Food Control 33 (2013) 6–14

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Effect of different autochthonous starter cultures on the volatile compounds profile and sensory properties of Galician chorizo, a traditional Spanish dry fermented sausage

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ARTICLE INFO

Article history:
Received 11 September 2012
Received in revised form
22 January 2013
Accepted 26 January 2013

Keywords:
Fermented sausages
Staphylococcus
Lactobacillus
Sensory evaluation
Volatile compounds
SPME-GC/MS

ABSTRACT

This study deals with the effect of different autochthonous starter cultures used in the manufacture of Galician chorizo, a traditional dry fermented sausage produced in the north-west of Spain, on its volatile compounds profile and sensory properties.

SPME-GC/MS was used in volatile compounds analysis and a trained group of panellists performed sensory evaluation of final products. In addition, physicochemical parameters and microbial counts were performed throughout the ripening in order to follow and control the process conditions.

The starter cultures studied, consisting of a mix of *Lactobacillus sakei* strain and a strain of *Staphylococcus equorum*, *Staphylococcus epidermidis* or *Staphylococcus saprophyticus*, had been previously isolated from traditional Galician fermented meat products and technologically characterized.

According to the data obtained in this study, no significant differences were found among batches inoculated with different starter cultures. However, sensory evaluation revealed that overall acceptance was higher for inoculated batches than for the uninoculated control batch.

In conclusion, this work reinforce the idea that using selected autochthonous starter strains in sausage manufacture makes possible the obtaining of a homogeneous production without renouncing the desired typical characteristic obtained in artisanal elaborations.

1. Introduction

Traditional fermented meat products are commonly produced and consumed in different countries throughout the world, being Spain, together with Italy and Germany, one of the European countries where the wider variety of meat products is manufactured and where these have a greater economic weight. Galician chorizo is a traditional dry fermented sausage from the northwest of Spain that has a broad acceptance by consumers and a great installation in the local markets. The typical manufacture process of Galician chorizo consists of a mixture of pork, fat, salt, garlic and sweet and spicy paprika stuffed into a natural casing, such as pig's small intestine, that subsequently undergoes a bacterial fermentation process followed by a ripening period.

The aroma of dry fermented sausages has been widely studied in the last years due to the importance and complexity of this meat product, since there are a high number of changes that occur during fermentation and drying responsible for the final flavour and odour (Flores, Durá, Marco, & Toldrá, 2004). The characteristic aroma of fermented and ripened sausages is conferred by many different non-volatile and volatile compounds (Stahnke, 1994), resulting not only from spices and other condiments, but also from the activity of endogenous meat enzymes (Hierro, De La Hoz, & Ordóñez, 1997, 1999), as well as some bacterial groups such as lactic acid bacteria (LAB) and staphylococci (Demeyer, Tan, & Privett, 1974). LAB are responsible for the tangy flavour of sausages due to the lactic acid produced during fermentation, whose intensity will be depending essentially on the starter culture applied and the carbohydrate substrate present (Demeyer, Todorov, van Nevel, & Vets, 1982). Staphylococci participate in the development of characteristic sausage flavour and odour, since various aromatic substances and organic acids are released from their protease and lipase activity, as well as in the generation and stability of a desirable red colour and in lipid oxidation limitation through their nitrate reductase activity (Talon, Walter, Chartier, Barrière, & Montel, 1999).

Nowadays, although many typical dry fermented sausages are still produced with artisanal technologies, the use of starter cultures, generally consisting of a combination of lactic acid bacteria (LAB) and *Staphylococcus* species, has become common in the

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http://dx.doi.org/10.1016/j.foodcont.2013.01.040

manufacture of several types of fermented products in order to ensure safety, contribute to colour and flavour development and extend shelf-life maintaining the typical characteristics obtained in artisanal elaborations (Coppola, Iorizzo, Saotta, Sorrentino, & Grazia, 1997; Papamanoli, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002; Rebecchi, Crivori, Sarra, & Cocconcelli, 1998). There are several lactic acid bacteria, mainly *Lactobacillus sakei* in Europe and *Pediococcus acidilactici* in USA (Leroy, Verluyten, & De Vuyst, 2006), and some staphylococcal species, almost exclusively *Staphylococcus xylosus* and *Staphylococcus carnosus*, developed as commercial starters for the manufacture of dry sausages (Corbière Morot-Bizot, Leroy, & Talon, 2007). However, it is considered that non-autochthonous starter cultures could have a negative impact on the sensory properties of the product resulting in losses of desirable particular sensory characteristics (Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). Therefore, an exhaustive characterization of selected autochthonous microbial strains, regarding their technological and safety properties and their interactions with raw materials and applied technology, is crucial for the achievement of the desired quality parameters specific for the product type (Cachaldora, 2011; Casquete, Benito, et al., 2011; Casquete, Martín, et al., 2011; Erkkilä et al., 2001; Talon & Leroy, 2011). Although a number of studies about different aspects of Galician chorizo were published, such as the effect of diverse preserving methods on its sensory properties (Fernández-Fernández, Romero-Rodríguez, & Vázquez-Odériz, 2005), its polycyclic aromatic hydrocarbons (PAHs) content (Lorenzo et al., 2011) or its bacterial population dynamics during the ripening (Fonseca, Cachaldora, Gómez, Franco, & Carballo, 2013), to our knowledge this is the first time that the effect of different starter cultures on the final properties of Galician chorizo is studied.

The aim of this study was to evaluate the effect of different autochthonous starter cultures, consisting of a mix of a *L. sakei* strain and a strain belonging to *Staphylococcus equorum*, *Staphylococcus epidermidis* or *Staphylococcus saprophyticus* species, on volatile compounds profile, as determined by SPME-GC/MS analysis, and sensory properties of Galician chorizo. In addition, the evolution of microbial counts and physicochemical parameters were studied throughout the whole ripening process.

2. Materials and methods

2.1. Starter cultures preparation

One *Lactobacillus* strain (*L. sakei* LS131) and three *Staphylococcus* strains (*S. equorum* SA25, *S. epidermidis* SA49 and *S. saprophyticus* SB12), previously isolated in our laboratory from Androlla and Botillo, two traditional Galician fermented sausages, were used for the inoculation of Galician chorizo as starter cultures. These starter cultures were chosen from a set of isolates after identification by 16s rRNA gene sequencing and technological characterization, since they showed suitable technological properties (ability to grow at temperature and pH values of fermenting sausage, and at high NaCl concentrations, as well as nitrate reductase, proteolytic and lipolytic activities) and safety aptitude (absence of amino acid decarboxylase and enterotoxigenic activities) (Cachaldora, Fonseca, Franco, & Carballo, 2013; García Fontán, 2004). *Lactobacillus* strain was purified on MRS agar and successively subcultured on MRS broth to a final volume of 500 mL with a concentration of at least 10^8 CFU/mL, and *Staphylococcus* strains were purified on BHI agar and successively subcultured on BHI broth to a final volume of 1000 mL with a concentration of at least 10^8 CFU/mL. Cell concentration was calculated by interpolation of absorbance values measured at 600 nm into the correspondent growth curve. Cells were obtained by centrifugation at 4000 g for 5 min at 4 °C, washed with sterile saline solution (0.85% NaCl) and centrifuged again under the same conditions. The pelleted cells were resuspended in 40 mL of sterile distilled water to be added to the different batches.

2.2. Sausage production and sampling procedures

In triplicate and following traditional techniques, four different batches of Galician chorizo were manufactured, three of them with addition of different starter strains. Batches were named according to the starter culture added: (i) CNT batch, non-inoculated control, (ii) EQU batch, inoculated with *L. sakei* LS131 + *S. equorum* SA25, (iii) EPI batch, inoculated with *L. sakei* LS131 + *S. epidermidis* SA49, (iv) SAP batch, inoculated with *L. sakei* LS131 + *S. saprophyticus* SB12. *L. sakei* LS131 was added to the mix in an amount of 10^6 CFU/g, while each *Staphylococcus* strain was added in an amount of 10^7 CFU/g. Galician chorizo formulation includes lean pork (80%), pork back fat (20%), sweet paprika (22 g/kg), NaCl (15 g/kg), garlic (4 g/kg), spicy paprika (1 g/kg) and water (40 mL/kg). The lean pork and the pork back fat were ground through a 10 mm diameter mincing plate and vacuum mixed together with the other ingredients for 3 min. The mix was maintained at 4 °C for 24 h and then stuffed into natural porcine casings with a diameter of 36–38 mm. The sausages were fermented for 9 days at 6 °C and 80% relative humidity and then transferred to a drying-ripening chamber where they were kept for 21 more days at 12 °C and 75% relative humidity. Samples at day 0 (mix before stuffing) and after 2, 5, 9, 14, 21 and 30 days of ripening were taken for subsequent analysis.

2.3. Physicochemical analysis

The moisture content of the samples was determined by dehydration at 105 °C until constant weight according to the ISO recommended standard 1442:1997. Water activity was determined using a Fast-lab (GBX, Bourg-de-Péage, France) water activity metre. The pH of samples was measured using a pH metre GLP21 (Crison Instruments, Barcelona, Spain). The acid index was determined in fat extracted with chloroform–methanol according to Folch, Lees, and Sloane Stanley (1957), by titrating with KOH and using phenolphthalein as indicator (AOAC, 2003). Non-protein nitrogen (NPN) content was determined by the Nessler method (Johnson, 1941) after protein precipitation with 0.6 M perchloric acid (De Ketelaere, Demeyer, Vandekerckhove, & Vervaeke, 1974).

2.4. Microbial counts

In triplicate, 10 g of sample taken from the mix before stuffing or from the central part of each sausage at the different sampling times was aseptically added to 40 mL of a sterile solution of 0.1% peptone water (Oxoid, Basingstoke, UK). This mixture was homogenized in a Masticator Classic (IUL Instruments, Barcelona, Spain) blender for 2 min at room temperature. For microbial plate counts, serial decimal dilutions in peptone water were prepared and poured or spread on different agar media. Total mesophilic aerobic bacterial counts were determined on standard plate count agar (PCA) (Oxoid) and incubating at 37 °C for 48 h. Staphylococci were enumerated on mannitol salt agar (MSA) (Oxoid) after incubation at 37 °C for 48 h. LAB were enumerated on de Man, Rogosa, Sharpe (MRS) agar incubated at 30 °C for 72 h in anaerobiosis using anaerobic jars with GENbox anaer (bioMérieux, Marcy-l'Etoile, France). *Enterobacteriaceae* counts were determined by pour plating in violet red bile glucose agar (VRBGA) (Oxoid) incubated with a double layer at 37 °C for 24 h.