Meat Science 96 (2014) 1469–1477

Contents lists available at ScienceDirect

Meat Science

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

Impact of *Debaryomyces hansenii* strains inoculation on the quality of slow dry-cured fermented sausages

Liliana Cano-García, Carmela Belloch, Mónica Flores *

Institute of Agrochemistry and Food Technology (IATA-CSIC), Avda. Agustín Escardino 7, E-46980 Paterna, Valencia, Spain

ARTICLE INFO

Article history:
Received 31 July 2013
Received in revised form 12 December 2013
Accepted 16 December 2013

Keywords:
Yeast
D. hansenii
Dry-cured sausages
Flavour
Aroma
Volatile compound

ABSTRACT

Debaryomyces hansenii strains, M4 and P2, isolated from natural fermented sausages were inoculated in slow fermented sausages to study their effect on processing parameters, microbial population, volatile compound and sensory characteristics. The inoculation of *D. hansenii* strains, M4 and P2, did not affect the ripening process as no differences in pH and Aw were detected. The dominance of the inoculated yeast strains along the process was followed by RAPDs of M13 minisatellite. The inoculated yeasts, P2 and M4, were recovered at the end of the ripening process although P2 appeared in higher counts than M4. The sausages inoculated with P2 resulted in a decrease in lipid oxidation values (TBARS) and a reduction of lipid-oxidation derived aldehydes in addition to a highest acid compound abundance. M4 inoculated sausages resulted in highest sulphur containing compound abundance. However, no differences in consumer acceptance were detected. Moreover, both yeast strains were responsible for the generation of ethyl methyl-branched ester compounds in the dry-cured sausages.

1. Introduction

Slow fermentation of large diameter fermented sausages is a process that requires long ripening times (Marco, Navarro, & Flores, 2008). These traditional low-acid dry-cured fermented sausages are much appreciated in Southern European countries due to their moderate pH decrease produced by microbial activity and by the development of specific sensory characteristics (Ravyts, De Vuyst, & Leroy, 2012).

The improvement and diversification of fermented flavour by selecting microorganism with unique flavour-developing capabilities are an opportunity to increase the competitiveness of the final dry-cured fermented sausage. In this case, the contribution of yeast strains to the sensory characteristics of dry-cured fermented sausages has been mainly attributed to the stabilization of the reddening reaction and development of a characteristic yeast flavour, due to their ability to degrade peroxides and the presence of lipolytic and proteolytic activities (Durá, Flores, & Toldrá, 2004; Olesen & Stahnke, 2000). Among spontaneous fermented sausages, *Debaryomyces hansenii* is often the dominated yeast strain in fermented sausages (Cocolin, Urso, Rantsiou, Cantoni, & Comi, 2006; Mendonça, Gouvêa, Hungaro, Sodré, & Querol-Simón, 2013).

The selection of yeast strains to improve sausage fermentation with respect to flavour may be affected due to yeast growth inhibition by ingredients, antifungal compounds or technological parameters (Olesen & Stahnke, 2000). Also, yeast metabolic capabilities to produce flavour compounds may differ among strains. The addition of *D. hansenii* yeast strains in dry-cured fermented sausages has been related to the increase in esters and acid compounds (Andrade, Córdoba, Casado, Córdoba, & Rodríguez, 2010; Flores, Durá, Marco, & Toldrá, 2004; Purriños, Carballo, & Lorenzo, 2013) an increase in branched alcohols and aldehydes (Andrade et al., 2010) and a decrease in linear aldehydes (Flores et al., 2004; Purriños et al., 2013) although Olesen and Stahnke (2000) did not find any volatile compound effect.

The differences in flavour development in dry-cured fermented sausages associated with a particular yeast strain have been followed throughout the ripening process using yeast media (Flores et al., 2004; Olesen & Stahnke, 2000), morphological and biochemical yeast characteristics (Flores et al., 2004; Iucci et al., 2007) but few studies have demonstrated the presence of the inoculated yeast strains by molecular characterization (Andrade et al., 2010; Mendonça et al., 2013). Also, the implantation of the inoculated yeast strains throughout the ripening process has not been followed.

The molecular characterization of the different yeast strains of *D. hansenii* isolated from traditional fermented sausages has demonstrated that there is a large variability within *D. hansenii* (Cano-García, Flores, & Belloch, 2013; Mendonça et al., 2013). Also, the aromatic potential is different among selected *D. hansenii* strains as it was shown by Cano-García, Rivera-Jiménez, Belloch, and Flores (2014) in a meat model system. In this study, two *D. hansenii* strains, M4 and P2, showed a remarkable aroma potential due to their ester and sulphur production capabilities. However, the metabolic activity of these yeasts might be affected by meat ingredients, technological parameters and also microorganism present since competition between microbial populations is occurring throughout the ripening process (Ravyts et al., 2012).

* Corresponding author. Tel.: +34 96 3900022; fax: +34 963636301.
E-mail address: mflores@iata.csic.es (M. Flores).

0309-1740/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.12.011

Therefore, our aim was to evaluate the effect of *D. hansenii* yeast strains, M4 and P2, on the sensory quality of slow dry-cured fermented sausages. In addition, the implantation of the inoculated *D. hansenii* strains was checked by molecular techniques throughout the whole ripening process.

2. Materials and methods

2.1. Preparation of yeast strain starter

Two *D. hansenii* strains, M4 and P2, previously isolated from naturally fermented sausages "salchichón de Requena" (Requena, Valencia, Spain) were selected by their aromatic potential (Cano-García, et al., 2013, 2014) to be inoculated in slow dry-cured fermented sausages. The yeasts were grown on GPY medium (glucose 2%, peptone 0.5%, yeast extract 0.5%, pH 6.0) to a concentration of 10^8 cfu mL^{-1}. The cells were separated by centrifugation (7000 rpm for 10 min at 4 °C) and washed with sterile saline solution (0.9% salt). The collected cells were concentrated in 1 mL of saline solution per gramme of the cells (wet weight) and the suspensions stored at −80 °C until further use.

2.2. Preparation of dry-cured fermented sausages

Three different batches of dry-cured fermented sausages were manufactured; a control batch without yeasts (batch C) and two batches inoculated with M4 (batch M4) and P2 (batch P2) yeast strains, respectively. Twenty two sausages were manufactured in each batch.

Sausage batches were prepared with lean pork (75%) and pork back fat (25%) with the following additives (g/kg): NaCl (27), lactose (20), dextrin (20), sodium caseinate (20), glucose (7), sodium ascorbate (0.5), sodium nitrite (0.15), and potassium nitrate (0.15). The meat was ground through a 10 mm hole diameter plate, vacuum minced with the remaining ingredients and inoculated with a starter culture C-P-77S bactoferm (Chr. Inc., Hansen, Denmark) containing *Lactobacillus pentosus* and *Staphylococcus carnosus*. For the yeast inoculated batches, appropriate volumes of P2 or M4 yeast strain suspension were added to the meat batter at final concentration of 10^6 cfu g^{-1} of yeast strain. The meat mixture was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) making sausages of approximately 0.7 kg as the final weight. All sausage batches were ripened in the same drying chamber and subjected to an initial stage at 15–20 °C and 75–85% HR for 22 h, followed by drying at 9 °C and 75–85% HR for 43 days. In order to control the ripening process, weight losses and pH were measured during processing (Olivares, Navarro, Salvador & Flores, 2010).

From each batch, a 500 g portion of meat mixture (0 day) and three sausages at 10, 27 and 43 days were randomly collected to study the effect of inoculated yeast strains and ripening time. 150 g portion of each sausage was minced and used for moisture, water activity and pH tests and a 10 g portion was taken for microbiological analysis. In addition, the remaining minced sausage was vacuum packaged and frozen at −20 °C for subsequent analyses (TBARS) after measuring sausage colour. Also, several sausage slices were wrapped in aluminium foil, vacuum packaged and stored at −80 °C for volatile compound analysis. Finally, sensory analysis was carried out at 43 days of the drying process. Results were expressed as the mean of three replicates per 100 g of dry matter at each processing time and batch.

2.3. Chemical analysis

The measurement of pH, water activity, colour evaluation (CIE L*, a*, b*), and moisture was performed as described by Olivares et al. (2010). Thiobarbituric acid reactive substances (TBARS) were quantified to determine the degree of lipid oxidation (Olivares, Navarro & Flores, 2011) using trichloroacetic acid as solvent instead of perchloric acid. The results were expressed as mg malonaldehyde (MDA)/kg in dry matter.

2.4. Microbial analysis

For microbial analysis, minced sausage samples were homogenized with sterile saline solution (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min. Further decimal dilutions of the homogenate were made in sterile saline solution and 0.1 mL of each dilution was spread onto the surface of appropriate agar media. Rose Bengal Agar with chloramphenicol (RBA) (Conda SA, Madrid, Spain) incubated at 28 °C for 72 h was used for yeast counts. Lactobacilli counts were done on (MRS) Man Rogosa Sharpe Agar (Scharlau Chemie SA, Barcelona, Spain) in anaerobic jars while Staphylococci were counted by surface plating on (MSA) Mannitol Salt Agar (Scharlau Chemie SA, Barcelona, Spain) and both were incubated at 37 °C for 48 h.

2.5. Strain characterization by molecular methods

Ten yeast strains were isolated from each inoculated batch at the different ripening times and then subjected to molecular characterization by minisatellite PCR amplification using the M13 primer. DNA was extracted as described in Querol, Barrio, and Ramón (1992) and M13 minisatellite PCR amplification was carried out as described elsewhere (Cano-García et al., 2013). PCR products (10 μL) were resolved by electrophoresis on 2% agarose gel in 1× TAE buffer at 90 V for 3 h, stained with Red Safe nucleic acid staining solution 20,000× (Intron biotechnology, Kyunggi-do, Korea) and visualized under UV light. DNA fragment sizes were determined using 100-bp DNA ladder. Minisatellite PCR patterns were compared with those previously obtained for these strains (Cano-García et al., 2013).

2.6. Analysis of volatile compounds

Frozen sausage slices were minced with 0.75 mg of BHT and 5 g weighted into 20 mL headspace vial (Gerstel, Germany). The vial was maintained at 37 °C during 30 min to equilibrate its headspace. Then, volatile compounds in samples were extracted by a Solid Phase Microextraction (SPME) device using an automatic injector Gerstel MPS2 (Gerstel, Germany). The extraction was done using an 85 μm CAR/PDMS sf fibre (Supelco, Bellefonte, PA, USA) by headspace exposure during 3 h at 37 °C and a previous vial equilibration for 30 min at 37 °C.

Identification and quantification of volatile compounds were done using a gas chromatograph (GC HP 7890 series II) equipped with an HP 5975C mass selective detector (MS Hewlett Packard, Palo Alto, CA, USA). The compounds absorbed by the fibre were desorbed in the injection port of the GC–MS for 15 min at 220 °C with the purge valve off (splitless mode) using He as carrier gas with a linear velocity of 34.3 cm s^{-1}. Compounds were separated on a DB-624 capillary column (J&W scientific, 30 m × 0.25 mm × 1.4 μm) and analyzed as described by Cano-García et al. (2013). Volatile compounds were identified by comparison with mass spectra from the library database (Nist'05) and with Kovats retention indices of authentic standards (Kovats, 1965) calculated using the series of n-alkanes (Aldrich, Germany). Quantification of each volatile compound was based on the abundance of total extracted area (TIC) or the area of a target ion when different compounds coeluted.

2.7. Sensory analysis

A consumer sensory analysis was performed at the end of the ripening process (43 days). Testing was carried out in a sensory laboratory equipped with individual booths (Olivares et al., 2011). Data acquisition was performed using Compusense *five* release 5.0 software (Compusense Inc., Guelph, Ontario, Canada). The casing was removed and sausages cut into slices of 4 mm thickness. Sausages from each batch (C, M4, P2 batches) were labelled with random three-digit codes and presented on a plate at room temperature with water and unsalted toasts to cleanse