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The effects of starter culture types on the technological quality, lipid oxidation and biogenic amines in fermented sausages

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ABSTRACT

The aim of this study was to evaluate the effect of different starter cultures on the quality, lipid oxidation and biogenic amines in fermented sausages. Six treatments (5 inoculated with 5 different commercial starter cultures and 1 without inoculation) were prepared. Our results revealed that all the inoculated batches had significantly lower pH values compared with the control ($P < 0.05$). The inoculation with starter cultures resulted in the increased lipid oxidation levels in the products. Furthermore, all the inoculated batches presented significantly higher scores for sensory attributes such as flavor, taste and acceptability in comparison to the control. Putrescine (88.64–455.39 mg/kg) and tyramine (223.85–444.67 mg/kg) were the two unique biogenic amines detected in all treatments. Putrescine was positively correlated ($r = 0.868$, $P < 0.05$) with pH, and most inoculated batches with lower pH values presented lower putrescine contents compared with control ($P < 0.05$). While, tyramine contents were found significantly higher in all inoculated batches, except the batches inoculated with SA2 (*Staphylococcus carnosus* + *Staphylococcus xylosus* + *Debaryomyces hansenii* + *Lactobacillus curvatus*) or SA7 (*Staphylococcus carnosus* + *Lactobacillus sakei*). Overall, the starter cultures significantly affected most quality parameters determined. On the basis of present results, the starter culture (SA7) containing *Staphylococcus carnosus* and *Lactobacillus sakei* emerged out to be more suitable for production of high quality products with lowered biogenic amines.

1. Introduction

In the industrial production of fermented sausages, starter cultures containing lactic acid bacteria (LAB)/or Gram-positive, catalase-positive cocci (GCC) are usually inoculated to initiate rapid acidification of the raw meat batter (Leroy & Vuyst, 2004). The use of starter cultures has been found to improve the technological quality, desirable sensory quality and safety of the finished product (Leroy, Verluyten, & Vuyst, 2006). During fermentation/ripening, the LAB or GCC play some roles in the biochemical changes in fermented sausages such as; proteolysis (Beriain, Lizaso, & Chasco, 2000; Casaburi et al., 2007; Tabanelli et al., 2012), lipolysis (Casaburi et al., 2007; Hierro, de la Hoz, & Ordonez, 1997; Zhao et al., 2011) and glycolysis (Viallon et al., 1996). These processes play a central role affecting the final quality characteristics of the finish products. However, it has been reported that the acidifying capacity or the fitness of commercial meat starter cultures when used in particular formulation of fermented sausage is questionable since a culture that performs well in a formulation for fermented sausage type is not necessarily efficient in another type (Leroy et al., 2006). Therefore, suitable cultures must be selected for each formulation of product type or technology of fermentation used in order to assure the final quality and safety of the products for consumers.

More to the point, biogenic amines (BA) are formed in many kinds of food, such as fermented foods and dry sausages etc. (Latorre-Moratalla, Bover-Cid, Veciana-Nogués, & Vidal-Carou, 2012; Ruiz-Capillas & Jimenez-Colmenero, 2004) as the result of decarboxylation of free amino acids by the decarboxylase enzymes originated from spoilage bacteria (e.g., *Pseudomonas* spp., *Bacillus and Enterobacteriaceae*), GCC and LAB etc. (Kucerova, Svobodová, Tůma, Ondráčková, & Plocková, 2009; Landeta, De las Rivas, Carrascosa, & Munoz, 2007; Ruiz-Capillas & Jimenez-Colmenero, 2004; Santos, 1998; Shalaby, 1996). Recently, the biogenic amines

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have been extensively studied due to: (i) their direct toxic effects on consumer's health: the intake of foods containing high concentrations of biogenic amines especially the tyramine can cause poisoning with symptoms such as migraine, headaches, gastric and intestinal problems (Stratton, Hutkins, & Taylor, 1991); (ii) their role as quality indexes and indicators of unwanted microbial activity in meat products (Eerola, Maijala, Roig-Sagues, Salminen, & Hirvi, 1996). Studies have found that many LAB strains have decarboxylase activity and they are the main producers of biogenic amines in fermented meat products (Bover-Cid & Holzapfel, 1999; Ruiz-Capillas & Jimenez-Colmenero, 2004; Kucerova et al., 2009). High *Lactobacillus* counts have been associated with the formation of high concentrations of histamine and tyramine (Kucerova et al., 2009; Maijala & Eerola, 1993) or some *Lactobacillus* strains such as *Lactobacillus curvatus*, *Lactobacillus brevis* and *Lactobacillus buchneri* etc. have been found as the important tyramine producers (Bover-Cid & Holzapfel, 1999; Tabanelli et al., 2012).

Recently, however, numerous studies have found that starter cultures play an important role in controlling and preventing the BA formation in fermented meat products due to their ability to lower pH environment which inhibit the growth of BA-producing bacteria (Baka, Papavergou, Pragalaki, Bloukas, & Kotzekidou, 2011; Latorre-Moratalla et al., 2007; Lu et al., 2010). Tabanelli et al. (2012) also found that a slow pH drop in fermented sausages was characterized by higher BA contents. However, it should be known that the acidifying activity capacity differs depending upon each LAB/GCC strains or type of starter cultures used (Leroy et al., 2006). Therefore, we hypothesized that different starter cultures containing different LAB/or GCC strains may also affect the quantity and quality of BA in the products. So far, though a number of studies have been conducted to evaluate the effects of starter cultures on the quality and biogenic amines in fermented sausages products (Baka et al., 2011; Casaburi et al., 2007; Lorenzo, Gomez, Purrinos, & Fonseca, 2016; Lu et al., 2010; Tabanelli et al., 2012), however, only two or three types of starter cultures were tested in these studies. Therefore, further studies are needed to provide a more detailed overview about the roles of starter cultures in quality determination and BA formation in fermented sausages since a large number of commercial starter cultures have recently been produced and available on the markets. In order to select the most suitable starter culture for production of high quality and healthier fermented sausages with lowered BA content for consumer, in the present study, five different commercial starter cultures containing different LAB/or GCC strains were used and investigated for their effects on the quality characteristics and BA contents in the products.

2. Materials and method

2.1. Materials

Fresh pork ham and back-fat were obtained from a local commercial processor (Jeonju, Korea) 24 h after slaughter. Five commercial starter cultures including: Starterkulturen Almi. 2 (SA2: *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Debaryomyces hansenii* and *Lactobacillus curvatus)*, Starterkulturen Almi 7 (SA7: *Staphylococcus carnosus*, *Lactobacillus sakei*), Starterkulturen 13 (S13: *Pediococcus pentosaceus*, *Staphylococcus carnosus*), Starterkulturen Almi 20 (SA20: *Pediococcus acidilactici*), Starterkulturen Almi Rohschinken (SAR: *Staphylococcus xylosus*, *Lactobacillus plantarum*), and a mould-culture (*Penicillium nalgiovensis*) were purchased from Almi Ges. m.b.H & Co GK (Oftering, Austria).

2.2. Formulation and processing of fermented sausages

In the present study, six formulations of fermented sausage treatments were prepared. Each treatment batch was prepared with 20 kg of meat batter. All batches were made with 80% pork ham and 20% pork fat, and together with the following additives: NaCl 20 g/kg, sugar 15 g/kg, black pepper 2 g/kg, polyphosphate 2 g/kg, sodium ascorbate 2 g/kg; nitrite 0.1 g/kg, nitrate 0.05 g/kg. To determine the effects of starter cultures on the quality of fermented sausages, the cultures: SA2, SA7, SA13, SA20 and SAR 0.2 g/kg each type was added to each treatment (T) which was then named as: T-SA2, T-SA7, T-SA13, T-SA20 and T-SAR, respectively. The batch made with only the meat mixture and additives without culture inoculation was served as the control. The trimmed meat and fat were then separately chopped through a 3 mm plate using a silent chopper (Model 7548, Biro MFG. Co, Ohio, USA). For each treatment batch, the chopped meat, fat and ingredients were placed in a meat mixer (model CE93, RUHLE GMBH, Grafenhausen, Germany) and then mixed for 10 min at 4 °C; thereafter, the starter culture was added and mixed for further 5 min. After mixing, the meat batters were immediately stuffed into 65-mm diameter collagen casings (Naturin Viscofan Co, Tajonar-Navarra, Spain) using a vacuum stuffer (Model VF610, Handtmann Co, Biberach, Germany), resulting in sausages of ca. 1.5 kg. All the sausages were then dipped in the mould-culture containing solution which was previously prepared according to the manufacturer's instruction. Finally, the sausages were then hung vertically on metal rods, set on the cart and transferred to an air-conditioned chamber where the temperature and relative humidity (RH) were set as follows: 15 °C/90% for 18 h, 22–23 °C/90% for 48 h, 14–15 °C/85% for 10 days and 14–15 °C/72% until the samples reached a weight loss of about 40–45%. At the end of the ripening/drying (40^{th} day), the samples were collected and used for the analyses. The weights of sausage samples taken before (immediately after filling into casing) and after ripening were used to determine the yield.

2.3. Proximate composition

The moisture, protein and fat contents were analyzed using a Food Scan™ Lab 78810 (Foss Tecator Co., Ltd., DK), according to the method developed by Anderson (2007). To determine these contents, approximately 180 g of homogenized sample (each) was distributed in the instrument's round sample dish and loaded into the instrument's sample chamber. Each sample was determined in triplicates.

2.4. pH and water activity (a_w)

The pH values of samples were determined in triplicates using a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) that was calibrated with 3 different standard pH solutions (4.0, 7.0 and 9.25). The pH was measured after homogenizing 3 g of each sample with 27 mL of distilled water for 30 s using a homogenizer. Water activity (a_w) of the fermented sausages was determined at 25 °C with a Novasina measuring instrument; model AW SPRINT-TH 300 (Pfaffikon, Switzerland). Calibration was done by using several saturated solutions of known a_w.

2.5. Lipid oxidation

The content of thiobarbituric acid reactive substances (TBARS) was determined to evaluate the lipid oxidation levels in samples between the treatments, using the method of Pikul, Leszczynski, and Kummerow (1989). Briefly, each sample (5 g) with 17.5 mL of 4% perchloric acid and 0.5 mL of 7.5% butylated hydroxyanisole (BHA) in ethanol was homogenized at 13,000×rpm for 20 s using a homogenizer (Polytron MR-2100, Kinematica AG, Switzerland). The volume of the homogenate was adjusted to 25 mL with 4%