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Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures

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Abstract

In this study, two strains of *Staphylococcus xylosus* isolated from traditional fermented sausages of Vallo di Diano (Southern Italy) were used in combination with an acidifying strain of *Lactobacillus curvatus* as starter culture for the production of fermented sausages. Two starter formulation were developed combining the proteolytic but not lipolytic (prt^+, lip^-) *S. xylosus* CVS11 with the *L. curvatus* AVL3 (starter S1) and the *S. xylosus* FVS21 (prt^-, lip^+) with the same strain of *L. curvatus* (starter S2). Proteolysis and lipolysis were observed during ripening by the increase in total free amino acids (FAA) and free fatty acids (FFA), respectively. Such activities were observed in both started and non started sausages (control). Moreover, the proteolytic and lipolytic activities were detected in products started by both formulations irrespective of the presence of such activities in the strains used. Therefore, it was not possible to conclude whether the effect of proteolysis and lipolysis during ripening of the started fermented sausages was due to the activity of the starter cultures or to the action of meat endogenous enzymes.

Keywords: *Staphylococcus xylosus*; Starter culture; Traditional fermented sausages

1. Introduction

Fermented sausages are the result of biochemical, microbiological, physical and sensorial changes occurring in a meat mixture during ripening under defined conditions of temperature and relative humidity (RH). A great variety of fermented sausages are produced in Italy and other European countries and many of them have been granted PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) labels (http://europa.eu.int/comm/agriculture/qual/en/pgi_03en.htm. Accessed 17.07.06.). Although many fermented sausages are commonly produced in industrial plants, there still are regions in Italy and other parts of Europe where these products are obtained through traditional technologies without added starter culture. In the latter case, the required microorganisms originate from the meat itself or from the environment, and constitute a part of the so-called "house-flora" (Santos, Gonzalez-Fernandez, Jaime, & Rovira, 1998).

The commercial starter cultures in Europe, are mainly produced in Northern European countries and are not always able to compete well with the microflora colonizing Southern European meat plants; therefore, their use often results in losses of desirable sensory characteristics (Leroy, Verluyten, & De Vuyst, 2006; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). For this reason such artisanal fermented sausages are often of superior quality compared to those inoculated with industrial starters and possess distinctive qualities due to the technology used and to the properties of the raw meat (Moretti et al., 2004).

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Therefore, appropriate starter cultures have to be selected according to the specific formulation of the batter and technology of fermentation since environmental factors will interact to select a limited number of strains that are competitive enough to dominate the process (Rebecchi, Crivori, Sarra, & Cocconcelli, 1998). Environmental factors possibly affecting strain selection are ripening conditions such as temperature, RH, pH, NaCl, raw meat and other ingredients. In order to make the ideal starter culture for any particular technology and recipe, it is necessary to understand the properties required, and to have tools to improve the properties of the culture (Hansen, 2002).

During sausage fermentation, complex biochemical and physical reactions take place that result in a significant change in the initial characteristics. These changes can be summarized as follows: decrease in pH, changes in the initial microflora, reduction of nitrates to nitrites and the latter to nitric oxide, formation of nitrosomyoglobin, solubilization and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena, and dehydration.

The main microbial groups of technological interest isolated in spontaneously fermented sausages are lactic acid bacteria (LAB), coagulase negative staphylococci (CNS) and Kocuria (Corbiere Morot-Bizot, Leroy, & Talon, 2006). In addition, depending on the product, other groups may play a role, such as molds, enterococci and yeasts. LAB and CNS are actively involved in the development of texture, colour and flavour and the LAB also have a positive effect on the hygienic properties of the product, inhibiting pathogenic and spoilage flora by acidification or by the production of antimicrobials (Villani et al., 1994).

Staphylococcus and *Kocuria* contribute to the development of colour by reducing nitrate to nitrite and participate in the development of flavours of dry fermented sausages (Demeyer, Verplaetse, & Gistelink, 1986; Schleifer, 1986). They influence the composition of non-volatile and volatile compounds mainly by degrading free amino acids and inhibiting the oxidation of unsaturated free fatty acids (Hammes & Hertel, 1998; Sondergaard & Stahnke, 2002).

The aims of this work were to assess the biochemical characteristics of *S. xylosus* strains in order to select starter cultures for fermented sausages from Vallo di Diano (Campania region) a traditional sausage production region of Southern Italy. A further aim was to employ the selected strains in the manufacture of fermented sausages in order to evaluate the effect of the starter culture on the physico-chemical, microbiological and sensory properties of typical fermented sausages.

2. Materials and methods

2.1. Isolation, identification and technological properties of strains

Strains of *Staphylococcus xylosus* isolated from traditional fermented sausages (“*salsiccia*”) from Vallo Diano (Campania region–Italy) were used in this study. The strains were isolated and identified as previously reported (Blaiotta, Pennacchia, Ercolini, Moschetti, & Villani, 2003; Casaburi, Blaiotta, Mauriello, Pepe, & Villani, 2005). Briefly, *Micrococcaceae* present in dry fermented sausages were enumerated on Mannitol Salt Agar (MSA, Oxoid, Milan, Italy) after 48 h at 30 °C. Colonies from countable plates were initially tested for morphology, gram-stain and catalase production. Gram-positive and catalase-positive cocci were purified by streaking on MSA and maintained on P-agar (Phillips & Nash, 1985) slants stored at 4 °C. They were subjected to the oxidation/fermentation test in OF medium and to anaerobic growth in semisolid thioglycollate medium (Evans & Kloos, 1972). Sensitivity to furazolidone, bacitracin and lysostaphin was determined as described by Kloos and Bannerman (1995). Production of pigment was observed on P-agar. Staphylococci were assayed for coagulase activity with coagulase plasma (Becton, Dickinson and Company, NJ, USA) and for novobiocin sensitivity (Kloos, Tornabene, & Schleifer, 1974). Other biochemical properties were studied using API Staph identification strips and API LAB Plus software according to the manufacturer’s instructions (API, Biomérieux System).

Species-specific PCR assays were used to confirm the identity of the strains as belonging to *S. xylosus* as previously reported (Blaiotta et al., 2003). Briefly, DNA extraction was carried out from a single colony using an InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA) and 5 μl (about 25 ng) were used for PCR amplifications. Two sets of primers targeting on xylulokinase (*xyl*B) and on 60 kDa heat-shock protein (*Hsp*60) genes of *S. xylosus*, were used (Blaiotta et al., 2003).

The strains belonging to *Staphylococcus xylosus* species were studied to evaluate catalase, nitrate reductase, proteolytic, lipolytic and antioxidant activities as described previously (Casaburi et al., 2005). Briefly, catalase activity was measured on resting cells according to Aebi (1974) after growth in YT broth containing 1% tryptone, 0.5% yeast extract, 3% NaCl, pH 7.0, for 24 h at 30 °C; nitrate reductase activity was determined by the agar plate method after incubation at 30 °C for 7 h by measuring the red haloes surrounding the wells; superoxide dismutase (SOD) activity was determined by a spectrophotometric method; lipolytic activity was measured by titration after growth at 30 °C for 7 days in YT supplemented with 4% (w/v) pork fat (YTF). The proteolytic activity was determined by the agar plate method against sarcoplasmic and myofibrillar proteins measuring the diameter of the clear zone surrounding the inoculated wells, after 48 h of incubation.

The strain of *Lactobacillus curvatus* AVL3, from the culture collection of Dipartimento di Scienza degli Alimenti, Università di Napoli “Federico II” was cultivated in MRS broth and maintained at −80 °C in 15% (v/v) glycerol.