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Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions

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ABSTRACT

In this work, two types of traditional Italian dry fermented sausages (Felino-type and Milano-type), were analyzed as a case study. The aim was to evaluate the effect of different environmental conditions in the drying chambers and different starter cultures on the characteristics of the industrially produced sausages. At the end of ripening, the sausages obtained were analyzed to determine microbial counts, biogenic amines (BA) accumulation and volatile aroma profile by using SPME-GC analysis. These profiles were compared with the results of a trained panel group. Felino-type sausages, inoculated only with *Staphylococci*, were characterized by a slow pH drop and by the presence of higher BA contents. Moreover, specific enzymatic activities of Staphylococci, such as phenylalanine metabolism, were observed and these drastically modified product volatile profiles. Also the different drying conditions applied were able to affect some sensory characteristics of the final products such as hardness and chewiness reflecting different water loss kinetics.

1. Introduction

Fermented sausages are the result of a complex microbiological activity which consists of a lactic fermentation (which takes place in the first days after casing) and several biochemical transformations characterizing the more or less prolonged ripening period; among the principal agents of these activities there are *Micrococci*, *Staphylococci* and lactic acid bacteria (LAB). In addition, in many fermented sausages produced in the Mediterranean area a crucial role is played by the moulds which grow on the casing during ripening (Demeyer, 2004; Hammes, Haller, Michael, & Gänzle, 2003; Talon, Leroy, & Lebert, 2007; Zambonelli, Papa, Romano, Suzzi, & Grazia, 1992).

Among the most important factors determining the characteristics and the quality of fermented sausages there are the choice of starter cultures and the environmental conditions characterizing fermentation and ripening (Toldrá, 2006, chap. 181).

Since the last decades the great part of fermented sausages produced in Europe are prepared using starter cultures. These cultures belong mainly to LAB (*Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*) and coagulase negative *Staphylococci* (CNS, such as *Staphylococcus xylosus* and *Staphylococcus carnosus*). In addition, when desired, white selected moulds (usually belonging to the species *Penicillium nalgiovense*) are inoculated on the surfaces of the sausages (Hugas & Monfort, 1997; Toldrá, 2006, chap. 181; Talon & Leroy, 2011).

The growth of these species is strongly influenced by the composition of the meat mixture (type of meat, salt, addition of sugars, nitrate/nitrite, spices, etc.) and by environmental conditions (temperature and relative humidity) applied after casing to allow the drying and ripening of sausages. In the Mediterranean sausages the temperatures are relatively low since the beginning (less than 25 °C) and decrease during ripening (down to 15–17 °C or less). Also relative humidity (RH) has to be stringently controlled to permit a correct kinetic of water loss of the sausages; the value of RH decreases during the ripening following the behaviour of temperature (Feiner, 2006). The selection and interactions of raw materials, microbial strains, technology and ingredients are crucial for the sensory properties, volatile profile, safety and shelf-life of products (Talon & Leroy, 2011; Toldrá, 2006, chap. 181; Hammes et al., 2003). The interaction between starter cultures and environmental conditions drives the cell metabolisms towards results that can be beneficial or detrimental for the quality of the sausages.

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In first instance these activities determine the formation of the organoleptic characteristics of the products, but they can also influence the safety and hygienic profile of the sausages by counteracting pathogen growth and toxic compound accumulation (Toldrá, 2006, chap. 181; Hammes et al., 2003). Among these latter, biogenic amines (BA) have received in the last years particular attention (Silla-Santos, 1996). The presence of the most active BA (histamine, tyramine, 2-phenylethylmine) has been reported at different extent in fermented meat (Ansorena et al., 2002). Though it is difficult to hypothesize the complete absence of BA in fermented meat, it is fundamental to reduce their accumulation to the lower level (Suzzi & Gardini, 2003). BA are a result of amino acid degradation, as well as many other compounds that are relevant for the flavour of salami (Fernández & Zúñiga, 2006; Olesen & Stahnke, 2004). Together with amino acids, the metabolism of free fatty acids, liberated by lipase action, is fundamental for the formation of molecules with a high sensory impact. Thus, the combination of microbial and endogenous enzymes activity and chemical reactions (*i.e.* lipid autoxidation) are responsible for the aroma formation in fermented sausages (Ordóñez, Hierro, Bruna, & de la Hoz, 1999).

This paper reports the results of a case study. Two types of traditional Italian dry fermented sausages were industrially produced by using different environmental conditions and starter cultures. The salamis considered were Felino-type (Coloretti, Chiavari, Armaforte, Carri, & Castagnetti, 2008) and Milano-type sausages (Demeyer, 2004, chap. 17), which differed for size and weight, fat/lean meat ratio, mincing degree and for some ingredients. Two starter cultures were used for each type of sausages. The second variable was the temperature and RH program of the drying phase, *i.e.* the first days of the ripening process. During this step meat fermentation begins and crucial events take place, influencing all the following ripening features. In this trial the effect of the drying process usually applied by the producer was compared with a modified process characterized by the presence of periods in which the controllers of drying chambers were switched off and, consequently, temperature and above all RH were left to fluctuate spontaneously.

The aim of this work was to evaluate the effect of the different starter cultures used and the different environmental conditions applied during drying on the characteristics of sausages. At the end of the ripening (41 d for Felino-type and 76 for Milano-type) the salamis were evaluated by a trained panel group and the results were compared with the results of an SPME–GC analysis of the volatile compounds characterizing each sausages. In addition, the sausages were sampled to determine the number of the most important bacterial groups and the BA accumulated.

2. Materials and methods

2.1. Sausage manufacture

The sausages used in this work have been manufactured in a local factory. Two different dry fermented sausages typically produced in Northern Italy were considered: Felino-type and Milano-type.

Felino-type sausages were produced by using lean pork (73% w/w), pork fat (27% w/w), NaCl (2.3% w/w), sodium ascorbate (0.05% w/w), dextrose (0.30% w/w), KNO_3 (0.015% w/w), $NaNO_2$ (0.010% w/w) and spices (black pepper and garlic). After mincing (7 mm) and mixing, the mixture was divided into two batches: batch A was inoculated with 0.025% of commercial starters powder (Kron Morelli srl, Lodi, Italy) containing a strain of *S. carnosus* and a strain *S. xylosus,* while batch B was added with a commercial mixture of 5 different strains of *S. xylosus* at a concentration of 0.025%. The meat mixture was immediately stuffed in natural casings to obtain sausages with an initial weight of approx. 1400–1500 g, a length of approx. 60 cm and a diameter of 50–60 mm. The sausages were kept 24 h at 6 °C before their entrance into the fermentation chambers.

Milano-type sausage were made up with pork shoulder (72% w/w) and streaky bacon (28% w/w), NaCl (2.6% w/w), sodium ascorbate (0.05% w/w), dextrose (0.50% w/w), KNO_3 (0.015% w/w), $NaNO_2$ (0.010% w/w), wine (1% v/w) and spices (white pepper powder and black pepper whole grain, 0.12% w/w). After mincing (3.5 mm) and mixing, the mixture was divided into two batches: batch C was added with 0.025% of commercial culture starter (Kron Morelli srl, Lodi, Italy) containing a *L. sakei* strain and the same *S. carnosus* and *S. xylosus* strains used in batch A, while batch D was inoculated with 0.025% of a commercial starter culture, containing *L. curvatus*, *L. sakei* and the same mixture of five *S. xylosus* strains used in batch B. After mincing and mixing of ingredients the meat mixture was kept 24 h at 4 °C before stuffing in synthetic casings. The sausages had a length of 14 cm, a diameter of 50–60 mm and an initial weight of about 360 g. Then, the sausages were transferred in the ripening chamber.

The initial viable cell concentration of each dried starter cultures was approx. 11 log CFU/g.

A selected strain of *P. nalgiovense* was inoculated by immerging the sausages in spore suspension before fermentation. For each batch about 1000 kg of meat mixture were used.

For each batch and for each type of sausage two different drying condition (traditional and modified) were followed, as showed in Fig. 1. The term “switch off” indicated that temperature and RH in the drying chamber fluctuate spontaneously, without any control. In this period (stewing) temperature marks small increases, while RH tends rapidly to 100%. After the application of the different drying programs, sausages were held in the ripening chamber at 13–15 °C and 80–86% RH up to 41 d for Felino-type sausages and 76 d for Milano-type sausages.

2.2. Weight losses, pH and microbial counts

During the ripening, the weight loss was measured for each type of sausage and the pH and water activity were detected by using a pH-meter Basic 20 (Crison Instruments, Barcelona, Spain) and an Aqualab CX3-TE (Labo-Scientifica, Parma, Italy), respectively. Each datum is the mean of the results obtained in three different sausages.

Microbiological analyses were performed at the end of each time of ripening (after 76 days for Milano-type and after 41 days for Felino-type). After aseptically removing the casing, approx. 10 g of sausage were 10-fold diluted with 90 mL of 0.9% (w/v) NaCl and homogenized in a Lab Blender Stomacher (Seward Medical, London, UK) for 2 min. Decimal dilutions were performed and plated onto selective media. For the yeast count, Sabouraud Dextrose Agar (Oxoid, Basingstoke, UK), with added 200 mg/L of chloramphenicol, was used and the plates were incubated at 28 °C for 72 h. Lactobacilli were enumerated by plating appropriate dilutions of the samples on MRS agar (Oxoid) incubated at 30 °C for 48 h in anaerobic conditions. *Micrococcaceae* and *Staphylococcaceae* were detected on Baird-Parker agar with Egg Yolk Tellurite Emulsion (Oxoid) incubated at 37 °C for 48 h. The colonies showing lecithinase activity were further tested in tubes containing coagulase rabbit plasma (Oxoid) to confirm coagulase positive strains. *Enterococci* were enumerated on Slanetz and Bartley medium incubated at 44 °C for 24 h. *Enterobacteriaceae* were enumerated by pour plating in violet red bile glucose agar (Oxoid) with a double layer at 37 °C for 24 h. Three replicates were carried out for each microbial count. Each count is the mean of the results obtained from analysing three different sausages.