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Production of cured meat color in nitrite-free Harbin red sausage by *Lactobacillus fermentum* fermentation

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Abstract

Lactobacillus fermentum was substituted for nitrite to produce cured pink color in a Chinese-style sausage. Treatments included inoculations (10^4, 10^6, and 10^8 CFU/g meat) followed by fermentation at 30 °C for 8 h and then at 4 °C for 16 h. Control sausage (with sodium nitrite, 60 mg/kg meat) was cured at 4 °C for 24 h without *L. fermentum*. The UV–Vis spectra of pigment extract from *L. fermentum*-treated sausage were identical to that of nitrosylmyoglobin (NO-Mb) formed in nitrite-treated control. The NO-Mb concentration and the colorimetric a^* value of sausage treated with 10^8 CFU/g meat of *L. fermentum* essentially replicated those in nitrite-cured meat. Free amino acid content in sausage treated with *L. fermentum* was greater and the pH slightly lower compared with the nitrite-cured control sample. This study showed that *L. fermentum* has the potential to substitute for nitrite in the sausage production.

Keywords: Sausage; *Lactobacillus fermentum*; Nitrite; Nitrosylmyoglobin

1. Introduction

Curing has been used for centuries to preserve meat and meat products (Møler, Jensen, Skibsted, & Chel, 2003). Current methods of meat curing involve the addition of nitrite, salt, ascorbate (or erythorbate), polyphosphates, and seasonings to meat. Of these, nitrite is the key ingredient. A primary function of nitrite is the production of the characteristic pink color of cured meats, which is desired by the consumer and is usually indicative of quality of cooked products (Shahidi & Pegg, 1991). By virtue of its strong antioxidant properties in meats, nitrite also inhibits lipid oxidation and contributes to desirable meat product flavor (Sanz, Vila, Toldrá, Nieto, & Flores, 1997). In addition, nitrite imparts antibacterial activity, particularly the inhibition of germination of spores and toxin formation by *Clostridium botulinum* (Cassens, 1997a; Martin, 2001).

Despite all of its desired properties, the safety of nitrite to human health has been questioned (Morita, Sakata, & Nagata, 1998). Nitrite can cause the formation of carcinogenic *N*-nitrosamines in cured products due to its reaction with secondary amines and amino acids in muscle proteins. Furthermore, residual nitrite in cured meats may form *N*-nitrosamines in the gastrointestinal tract (Shahidi & Pegg, 1991; Cassens, 1997b). Thus, the meat industry continues to search for alternative methods to produce nitrite-free meats that maintain the color characteristics of nitrite-cured meat products.

Color formation and color stability are important sensory attributes of meat products which influence the products' acceptability by consumers. The pigment responsible for the characteristic pink color of cured meat is a ferrous complex of myoglobin containing nitric oxide (NO), namely, nitrosylmyoglobin or NO-Mb. The complex is

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formed by the reaction of myoglobin with NO generated from nitrite (Morita et al., 1998). Nitrosohemachrome is a denatured, stable form of NO-Mb in cooked, cured meats (Martin, 2001).

Nitrosylated hemin has been developed for nitrite-free "cured" meats. When pre-synthesized nitrosylmyoglobin, together with proper antioxidant and antimicrobial agents, were added to fresh meat, the resulting meat products reportedly resembled nitrite-cured meats (Pegg, Shahidi, Gogan, & Desilva, 1996; Shahidi & Pegg, 1992). Another alternative method to produce pink, cured color is through microbial conversion of myoglobin. Several lactic acid bacteria have the ability to reduce Mb(Fe^{3+}) to Mb(Fe^{2+}) and change the muscle color from brown to bright red. Among them, *Kurthia* spp. and the strain *Lactobacillus fermentum* (JCM1173) have been reported to be capable of converting Mb(Fe^{3+}) to cured meat pigment NO-Mb(Fe^{2+}) (Arihara et al., 1993). Furthermore, *Staphylococcus xylosus* (FAX-1) has been observed to form NO-Mb(Fe^{2+}) both in model systems and in smoked sausages (Møler et al., 2003).

In the present study, *L. fermentum* (AS1.1880) was used to manufacture Harbin red sausage, a Chinese-style sausage containing specific ingredients and processed with a series of cooking and smoking steps. Our objective was to reproduce the characteristic pinkish cured color without the addition of nitrite or nitrate by means of *L. fermentum* strains AS1.1880 treatments.

2. Materials and methods

2.1. Bacterial culture preparation and inoculation

L. fermentum (strain AS1.1880) was obtained from Microbial Research Institute of Chinese Academy of Science (Beijing, China). Mann Rogosa Sharp (MRS 7543) agar was used for cultivating the bacteria culture at 30 °C prior to inoculation. MRS broth was prepared with peptone (10.0 g/L), beef extract (8.0 g/L), yeast extract (4.0 g/L), glucose (20 g/L), K_2HPO_4 (2.0 g/L), ammonium citrate (2.0 g/L), sodium acetate (5.0 g/L), $MgSO_4$ (0.2 g g/L), $MnSO_4$ (0.05 g/L), and Tween 80 (1.0 g/L), final pH 6.5 ± 0.2 (Hopebio-Technology Co. Ltd., Qingdao, China). A single colony in slant MRS culture was inoculated into 5 mL of MRS broth, and cultivated at 30 °C for 16 h. The culture was inoculated into MRS broth twice until the bacterial count reached 10^9 CFU/mL.

2.2. Preparation of sausage

Two batches of sausages (3 kg for each treatment per batch) were manufactured in a pilot plant with the following standard formulation: lean pork trimming (ground through a 0.3-cm orifice plate) 720 g/kg, pork back fat 170 g/kg, corn starch 60 g/kg, NaCl 27 g/kg, white pepper 2 g/kg, garlic 10 g/kg, monosodium glutamate 2.5 g/kg, glucose 5 g/kg, sodium erythorbate 0.5 g/kg, and alkaline phosphate 3 g/kg. For treatments 1, 2, and 3, *L. fermentum* starter culture was inoculated at levels of 10^4, 10^6, and 10^8 CFU/g meat, respectively, into ground lean meat. After incubation at 30 °C for 8 h, salt was added, and the mixture was held 4 °C for 16 h. The fermented, salted lean meat was subsequently chopped with all the seasonings and stuffed in porcine natural casing (~3.5 cm in diameter). For control (without *L. fermentum*), the lean pork was cured with all the ingredients except that 60 mg/kg of sodium nitrite was also added. The control batter was cured at 4 °C for 24 h. The sausages were roasted in a smokehouse at 65 °C for 1 h followed by cooking at 85 °C for 35–40 min until the internal temperature reached 74 °C. After cooking, the sausages were smoked for 8 h at 55 °C using wood chip as the source of smoke (Li, 2002).

2.3. Extraction of NO-Mb and UV–Vis spectral analysis

NO-Mb was extracted according to the method of Møler et al. (2003) with slight modifications. Cured raw meat samples (10 g) were minced and homogenized with 90 mL of 0.02 mol/L, pH 6.0 cold phosphate buffer using a high-speed tissue grinder (Model DS-1, Shanghai Specimen and Model Factory, China) at 4360*g* for 2 min. The homogenates were set in a 4 °C dark room for 1 h to allow pigment extraction. The slurries were subsequently centrifuged at 4360*g* (2 °C) for 20 min. The supernatants were filtered through a 0.45 μm Millipore membrane, and the filtrates in tightly capped bottles were kept in the dark on ice and analyzed within 2 h. Spectral analysis of the pigment extracts was conducted according to the method of Morita et al. (1998). The absorption scans were made from 350 nm to 650 nm at 1 nm increments using a UV–Vis spectrophotometer (SP-754PC, Shanghai Spectrum Instruments, Co., Ltd., China). The measurement was repeated two times each with a new meat sample.

2.4. Colorimetry

The internal color of sausages was measured using a Color Difference Meter (Model WSC-S, Shanghai Physics and Optics Instrument Co., Shanghai, China). The instrument was calibrated using a white standard plate ($L^* = 90.26$, $a^* = -1.29$, $b^* = 5.18$). The values, expressed as L^* (lightness) and a^* (redness) units, were obtained from five different cut areas of each sausage. For each treatment, three links of sausage were used for the analysis.

2.5. pH

Aliquots of 10 g of minced sausage samples were dispersed in 90 mL of distilled deionized water with a tissue grinder (DS-1, Shanghai Specimen and Model Factory, China). The pH of the slurry was measured using an Orion 230 A Plus pH meter with a glass pH electrode (Sensorex Co., Garden Grove, CA, USA). The measurement was carried out in triplicate.