Food Control 68 (2016) 358–366

Contents lists available at ScienceDirect

Food Control

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

Biogenic amine inhibition and quality protection of Harbin dry sausages by inoculation with *Staphylococcus xylosus* and *Lactobacillus plantarum*

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ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form
9 April 2016
Accepted 13 April 2016
Available online 14 April 2016

Keywords:
Harbin dry sausage
Staphylococcus xylosus
Lactobacillus plantarum
Physicochemical property
Biogenic amines
Sensory evaluation

ABSTRACT

This study was conducted to evaluate the effects of inoculation with *Staphylococcus xylosus, Lactobacillus plantarum,* or a mixture of strains (*L. plantarum* + *S. xylosus*) on the formation of biogenic amines (BAs) and quality characteristics in Harbin dry sausage. Microbial analysis shows that total aerobic bacteria and lactic acid bacteria (LAB) counts were higher in the inoculated sausages, especially in those inoculated with a mixture of strains, but the growth of enterobacteriaceae was inhibited ($P < 0.05$). A sharp decrease in the pH value of the sausages was observed, and the moisture content and water activity were significantly decreased during fermentation. Sausages inoculated with a mixture of bacterial strains had the lowest pH, moisture content and water activity ($P < 0.05$). Inoculation of dry sausages with *S. xylosus* or *L. plantarum,* especially a mixture of strains (*L. plantarum* + *S. xylosus*), significantly delayed lipid oxidation, improved sensory characteristics, and inhibited BA accumulation. Six types of BAs (cadaverine, putrescine, tryptamine, 2-phenylethylamine, histamine, and tyramine) were inhibited by the presence of *L. plantarum* and *S. xylosus*, and a mixture of them had the most inhibitive effect. Correlation analysis showed that the BA concentrations correlated well with enterobacteriaceae counts, and some BAs were negatively correlated with LAB counts. These results demonstrate that *S. xylosus* and *L. plantarum* could be used as starter cultures in Harbin dry sausage production to inhibit BA accumulation and improve quality characteristics.

1. Introduction

Biogenic amines (BAs) are organic, basic, nitrogenous compounds of low molecular weight, formed by decarboxylation of amino acids via microbial actions (Komprda et al., 2007; Nie, Zhang, & Lin, 2014). Commonly, BAs are involved in natural biological processes such as synaptic transmission, blood pressure control, allergic response, and cellular growth control. However, an excess of BAs in the body is hazardous to the nervous and cardiovascular systems, leading to physical discomforts such as dizziness, headache, hypertension, heart palpitations, and respiratory disorders (Lorenzo, Martínez, Franco, & Carballo, 2007). In addition, BAs are the precursors of nitrosamines, which are carcinogenic. For example, diamines, such as putrescine and cadaverine, can not only strengthen histamine toxicity, but can also contribute to the formation of heterocyclic carcinogenic nitrosamines.

Fermented sausage is one of the most common fermented meat products, and it is popular among consumers due to its unique flavour and texture. Lactic acid bacteria (LAB) and coagulase negative staphylococci (CNS) are the most common microorganisms in fermented meats, which are involved in the development of colour, texture, and flavour. Among them, *Lactobacillus* and *Staphylococcus* strains have been widely studied and used as starter cultures in meat products (Bedia, Méndez, & Bañón, 2011; Fadda, López, & Vignolo, 2010; Leroy, Verluyten, & De Vuyst, 2006). However, there is also a risk in using these bacterial starter cultures due to excessive accumulation of BAs during sausage fermentation. Free amino acids (FAAs), microbial decarboxylase, and low pH conditions are conducive to forming BAs (Nie et al., 2014). Starter cultures of LAB and *Staphylococcus* have proteolytic activity, which promotes the hydrolysis of meat proteins to FAAs, providing substrates for decarboxylic reactions (Fadda et al., 2010). Furthermore, starter cultures of *Lactobacillus farciminis* and

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http://dx.doi.org/10.1016/j.foodcont.2016.04.021
0956-7135/

Lactobacillus alimentarius, for example, possess decarboxylase activity that can also catalyse decarboxylic reactions (Suzzi & Gardini, 2003). Thus, more attention should be paid to the bacterial starter cultures used in fermented foods, especially in fermented meat products. Inoculation with starter cultures containing bacteria that lack decarboxylase activity is one of most effective methods for reducing the accumulation of BAs in fermented meat products. Formation of BAs can be directly blocked in the initial fermentation using amine-negative strains. Specifically, bacteriostatic starter cultures inhibit the growth of pathogenic microorganisms such as enterobacteriaceae, which can produce BAs, to decrease BA formation (Komprda et al., 2004; Simion, Vizireanu, Alexe, Franco, & Carballo, 2014; Zhang, Lin, & Nie, 2013). Additionally, the BAs that are produced can be further degraded into aldehydes, hydrogen peroxide, and nontoxic amines by the bacteria's biogenic amine oxidase (Leuschner, Heidel, & Hammes, 1998). Recently, studies have been conducted on the inhibition of pathogenic microorganism growth via bacteriostatic starter cultures to inhibit BA accumulation in different fermented meat products. Shukla, Park, Lee, Kim, and Kim (2014) found that starter cultures of *Lactobacillus sakei* and *Staphylococcus xylosus* could reduce the accumulation of putrescine, cadaverine, tyramine, histamine, and tryptamine in traditional Chinese smoked horsemeat sausage. Moreover, an amine-negative mixed starter culture containing *Lactobacillus plantarum* and *Saccharomyces cerevisiae* reduced putrescine and cadaverine levels in fermented silver carp sausage (Nie et al., 2014). Mah and Hwang (2009) found that *S. xylosus* could degrade histamine and tyramine in salted and fermented anchovy-*Myeolchi-jeot*.

Harbin dry sausage is a traditional Chinese fermented meat product, which is mainly produced in northeast China. Due to its unique flavour, texture, and short production cycle (approximately 15 d), Harbin dry sausage is popular among consumers. In our previous studies, we isolated and identified the main microorganisms in Harbin dry sausages, which included 14 strains of LAB, 11 strains of *Staphylococcus,* and 10 strains of yeast (Zhao & Kong, 2010a; 2010b; 2010c). In our previous study (unpublished), 14 kinds of lactic acid bacteria strains were tested, and *L. plantarum* possessed the greatest capability to degrade biogenic amines; meanwhile *S. xylosus* also showed significantly high abilities to reduce biogenic amines as well. The objective of this study was to evaluate the effect of starter cultures of amine-negative *S. xylosus* and *L. plantarum* on the accumulation of BAs in Harbin dry sausages. Six types of BAs were detected and several physicochemical properties, including pH, moisture content, water activity, and thiobarbituric acid-reactive substance (TBARS), were analysed. Meanwhile, bacterial counts and sensory quality were evaluated.

2. Materials and methods

2.1. Chemicals

Acetonitrile, methanol, sodium acetate anhydrous, Brij-35, 2-mercaptoethanol, *o*-phthalaldehyde (OPA), acetic acid, boric acid, potassium hydroxide, hydrochloric acid, perchloric acid, thiobarbituric acid (TBA), trichloroacetic acid, and trichloromethane were purchased from Solabio Corporation (Beijing, China). Acetonitrile and methanol are chromatographic grade, while all other chemicals are analytical grade. BA standards, including cadaverine, dihydrochloride, putrescine hydrochloride, tyramine, dihydrochloride, 2-phenylethylamine hydrochloride, histamine, and tryptamine, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Bacterial cultures

S. xylosus was isolated from Harbin dry sausage and identified by sequencing of 16S rDNA (Zhao & Kong, 2010b). *L. plantarum* was obtained from Key Laboratory (Northeast Agricultural University, Harbin, China). They were stored at the College of Food Science, Northeast Agricultural University, China. *L. plantarum* was kept on de Man Rogosa and Sharp (MRS) agar plates and *S. xylosus* was kept on Mannitol Salt Agar (MSA) plates at 4 °C. Thereafter, *L. plantarum* and *S. xylosus* were incubated in MRS and MSA broth, respectively, for 24 h at 30 °C and then maintained at 4 °C within 24 h. MRS broth was prepared according to Chen, Kong, Sun, Dong, and Liu (2015). MSA broth was prepared with beef extract (1.0 g/L), peptone (10.0 g/L), D-mannitol (10.0 g/L), and sodium chloride (75.0 g/L) at a final pH of 7.4 ± 0.2. *S. xylosus* was subcultured twice in MSA broth at 30 °C for 20 h, and *L. plantarum* was subcultured twice in MRS broth at 30 °C for 24 h. The cells were collected by centrifugation (9000 × *g*) with a centrifuge (Allegra™, 64R, Beckman, Germany) for 10 min at 4 °C, washed twice with saline solution, these strains were used as starter cultures.

2.3. Preparation of Harbin dry sausage

Four batches of Harbin dry sausages were manufactured: (1) a control batch without starter cultures; (2) sausage inoculated with *L. plantarum*; (3) sausage inoculated with *S. xylosus*; and (4) sausage inoculated with a mixture of bacterial strains (*L. plantarum* + *S. xylosus*). The sausages were prepared with lean pork (90%) and pork back fat (10%) minced through a 1.5 cm orifice plate and to this sausage mixture was added 2.5% sodium chloride, 5% glucose, 0.01% sodium nitrite, 0.3% monosodium glutamate, 1% wine, and 0.3% mixed spices, which contained cassia bark, *Pericarpium Zanthoxyli*, aniseed, fennel, angelica, *Amomum Villosum*, pepper, round cardamom, and clove (Chen et al., 2015). After mixing thoroughly, the meat batter was inoculated with *L. plantarum* or *S. xylosus* suspensions to a final concentration of 10^7 CFU/g meat. Each mixture was thoroughly mixed and stuffed into a natural porcine casing (3-cm diameter), resulting in sausages with a final weight of approximately 0.15 kg each. All batches were subjected to air drying at 25 ± 2 °C for 24 h (30%–50% relative humidity) and subsequently transferred to an incubator at 25 ± 2 °C and 75%–80% relative humidity for fermentation. Samples were collected on days 0, 3, 6, and 9; four sausages per batch were used for analysis.

2.4. Bacterial counts

Microbiological analyses were performed immediately after the stuffing and during the fermentation period as described by Wang et al. (2015a) with slight modifications. After aseptically removing the casing, approximately 10.0 g of sausage were aseptically diluted 10-fold with 90.0 mL of sterile isotonic saline and homogenized in a stomacher (Seward Medical, London, UK) for 2 min. Serial decimal dilutions were prepared in sterile isotonic saline. Appropriate decimal dilutions of the samples were prepared using isotonic saline, and 0.1 mL of each dilution was plated in triplicate on various selective agars. Total aerobic counts (TACs) were measured using plate count agar after incubation at 37 °C for 48 h. LAB counts were enumerated on MRS agar after incubation at 30 °C for 48 h. Enterobacteriaceae counts were cultured on crystal-violet neutral-red bile dextrose agar plates at 37 °C for 48 h.

2.5. pH, moisture content, water activity, and TBARS values

The pH value was measured using an electronic pH meter