2.17 ± 0.02	ND	ND	ND
Batch c	2.48 ± 0.09	3.36 ± 0.03	1.76 ± 0.05	ND	ND	ND
Gram negative bacteria						
Batch a	4.36 ± 0.17	3.61 ± 0.02	3.68 ± 0.21	2.63 ± 0.04	1.56 ± 0.11	ND
Batch b	4.28 ± 0.08	2.51 ± 0.11	2.50 ± 0.08	ND	ND	ND
Batch c	4.27 ± 0.03	2.50 ± 0.05	2.50 ± 0.10	ND	ND	ND
Moulds and yeasts						
Batch a	4.22 ± 0.21	5.93 ± 0.06	5.88 ± 0.08	6.13 ± 0.13	5.87 ± 0.09	5.41 ± 0.06
Batch b	4.23 ± 0.14	5.92 ± 0.12	5.85 ± 0.22	5.96 ± 0.11	5.42 ± 0.06	4.76 ± 0.13
Batch c	4.25 ± 0.09	5.63 ± 0.07	6.19 ± 0.02	6.09 ± 0.12	5.53 ± 0.10	4.89 ± 0.08

Batch a: control with NaCl 2.8–3%; batch b: NaCl 2.8–3%, glucose 0.2% and starter; batch c: NaCl 2.8–3%, glucose 0.2%, nitrate 50 ppm and starter

Values are expressed in log c.f.u./g. Each number is the mean ± standard deviation of two samples taken from the same batch

ND not detected

mid-ripening and then a count of 2.63 c.f.u./g at the end of ripening. During storage, the Gram-negative bacterial count presented a similar pattern to that observed in Enterobacteriaceae numbers. In this study, Enterobacteriaceae and Gram-negative bacteria were present in all batches at the beginning phase, which is a regular finding due to their well-know sensitivity to acidic environments (Adams and Nicolaides 1997; Zdolec et al. 2008). Our results showed that Enterobacteriaceae and Gram-negative bacteria had behaviours similar to those observed in other studies (Casaburi et al. 2007; Cenci-Goga et al. 2008; Zdolec et al. 2008) and confirmed the observation of Giraffa (2002) that established Enterobacteriaceae can survive and grow during fermentation of meat and dairy products, especially in foods without the use of competitive starter cultures. In our experiment the great reduction of Enterobacteriaceae, below the detection limit at the end of ripening, was a good indicator of microbiological safety and quality of sausages and of their preservation conditions (Pidcock et al. 2002) and confirmed the study of Lucke (1986) that established that Enterobacteriaceae are rarely found in long-ripening-period meat products due to their high sensitivity to acidity and desiccation (Gonzalez and Diez 2002).

LAB and CNS

In all batches the initial count of LAB was 3.5–4 log c.f.u./g but rapidly dominated the microflora from the end of drying, increasing to 8.5–9 log c.f.u./g. During ripening, the addition of starter increased the LAB count significantly, resulting in a presence ten times more elevated in starter-inoculated samples than control, as shown in Table 3. The difference among the control and treatments was preserved, also, during the whole storage period. The significant increase of LAB count confirmed the good adaptation of native lactic strains to meat substrate, suggesting a higher dominance and resistance to specific manufacturing conditions, as already demonstrated in previous studies (Bonomo et al. 2008a; Urso et al. 2006; Zdolec et al. 2008). As to CNS, the initial count of 3–3.5 log c.f.u./g in all batches, increased more rapidly during the first phases of ripening in the starter-inoculated samples reaching 9 log c.f.u./g and, then, the final

population (6.6–6.8 log c.f.u./g) in treatments was significantly lower but it was greater than control, and it had a steady trend during all storage (Table 3). These results suggest the poor competitiveness of CNS due to intensive growth and dominance of LAB, as reported by other authors (Samelis et al. 1998; Zdolec et al. 2008).

Moulds and yeasts

Counts of about 4.2 log c.f.u./g were found in the meat mixture of all batches and were higher in the final products, with an increase of about 2 log c.f.u./g. During storage, their presence decreased and, at the end, moulds and yeasts numbers were lower, especially in the starter-inoculated samples with a value of 4.7–4.9 log c.f.u./g.

Chemical-physical analyses

Moisture, pH, water activity and colour changes

The results of moisture determinations, pH and a_w are reported in Table 4. The moisture content, at the end of ripening, ranged between 49 and 54% in all samples, with and without starter addition. No significant differences were observed among control and starter-inoculated batches during all ripening phases and also the whole storage period. The a_w showed high values at the beginning and in the mid-ripening and then, at the end of the process, ranged between 0.67 and 0.70. At the end of storage, starter-inoculated batches still showed values lower than the control, as during ripening. A different trend was noted for pH, the initial value of 5.60 in the control and of 6.18 and 5.60 in the batches b and c respectively, reached a very low value in the mid-ripening, of about 5.1–5.2 and then, at the end of ripening, pH increased slightly in the samples added with starter, while in the control the value increased of 0.7. In our study we found the usual pH trend observed by other authors (Kozačinski et al. 2006; Zdolec et al. 2007, 2008; Casaburi et al. 2007); it is manifested as a fast decrease during the first phases of ripening and a slight increase towards the end of ripening. However, the addition of starter caused a more rapid acidification that was reduced strongly at the end of storage.

Table 4 Measurement of moisture, pH and water activity (a_w) of traditional fermented sausages at four different ripening times: before stuffing ($t = 0$) and, during processing, at the end of drying ($t = 7$ days), at the mid-ripening ($t = 15$–20 days) and at the end of ripening ($t = 28$–30 days)

Time (days)	Batch		
	a	b	c
Moisture %[a]			
0	68.9 ± 0.20	67.2 ± 0.11	69.4 ± 031
7	60.7 ± 0.22	65.8 ± 0.15	61.8 ± 0.20
15–20	57.3 ± 0.18	59.8 ± 0.23	59.8 ± 0.30
28–30	52.7 ± 0.10	53.8 ± 0.17	49.9 ± 0.24
pH[b]			
0	5.60 ± 0.12	6.18 ± 0.08	5.60 ± 0.07
7	5.21 ± 0.20	5.12 ± 0.05	5.20 ± 0.09
15–20	5.24 ± 0.06	5.19 ± 0.03	5.11 ± 0.09
28–30	5.48 ± 0.07	5.20 ± 0.13	5.29 ± 0.15
a_w[c]			
0	0.97 ± 0.001	0.96 ± 0.003	0.97 ± 0.002
7	0.79 ± 0.001	0.88 ± 0.003	0.91 ± 0.001
15–20	0.80 ± 0.002	0.85 ± 0.001	0.82 ± 0.001
28–30	0.70 ± 0.001	0.68 ± 0.003	0.67 ± 0.002

[a] Values represent the mean ± standard deviation of two measurements

[b] Values represent the mean ± standard deviation of three measurements

[c] Values represent the mean ± standard deviation of two measurements

The changes of colour parameters are shown in Fig. 1. Our results indicated that there was an important and significant effect of starter addition on the colour parameters only in presence of nitrate. The higher variations of values were seen especially in the L^* parameter evaluation. At the end of ripening, batch c showed a colour more luminous than control with a L^* parameter value of 44% and 32–34% for sample c and for the other batches, respectively; this difference was completely reduced at the end of storage. Moreover, batch c showed an increase in chromatic intensity during the whole ripening process, as proved by hue angle values lower, and, at the end, it had a value of 48°. The parameter chroma, instead, had high values during ripening in all batches and at the end showed lower value of about 50; and, then, this colour brilliancy increased slightly at the end of the storage period.

Free amino acids (FAA) and free fatty acids (FFA)

Amino acids play a role in flavour development, so it is particularly important to determine their release during ripening of fermented sausages (Beriain et al. 2000; Casaburi et al. 2007). In this study the free amino acid concentration was determined during ripening to evaluate the effect of the autochthonous starter culture on proteolysis, by using a spectrophotometric approach according to Cd-ninhydrin method of Folkertsma and Fox (1992). The concentration of FAA during ripening is shown in Fig. 2. At the beginning and the first phases of ripening, the FAA concentrations were very similar in all cases, the at the end of ripening, we observed a significant increase of free amino acids in the starter-inoculated samples with a concentration of 1.87 g/kg, although slight variations in the

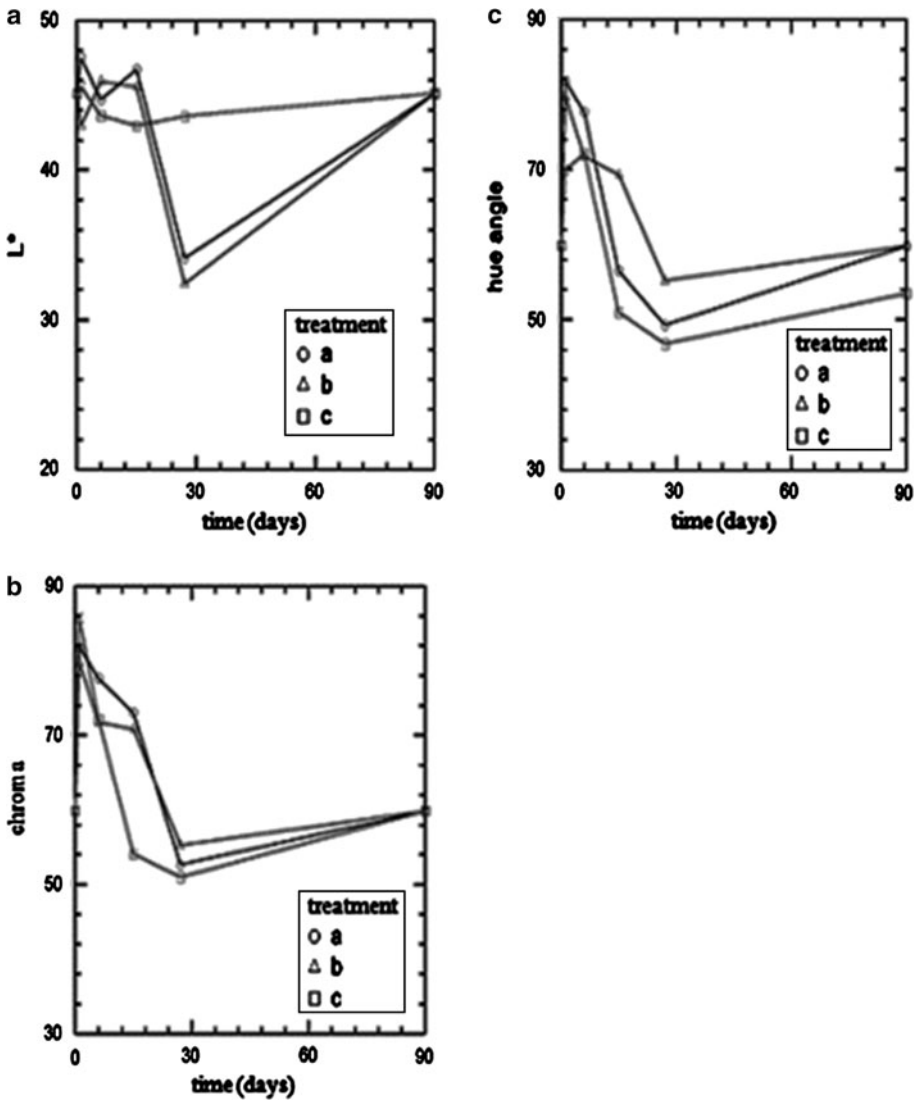

Fig. 1 The changes of colour parameters during experimental sausage ripening and storage with and without starter addition. **a** Control with NaCl 2.8–3% and glucose 0.2%; **b** NaCl 2.8–3%, glucose 0.2% and starter; **c** NaCl 2.8–3%, glucose 0.2%, nitrate 50 mg/l and starter

different ripening phases were detected, while, in the control sample, the FAA concentration was relatively constant with a final value of 0.98 g/kg. The FAA profile observed in our study is in accordance with results obtained by Casaburi et al. (2007). Together with proteolysis, lipolysis is directly involved in flavour formation during ripening of fermented sausages. FFA, released as a result of endogenous or microbial lipases, are the substrates for the biochemical reactions leading to the formation of aroma compounds and flavour of the final product (Countron-Gambotti and Gandemer 1999; Ordonez et al. 1999). Several authors have proposed the use of microbial starter cultures with lipolytic activity to influence the development of aroma in fermented sausages (Johansson et al. 1994; Samelis et al. 1993; Stanhke 1995). In this work, the FFA concentration was determined to evaluate the effect of the autochthonous starter on the lipid fraction of sausages from Basilicata region during ripening. All samples showed an increase of FFA during ripening in agreement with other studies (Comi et al. 1999; Hernandez et al. 1999; Lizaso et al. 1999; Molly et al. 1996; Casaburi et al. 2007). In all batches, the final concentrations were ranged between 3,250 and 4,550 mg/kg. Moreover, the results indicated that there was a greater release in the batch b during each ripening phase, but no significant difference compared to other samples; and, then, this FFA release was relative constant during the whole storage period.

Conclusions

In conclusion, the results presented in this study established that the use of autochthonous starter cultures affects significantly the organoleptic characteristics of fermented sausages by specific and important activities that contribute to improve the quality and safety of these traditionally

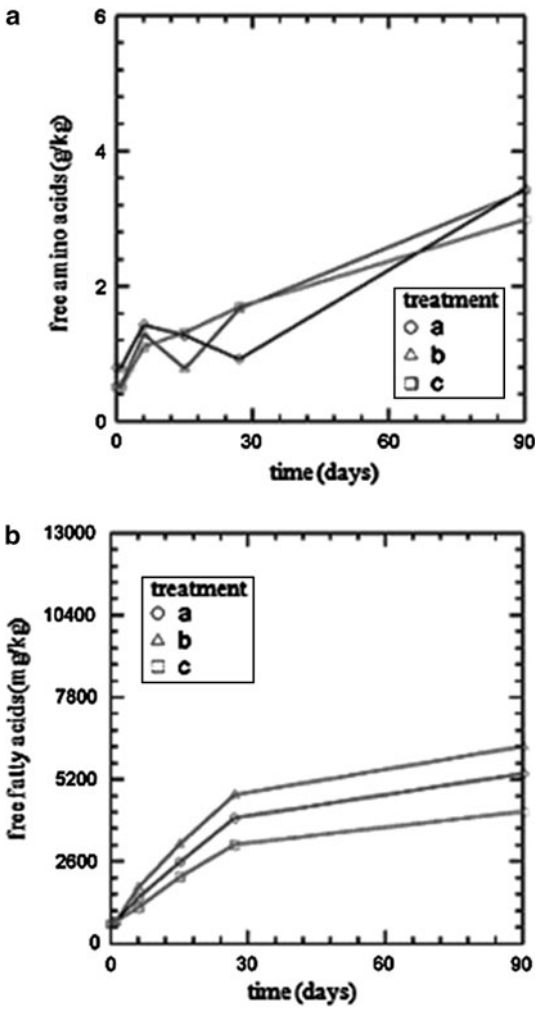

Fig. 2 Concentration of free amino acids and free fatty acids during experimental sausage ripening and storage with and without starter addition. **a** Control with NaCl 2.8–3% and glucose 0.2%; **b** NaCl 2.8–3%, glucose 0.2% and starter; **c** NaCl 2.8–3%, glucose 0.2%, nitrate 50 mg/l and starter

manufactured products. A native starter represents an alternative more efficient than the implementation of commercialized starters that not do always give satisfactory results, producing losses of desirable properties. Strains, isolated from indigenous microflora, have high adaptation properties in this kind of product and, so, the close relation to the environmental parameters, endowing them with specific abilities and important activities that make the traditional products unique. This experimental test demonstrated that the addition of starter led to a final product with better quality and microbiological features with inhibition of spoilage microorganisms and a good presence of lactic acid bacteria and staphylococci. The final product had improved chemical-physical properties with a good and rapid acidification, a brilliant and luminous red colour and an important contribution of proteolysis and lipolysis to the formation of flavour and aroma compounds. The use of autochthonous starter cultures allows to obtain products with organoleptic characteristics expected and steady in time and represent an important and efficient tool to standardize the production process improving the safety and quality, but preserving the typical character of the products.

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