PRODUCTION OF CURED MEAT COLOR IN NITRITE-FREE HARBIN RED SAUSAGE BY *LACTOBACILLUS FERMENTUM* FERMENTATION

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Introduction

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 (Shahidi et al., 1991). 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. An 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 *Lactobacillus fermentum* strain AS1.188 have been reported to be capable of converting Mb(Fe^{3+}) to cured meat pigment NO-Mb(Fe^{2+}) (Arihara et al., 1998).

In the present study, *L. fermentum* (AS1.188) 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* strain AS1.188 treatments.

Materials and Methods

Lactobacillus fermentum (strain AS1.188) was obtained from Microbial Research Institute of Chinese Academy of Science (Beijing, China). The culture was inoculated into MRS broth twice until the bacterial count reached 10^9 CFU/mL. Sausages were manufactured in a pilot plant with the standard formulation. 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 at 4 °C for 16 h. The fermented, salted lean meat was subsequently chopped with all the seasonings and stuffed in porcine natural casing. For control, the lean pork was cured with all the ingredients except *L. fermentum*, and 60 mg/kg of sodium nitrite was also added. The control batter was cured at 4 °C for 24 h.

NO-Mb was extracted according to the method of Møler et al. (2003). The internal color of sausages was measured using a Color Difference Meter (Model WSC-S, Shanghai Physics and Optics Instrument Co., Shanghai, China). Residual nitrite in cooked sausages was measured by a modified colorimetric method (Rincón et al., 2003). The FAA (free amino acid) after methylation were determined using an amino acid analyzer (Model L-8800, Hitachi Co., Tokyo, Japan). The FFA (free fat acid) methal derivatives were subjected to gas chromatography analysis (Model 6890N, Aligent Technologies Co., Ltd., USA.).

Statistical analysis was performed using Statistical Package for Social Science (SPSS, Windows version 12.0, SPSS Inc., Chicago, IL, USA). Significant differences ($P < 0.05$) between means were identified using Least Significant Difference procedures.

Results and Discussion

UV-visible spectra. Each derivative myoglobin has a characteristic absorbance spectrum, which can be used to determine the proportion of each pigment in meat samples (Millar et al., 1996). The spectrum of NO-Mb exhibits absorption peaks at 421, 540 and 579 nm, which are different from the other myoglobin derivatives. The absorbance spectra of the sausage pigment extracts from the present study showed three absorbance maxima (421, 548, and 580 nm) (Fig. 1) that almost matched those of NO-Mb reported by Millar et al. (1996). Therefore, the main pigment extracted in this experiment could be regarded as NO-Mb. Comparison of control and treated sausages indicated a striking pattern similarity between the sample spectra. However, the magnitude of absorbance differed, depending on the concentration of *L. fermentum* inoculated, i.e., it

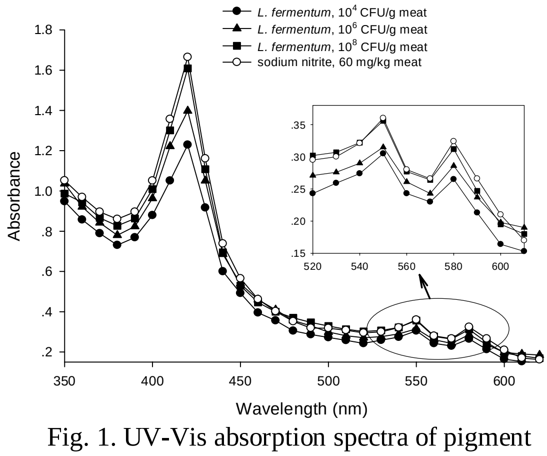

Fig. 1. UV-Vis absorption spectra of pigment extracts from cooked sausages.

increased (P < 0.05) with the dosage of *L. fermentum*. The 10^8 CFU/g meat inoculation level (treatment 3) produced a NO-Mb spectrum that essentially replicated that of the nitrite-cured control (60 mg/kg of nitrite applied to raw meat). The result demonstrated that *L. fermentum* AS1.188 was capable of producing NO-Mb in this sausage product without the addition of extraneous sodium nitrite.

Colorimetry. All cooked sausages developed pinkish color characteristic of nitrite-cured meats. However, there was no difference (P > 0.05) in the L* value between control (60 mg/kg of nitrite) and all *L. fermentum* treatments (1, 2, and 3) (Table 1). The a* values of treatment 1 (10^4 CFU/g meat) and treatment 2 (10^6 CFU/g meat) were significantly lower (P < 0.05) than treatment 3 (10^8 CFU/g meat) and the control. These colorimetric results were in excellent agreement with those obtained from the analysis of extracted NO-Mb pigment (Fig. 1). It is known that sodium nitrite is responsible for the formation of characteristic pink color in cured meat. Thus, the observed pinkish color development as indicated by the a* value supported the conclusion that *L. fermentum* fermentation contributed to the formation of NO-Mb in cured meat without the addition of nitrite. This conclusion was supported by the lack of any measurable pink color if the sausage was processed with neither added nitrite nor *L. fermentum*. The data also suggested that the *L. fermentum* application level was important; a 10^8 CFU/g inoculation was necessary to produce the pink color intensity comparable to that produced by 60 mg/kg of nitrite.

pH and residual nitrite. The pH of control sausage (6.27) did not change from its raw batter (pH 6.30). However, as expected, the pH values of sausages containing *L. fermentum* were lower (P < 0.05) than control sausage apparently resulting from lactic fermentation (Table 1). Although nitrite was not added to *L. fermentum*-treated sausages, small amounts (2.33–2.66 mg/kg) of nitrite were detected in these cooked sausages. Notwithstanding, these levels of nitrite were significantly lower (P < 0.05) than that in control sausages (10.76 mg/kg), which contained 60 mg/kg added nitrite in the product formulation.

FAA and FFA. Fermented sausages with *L. fermentum* had a higher FAA content than that of cured with nitrite. Proteolytic activities in *L. fermentum* resulted in a net increase of FAA in all sausage samples. There was no significant difference in FFA content between fermented and control sausages. Almost all unsaturated fatty acids (except C18:1) in *L. fermentum* (10^8 CFU/g) treatment were slightly higher than those cured with nitrite.

Table 1 Color differences, pH, and residual nitrite in sausages

Sample*	L-value	a-value	b-value	pH	$NaNO_2$ (mg/kg)
1	50.29 ± 0.48^a	6.75 ± 0.47^b	6.93 ± 0.07^c	6.18 ± 0.02^b	2.66 ± 0.08^b
2	49.71 ± 0.51^a	7.68 ± 0.32^b	7.06 ± 0.16^{bc}	6.04 ± 0.01^c	2.53 ± 0.05^b
3	50.31 ± 0.34^a	9.20 ± 0.71^a	7.39 ± 0.15^a	5.73 ± 0.04^d	2.33 ± 0.07^b
4	50.02 ± 0.91^a	9.25 ± 1.02^a	7.16 ± 0.23^{ab}	6.27 ± 0.03^a	10.76 ± 0.10^a

*1 = *L. fermentum* AS1.188, 10^4 CFU/g meat; 2 = *L. fermentum* AS1.188, 10^6 CFU/g meat; 3 = *L. fermentum* AS1.188, 10^8 CFU/g meat; 4 = sodium nitrite, 60 mg/kg meat. a-d Means in the same column with different superscript letters differ significantly (P < 0.05).

Conclusions

This study showed that nitrosylmyoglobin could be generated in Harbin red sausage when *L. fermentum* AS1.188 was inoculated into the meat batter, and the formation of a characteristic pink color with an intensity comparable to that in nitrite-cured sausage could be achieved by the use of 10^8 CFU/g of the culture. The application of *L. fermentum* also has the possible health benefits because of reducing nitrite levels in products. Although *L. fermentum* has the potential to substitute for nitrite in the production of sausage, the combination of *L. fermentum* and low levels of sodium nitrite seems to be more attractive because it would provide an assurance of product success in case of variation in *L. fermentum* activity. Additional research is required to determine the oxidative stability and microbial shelf-life of the nitrite-free, fermented sausage.

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