PAPER

Microbiological and physicochemical profile of traditional *Salsiccia toscana* during storage

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Abstract

The aim of this study was to evaluate the evolution during refrigerated storage of the main microbiological and physicochemical parameters of a traditional Italian fresh sausage, *salsiccia toscana*. The following parameters were analyzed at Days 0, 6 and 9 of storage: total aerobic mesophilic and psychrotrophic counts, *Brochothrix thermosphacta*, lactic acid bacteria, *Pseudomonadaceae*, *Enterobacteriaceae*, *Escherichia coli*, *Micrococcaceae*, yeasts and moulds, *Salmonella* spp., *Listeria monocytogenes*; pH, A_w, moisture, protein, total lipids, ash, fatty acid composition, Thiobarbituric Acid Reactive Substances, colour measurement. The results revealed a statistically significant increase in the main microbiological parameters between Days 0 and 9 of refrigerated storage, with total bacterial counts exceeding 10^7 CFU/g after nine days and *Brochothrix thermosphacta* representing the main spoilage microorganism. Among physicochemical parameters, discolouration was noted during the time of storage, with a significantly paler colour on the surface of sausages with casings after nine days of storage. Considering that the initial bacterial counts were higher than 10^5 CFU/g for *Brochothrix thermosphacta*, *Pseudomonadaceae*, lactic acid bacteria and *Enterobacteriaceae*, and that the product is traditionally purchased loose without any packaging, it is fundamental to improve the good manufacturing practices, particularly measures to control processing temperatures.

Introduction

Salsiccia toscana is a very popular fresh sausage from the Toscana region, Italy, and is included in the list of national traditional agricultural and food products (Italian Regulation, 2011). It is based on a simple mixture of pork meat, pig fat, salt, pepper, spices, water and additives, stuffed in natural pig gut casings. It is shaped like a cylindrical tube about 10 cm long, and is pink in colour with a soft texture. The product is usually stored unpacked in the butcher's shops and purchased loose. The quality and the very particular taste of *salsiccia toscana* depend on the characteristics of the raw materials and on the type of processing method that has been passed on from one generation of producers to the next. In the past, *salsiccia toscana* was produced only in winter, but now this sausage is also prepared in summer. This extension of the manufacturing season has increased annual production to approximately 2 million kilograms (ARSIA, 2001). Like many other traditional foods, *salsiccia toscana* is mostly produced by small family units or butcher's shops. In the Toscana region, this product is very popular and frequently consumed under-cooked or even raw.

Pig meat and fresh sausages are reported to be frequently contaminated by pathogen microorganism *Salmonella* spp. (EFSA, 2011). In particular, some researchers suggest that uncooked fresh sausages could be an important source of *Salmonella* infection in humans (Giovannini *et al.*, 2004; Muermann *et al.*, 2011). Giovannini *et al.* (2004) sampled 227 fresh sausages in the Abruzzo region of Central Italy and registered 17.6% to be *Salmonella* positive. Similarly, Bonardi *et al.* (2002) examined 129 samples of fresh pork sausages produced in the Province of Parma in North Eastern Italy and found *Salmonella* in 13.2% of the samples.

Listeria monocytogenes has also been isolated from Italian fresh sausages: Bonardi *et al.* (2002) found *Listeria monocytogenes* in 49.6% of samples of fresh Italian sausages, whereas De Cesare *et al.* (2007) tested 288 samples from Northern Italy and reported 38.9% to be positive with more than 100 MPN/g in 20.5% of the positive samples. The perishable nature of *salsiccia toscana* is closely related to its processing. In fact, fresh meat is characterised by high water content and a rich nutrient matrix available on the surface. Furthermore, during the chopping process, oxidation and microbial contamination are facilitated by the loss of intracellular exudates and the increase of surface exposure to air. Microbial spoilage is responsible for the development of off-odours, which make the product unacceptable. The sensory changes are related to the type of microbial flora contaminating the meat and to the conditions of meat storing and processing. The spoilage flora of minced meat stored in air is dominated by *Pseudomonadaceae* and, to a lesser extent, by *Enterobacteriaceae*. Different treatments may select other microorganisms such as lactic acid bacteria (LAB), *Brochothrix thermosphacta* and yeasts (García-Lòpez *et al.*, 1998).

Among organoleptic characteristics, the colour of meat or meat products, related to the chemical state of the muscle pigment myoglobin, is a very important quality attribute that influences consumer acceptance. Indeed, consumers prefer bright red fresh meats and fresh meat products (Cornforth, 1994), whereas they find unattractive the dull purple colour associated with the deoxy form of myoglobin, as well as the dull brown colour given by the stable oxidised form, metmyoglobin (Gill, 2003).

Many studies on the evolution of the physicochemical, microbiological and sensory profile of fresh sausages during refrigerated storage have been carried out to evaluate different types of modified atmosphere packaging (Tremonte *et al.*, 2005; Martinez *et al.*, 2006; Chiavaro *et al.*, 2008; Sarli *et al.*, 2009; Ruiz-Capillas and Jiménez-Colmenero, 2010; Torrieri *et al.*, 2011), or the use of some antibacterial substances, such as sodium lactate and chitosan (Bingöl and Bostan, 2007; Soultos *et al.*, 2008), or the combination of commercial additives and spices (Kamdem *et al.*, 2007). However, even if *salsiccia toscana* is one of the most popular traditional sausages produced in Italy, there is little

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information about its spoilage pattern and physicochemical profile during the usual time of refrigerated storage.

The aim of this study was to evaluate changes in selected microbiological and physicochemical properties during refrigerated storage at different time intervals. Samples were analysed immediately after production (Day 0), at Day 6 (*i.e.*, the commercial term commonly indicated by manufacturers), and Day 9, to evaluate also a shelf life extension beyond that currently applied in butcher's shops in the Toscana region.

Materials and methods

Sausage production

Salsiccia toscana was prepared under commercial conditions in a butcher's shop inside a discount supermarket in Pisa, Central Italy. The sausage recipe was: pork meat 64%, pig fat 21%, salt (sodium chloride) 2%, pepper 0.3%, minced garlic 0.1%, water 10%, and 2 ready-to-use additive mixtures. Mix 1 (1%) contained salt, sodium citrate (E331), ascorbic acid (E300), sodium ascorbate (E301), natural flavours, spices and carmine (E120), and Mix 2 (1.5%) contained natural flavours and dextrose. The minced batter was stuffed into natural pork casings (40 mm diameter). Immediately after production, 6.35 kg of sausages (from a production batch of 30 kg) were randomly allocated in trays and stored in a refrigerator at 4°C for nine days. For study purposes, three different batches were used and data are the means of two and three replicates for each batch for microbiological and physicochemical analyses, respectively. In each batch, the analyses, with the exception of colour measurements, were carried out on a pool of five sausages obtained after the removal of the casings. Colour was measured on three sausages for each step of analysis (Days 0 and 9).

Microbiological analyses

Microbiological analyses were performed at Day 0, and after six and nine days of storage. Standard plate enumeration methods were used to determine the microbial populations of *salsiccia toscana*. A core of 25 g was aseptically removed from a pool of five sausages and blended with 225 mL of Maximum Recovery Diluent (MRD, Oxoid, Basingstoke, UK) using a stomacher (PBI International, Milano, Italy) to achieve an initial 1:9 sausage emulsion. Dilutions up to 10^{-8} were prepared in MRD and plated in duplicate onto various media for enumeration of bacteria. Mean bacterial counts were determined from plates bearing 30-300 colonies. All media and media supplements were obtained from Oxoid.

Total aerobic mesophilic and psychrotrophic counts were determined on Plate Count Agar (PCA) using 1 mL of inoculum with pour plates, incubated at 30°C for three days and at 4°C for ten days. *B. thermosphacta* was determined on Streptomycin Thallous Acetate (STA) selective medium (STA agar base with STA selective supplement) (0.1 mL spread plates) after incubation at 25°C for two days; for confirmation of *B. thermosphacta*, five colonies per plate were tested for oxidase reaction (Oxidase Identification Kit, Oxoid). Lactic acid bacteria (LAB) were determined by anaerobic growth (Oxoid Gas Generating Kit) on MRS Agar, using 1 mL of inoculum with pour plates incubated at 25°C for three days. *Pseudomonas* spp. were determined on Pseudomonas CFC Agar (Pseudomonas Agar base with CFC supplement) (0.1 mL spread plates) incubated at 25°C for three days. *Enterobacteriaceae* were determined on Violet Red Bile Glucose Agar (0.1 mL spread plates) after incubation at 25°C for one day. *Escherichia coli* was determined on Tryptone Bile X-Glucuronide Medium (0.1 mL spread plates) after incubation at 30°C for three days. *Micrococcaceae* were enumerated on Mannitol Salt Agar (0.1 mL spread plates) after incubation at 37° C for one day. Yeasts and moulds were determined on Yeast Extract Glucose Chloramphenicol Agar (1 mL pour plates) after incubation at 25°C for five days.

The presence of *Salmonella* spp. and *Listeria monocytogenes* was evaluated following the UNI EN ISO 6579:2008 and the UNI EN ISO 11290-1:2005 standards, respectively (ISO 2005, 2008).

Measurements of pH and activity water

Sausage pH was measured using a Hanna pH211 pH-meter provided with a Hanna FC 200B pin electrode and an automatic temperature compensator (Hanna Instruments, Padova, Italy). Activity water (A_w) was measured on chopped sausage samples at 20°C with a Rotronic PBI AWYD device (PBI International, Milano, Italy).

These measures were performed at Day 0, and after six and nine days of storage.

Chemical analyses and fatty acid composition

For all chemical analyses, samples were ground in an electric mini food chopper Moulinex 390 (Moulinex, Paris, France). Moisture, ash and protein were determined as described by the AOAC (1990).

Total lipids from sausage samples were extracted with chloroform/methanol (2:1 v/v) according to the method of Folch *et al.* (1957). After extraction, total lipids were trans-esterified according to ISO 15884 methodology with slight modifications (Serra *et al.*, 2009). Briefly, 30 mg of total lipids, accurately weighed, were added to 2.5 mL of hexane containing the internal standards C9:0 e C19:0 methyl esters (0.5 mg/mL) and to 0.1 mL of a 2N solution of KOH in methanol. After vigorous agitation, samples were allowed to stand for five minutes at room temperature. Then 0.25 g of $NaHSO_4 \times H_2O$ was added and the solution was centrifuged for three min at 3000×g at 4°C. One µL of upper phase was injected into an FID gas-chromatograph apparatus (Thermo-Finnigan, GC 800 top, Waltham, MA, USA) equipped with a high polar capillary column (Chrompack CP-Sil 88 Varian, Middelburg, The Netherlands; 100 m × 0.25 mm i.d.; film thickness 0.20 µm). Helium was used as the carrier gas at a flow of 1 mL/min. The split ratio was 1:80. The injector temperature was set at 270°C, whereas the detector temperature was set at 300°C.

Acid methyl esters were identified by comparison with commercial standard mix FAME (GLC-674 Nuchek, Elysian, MN, USA) with the addition of single fatty acid (Nuchek, Elysian, MN USA; Larodan, Malmö, Sweden) obtaining a complete standard of 105 fatty acids. For each fatty acid, response factors to flame ionisation detector and inter- and intra-assay coefficients of variation were calculated by using a reference standard butter oil (CRM 164, Community Bureau of Reference, Brussels, Belgium).

The chemical analyses were performed at Day 0 and after six and nine days of storage.

Thiobarbituric acid reactive substances measurement

Lipid oxidation was evaluated by determining the Thiobarbituric Acid Reactive Substances (TBARS) that measures the amount of malonaldehyde produced in the fat during storage. TBARS values of meat were determined by an aqueous extraction method. The absorbance was read at 532 nm against a blank containing percloric acid and thiobarbituric acid. TBARS was calculated by multiplying the absorbance by a constant coefficient, which was calculated from standard curves and known dilutions, and were expressed as mg of malonaldehyde per kg of meat (Pikul *et al.*, 1989).

TBARS was measured at Day 0 and after nine days of storage.

Colour

Colour was measured both on the sausage surface, with and without casing, and on the internal sausage surface using a Minolta CR300 chroma meter (D65 Illuminant, 0° Incidence angle) (Minolta Camera Co. Ltd., Osaka, Japan) calibrated against a standard white tile in the CIEL*a*b* system, which measures the values of coordinates Lightness (L*), Chroma (C) and Hue (H) (Renerre, 1982). For each colour determination, values were recorded and averaged from three locations on the samples; colour was expressed as Lightness (L*), Chroma (C) and Hue (H) (Cassens *et al.*, 1995). Colour was measured at Day 0 and after nine days of storage.

Statistical analysis

The one-way ANOVA test (SAS, 1995) was used to compare the quality characteristics of sausages at Day 0 and samples stored for six and nine days. Results from microbial counts had been previously converted in log CFU/g. Statistical analyses were performed using the R software ver. 2.13.1 (R Foundation for Statistical Computing, Wien, Austria). The statistical significance of the changes throughout the test period was analysed with one-way ANOVA test and Tukey HSD test for *post-hoc* comparisons.

Results and discussion

Microbiological analyses

The results of the viable counts of the targeted microbial groups from sausage samples at different storage times are reported in Figure 1, with the exception of *Escherichia coli* that was not detected in any sample. During the refrigerated storage, all the microbial groups gradually increased up to Day 9. At Day 0, the highest counts were those of total mesophilic and psychrotrophic aerobic bacteria with loads of approximately 10^6 CFU/g. *B. thermosphacta*, *Pseudomonadaceae*, LAB and *Enterobacteriaceae* displayed loads of approximately 10^5 CFU/g; yeasts showed loads over 10^3 CFU/g, while *Micrococcaceae* and moulds displayed loads below 10^3 and 10^2 CFU/g, respectively.

These findings revealed an important bacterial contamination of the product with a noticeable variation in microbial counts among the batches, as shown by the quite high values of standard deviations. Interestingly, the first phase of processing in the butcher's shop, including meat chopping, is generally performed at an ambient temperature of up to 20°C and this may explain the high levels of some groups of bacteria in samples at Day 0. This, together with poor standardisation of the production process, may also be the reason for some variability between batches. Although other authors (Tremonte *et al.*, 2005) found lower initial total mesophilic counts ($<10^5$ CFU/g) in fresh sausages, Chiavaro *et al.* (2008) registered total viable counts of over 10^6 CFU/g in fresh sausages at Day 0 of storage and Kamdem *et al.* (2007) observed high levels of total mesophilic bacteria and LAB in the meat mixtures used to produce experimental samples of *salsiccia toscana* (5.69±0.50 log CFU/g and 4.86±0.45 log CFU/g, respectively). In the same paper, Kamdem *et al.* (2007) reported lower *Enterobacteriaceae* loads than those observed in our study. However, the finding of high levels of *Enterobacteriaceae* is not unusual; Bonardi *et al.* (2002) found total coliform levels of approximately 10^5 CFU/g in 16.3% of the examined *salsiccia* samples.

After six days of refrigerated storage, analysis of *salsiccia toscana* showed an increase in all targeted microbial groups: total mesophilic and psychrotrophic aerobic bacteria increased to values close to 10^7 CFU/g, while *B. thermosphacta*, *Pseudomonadaceae*, *Enterobacteriaceae* and LAB reached values of approximately 10^6 CFU/g. Yeasts and *Micrococcaceae* were over 10^4 CFU/g while moulds remained substantially unchanged. After nine days of storage, total mesophilic and psychrotrophic aerobic bacteria and *B. thermosphacta* grew to over 10^7 CFU/g, while the other groups reached values close to 10^7 CFU/g, except for yeasts, *Micrococcaceae* and moulds, with loads of approximately 10^5, over 10^4 and approximately 10^3 CFU/g, respectively.

Results of the statistical analysis showed that there were significant differences in total mesophilic and total psychrotrophic bacterial counts and *Micrococcaceae* counts between Day 0 and Day 6, and between Day 0 and Day 9, while the difference between Day 6 and Day 9 was not statistically significant. Significant differences between Day 0 and Day 9 were registered for *B. thermosphacta*, *Pseudomonadaceae*, LAB, *Enterobacteriaceae* and yeasts. No statistically significant differences were found among mold counts that were constantly low at the three storage time points (Days 0, 6 and 9). Similar microbial trends have been reported by other authors. In particular, Tremonte *et al.* (2005), in control samples packaged without gas, obtained similar increases in total mesophilic bacteria counts, even if LAB and *Pseudomonadaceae* prevailed against *B. thermosphacta*. By contrast, in our study *B. thermosphacta* represented the main spoilage microorganism. Our data also showed an important increase in *Enterobacteriaceae*, in agreement with data obtained by Tremonte *et al.* (2005) in pork sausages stored under air at 4°C. *Enterobacteriaceae* probably took advantage of fluctuations in storage temperature, a phenomenon that is common in domestic refrigerators and that is also found in refriger-

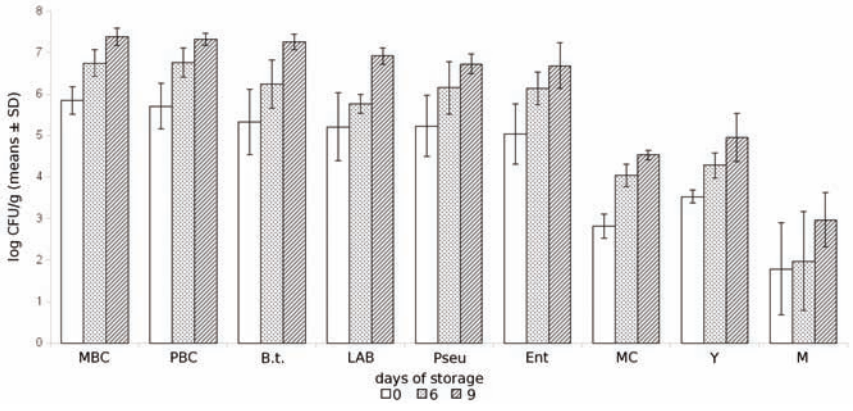

Figure 1. Viable counts of the different spoilage-related microbial groups (log CFU/g) detected on *salsiccia toscana* on samples at 0 days and after a refrigerated storage for 6 and 9 days (means±SD). MBC, mesophilic bacterial count; PBC, psychrotrophic bacterial count; B.t., *Brochothrix thermosphacta*; LAB, lactic acid bacteria; Pseu, *Pseudomonadaceae*; Ent, *Enterobacteriaceae*; MC, *Micrococcaceae;* Y, yeasts; M, moulds.

ated display benches and cabinets in butcher's shops. Chiavaro *et al.* (2008) registered values and evolution trends for yeasts and *Micrococcaceae* similar to ours in control samples packed under oxygen-permeable film and stored in a domestic refrigerator.

Finally, considering the pathogen microorganisms examined, *Listeria monocytogenes* was not detected in any sample, while *Salmonella* spp. was isolated from one sample at Day 0. In particular, *Salmonella* spp. was found in a pool sample from the third batch that revealed the highest microbial counts of the trial. Though this finding can only confirm the hygiene status of the examined products, since data were obtained from a single production cycle, this study confirms that fresh sausages consumed raw or undercooked may be responsible for infection in humans, both directly and through cross-contamination of other foods during meal preparation. On the other hand, the absence of *Salmonella* spp. in samples examined after six and nine days of storage is not so surprising, since this is probably due to a low initial bacterial load.

Values of pH and A_w

The pH of samples at Day 0 was 5.87±0.05 and remained substantially unchanged up to the end of the study with no statistical differences reported: Day 6, 5.88±0.05; Day 9, 5.86±0.05. Similar pH values of around 5.80 were reported for fresh sausages in other studies (Tremonte *et al.*, 2005; Torrieri *et al.*, 2011). During eight days of storage of *salsiccia toscana*, Kamdem *et al.* (2007) observed a different pH evolution ranging from an initial value of 5.90 to final values of between 5.62 and 5.67. The authors attributed this decrease to the development of LAB. In the present study, during nine days of refrigerated storage, LAB, in spite of their increase during storage, were not able to produce a significant increase in acidity. On the other hand, Tremonte *et al.* (2005) showed an increase in pH values in the control samples, packaged without gas, with respect to the initial values, during a 6- and 12-day period of storage at 4°C.

The A_w value of samples at Day 0 was 0.956 ±0.01 and there was no significant change during storage: Day 6 of storage 0.958±0.01; Day 9 0.957±0.03. Chiavaro *et al.* (2008) observed an initial A_w value of 0.974±0.03 in fresh pork sausage overwrapped with polyvinyl chloride oxygen-permeable film and, after nine days of refrigerated storage, reported a slight decrease to 0.965±0.04; however, the difference can be explained by the use of different sausage recipes.

Chemical analysis and fatty acid composition

As expected, there was no change in the chemical composition of samples during the storage time, as a consequence of the short period of storage (Table 1). Total fat content was lower than that reported for other traditional Mediterranean pork sausages, such as those produced in Greece (Ambrosiadis *et al.*, 2004). This was probably due to differences in

Table 1. Chemical composition of *salsiccia toscana* measured on samples at 0 days (T0) and after a refrigerated storage for 6 (T6) and 9 (T9) days (means ±SD).

	Storage time		
Chemical composition	T0	T6	T9
Dry matter, g/100 g	42.67±3.01	43.03±3.23	43.31±4.45
Crude protein, g/100 g	21.12±0.94	21.1±1.22	21.4±2.82
Ash, g/100 g	1.72±0.24	2.18±0.64	1.93±0.54
Total lipids, g/100 g	16.18±2.43	16.38±3.07	16.64±2.62
Energy content, Kcal/100 g	236.98±20.60	240.54±19.35	243.08±7.26

Table 2. Classes of fatty acid composition of *salsiccia toscana* measured on samples at 0 days (T0) and after a refrigerated storage for 6 (T6) and 9 (T9) days (means ±SD).

	Storage time		
	T0	T6	T9
Saturated fatty acids	5.06±0.93	5.27±1.97	6.06±1.17
Unsaturated fatty acids	7.57±1.59	7.82±2.72	8.72±1.19
Monounsaturated fatty acids	6.12±1.16	6.64±2.14	6.8±0.91
Polyunsaturated fatty acids	1.45±0.44	1.18±0.62	1.92±0.49
Polyunsaturated fatty acids n6	1.39±0.42	1.13±0.59	1.84±0.45
Polyunsaturated fatty acids n3	0.06±0.02	0.05±0.02	0.08±0.05

Table 3. *Salsiccia toscana* colour traits and thiobarbituric acid reactive substances values measured on samples at 0 days (T0) and after refrigerated storage for 9 (T9) days (means ±SD).

	Storage time	
	T0	T9
Sausages surface with casing		
L	51.90±0.64	51.93±1.73
H*	32.90±2.97[a]	41.43±1.23[b]
C*	13.73±1.52	13.08±1.09
Sausages surface without casing		
L*	49.95±2.18	52.49±2.68
H*	36.03±5.14	42.07±3.72
C*	18.40±0.40	16.50±2.61
Internal sausages surface		
L*	50.13±4.79	52.50±4.93
H*	35.93±2.97	39.87±5.00
C*	20.60±1.61	18.16±4.22
TBARS, mg malonaldhehyde/kg meat	0.43±0.06	0.58±0.04

TBARS, thiobarbituric acid reactive substance; [a,b] different letters in the same row show significantly different values ($P \leq 0.05$).

the lean meat:fat ratio in the recipe. Similarly, there was no difference in the composition of the main classes of fatty acids during the storage time, reflecting the fatty acid profile of pork meat fat (Table 2): unsaturated fatty acids accounted for nearly 60% of total fatty acids and the ratio of unsaturated:saturated fatty acids was nearly 1.5 (Woods and Fearon, 2009).

Thiobarbituric acid reactive substances

The TBARS values of the sausage samples, used as an index of lipid oxidation during storage and measured at each assessment time point, are shown in Table 3. Values remained substantially unchanged during storage, showing that the addition of spices and ascorbic acid to the sausage mixture limited the rate of lipid oxidation over time (Chiavaro *et al.*, 2008; Kamdem *et al.*, 2007).

Colour

Colour parameters, measured on the sausage surface with and without casing, and on the internal surface of the sausage at Days 0 and 9 of storage, are reported in Table 3.

As expected, the sausage surface showed an increase in the hue-angle value during the storage period and, consequently, a less intense red colour (Kamdem *et al.*, 2007). This trend was particularly evident on the surface with casing that showed a distinct change in colour over the time period studied, as shown by the significantly paler colour after nine days of storage than that measured at Day 0. Some authors also noticed that the sausage surface became discoloured during the time of storage (Kamdem *et al.*, 2007).

Colour traits measured on the sausage surface after removing the casing confirmed the progressive discolouration of meat during the time of storage, but values did not reach statistical significance and there was less change in colour as measured on the internal surface during the time of storage. It is interesting to note that, in spite of the absence of nitrates and nitrites (often used to give sausages their red colour), the use of spices preserved the meat colour in the inside of the sausages. In fact, the change in colour of the meat seems to proceed from the outside to the inside, in a centripetal fashion, during the time of storage.

Conclusions

Salsiccia toscana is a highly perishable product due to its characteristics, particularly A_w and pH. The results of this study showed that physicochemical properties were substantially maintained during the time of storage, in comparison with the product examined at Day 0 of storage. An increase in microbiological load was also seen, reaching values of over 10^7 CFU/g after nine days of storage. The deterioration in the microbiological profile during the time of storage was associated with a progressive discolouration of the sausages. Although no significant differences were observed in these parameters between Days 6 and 9, producers should not be recommended to extend the shelf life of this product considering that, generally, microbial spoilage of foods occurs when total aerobic counts reach 10^7 CFU/g (ICMSF, 1984). Furthermore, after nine days of storage some off-odours could be perceived, even if these were not sufficient to cause the product to be rejected. Similarly, Martinez *et al.* (2006) found that fresh pork sausages that were overwrapped in oxygen-permeable film had a score of 4, corresponding to presence of moderate off-odours, out of a scale of 5 after eight days of refrigerated storage. Torrieri *et al.* (2011) extended the shelf life of fresh pork sausages up to nine days by only using a 20% O_2 and 70% CO_2 modified atmosphere packaging. Indeed, the extension of the shelf life of *salsiccia toscana* could be more easily obtained by the application of some packaging systems, such as those that producers of high-quality fresh sausages sometimes use already. But these systems should be designed for the single specific product and an in-depth analysis is required in order to select the optimal atmosphere composition. Therefore, although there are already examples of MAP applied by producers of high-quality fresh sausages, more data are needed on the application of these packaging systems on *salsiccia toscana* specifically. Furthermore, in small butcher's shops, like the one in this study, *salsiccia toscana* is usually sold loose to the consumers. If producers wish to continue with this cheaper sales approach, this type of fresh sausage must have a longer durability profile. This could be extended by making big improvements to the manufacturing practices, since these have to be conducted under highly hygienic conditions, particularly in the first phase of processing, when the meat chopping should be performed at a refrigerated temperature in order to limit the microbiological counts.

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