

Effects of manufacturing conditions on zinc protoporphyrin IX formation in fermented dry sausage with *Lactococcus lactis* subsp. *cremoris* GB(A)-1

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Background and Objectives: Food color is a quality and purchase criterion for consumers. Nitrates/nitrites impart a bright red color to meat products. However, some consumers concern regarding the possible carcinogenicity of these compounds. Zinc protoporphyrin IX (ZnPP) that forms in dry-cured meat products during prolonged processing is a red pigment that may be an alternative to coloring technology in meat products. We have shown that *Lactococcus lactis* subsp. *cremoris* GB(A)-1 (hereafter GB(A)-1) readily promotes ZnPP formation in meat; dry sausage inoculated with GB(A)-1 turned bright red. However, the optimal condition for promoting the formation of ZnPP by GB(A)-1 in fermented dry sausage is still unclear because both ZnPP formation and bacterial growth are affected by various factors, including temperature, pH, and time. Therefore, this study examined the effects of manufacturing conditions on the formation of ZnPP in dry sausages inoculated with GB(A)-1 as a step toward the practical use of a new meat coloring technology.

Materials and Methods: The dry sausage mixture contained minced pork shoulder, 3% salt, and 1% glucose. The mixture was divided into two portions. One portion was inoculated with at 1.0×10^6 GB(A)-1/g (GB group). The other portion was added with ultra-pure water instead of GB(A)-1 (non-inoculated group). Each mixture was stuffed into cellophane casings and matured in an incubation chamber using three conditions (A, B, and C). Condition A was a 7-day resting period at 1°C, 8-h fermentation at 18°C, 21-day drying period with a gradual increase of temperature from 4°C to 14°C, and ripening period at 14°C until day35. Condition B was a 7-day resting period at 1°C, 6-h fermentation at 23°C, 7-day drying period with a gradual decrease of temperature from 22°C to 14°C, and ripening period at 14°C until day35. Condition C had no resting period with the same fermentation, drying, and ripening periods as in condition B. ZnPP and PPIX were extracted from the sausage homogenate using acetic acid/ethyl acetate so- lution and then quantified by high-performance liquid chromatography at excitation/emission wavelengths of 420/590 nm and 400/630 nm, respectively. Visual images of the dry sausages were captured using a digital camera. ZnPP autofluorescence of sausage cross-sections was recorded using a digital camera through a sheet-type bandpass filter following irradiation with purple LED light. The pH of the sausage homogenate was measured using a pH meter.

Results and Discussions: In the sausages matured in conditions A and B, the amount of ZnPP formed did not differ the GB and noninoculated groups. On the other hand, in the sausages matured in condition C, the ZnPP formed in the GB group was approximately double of the ZnPP formed in the non-inoculated group, and cross-sections in the GB group were bright red compared with the non-inoculated group. The amount of ZnPP increased during days 0-7 of production and then remained almost constant. The protoporphyrin IX precursor of ZnPP also increased slightly during days 0-7 of production. Conditions B and C were identical, except for the resting period, suggesting that the 7-day resting period at 1°C suppressed the ZnPP-promoting effect of GB(A)-1, regardless of the temperature following this period. A higher fermentation temperature at the initial stage of production may be essential for promoting the formation of ZnPP by GB(A)-1 in fermented dry sausage manufacturing. The pH of the GB group sausages matured in condition C rapidly decreased from 5.94 to 4.75 in the first 7 days. Sausages matured in conditions A and B showed almost no decrease in pH. In the non-inoculated group, ZnPP autofluorescence of cross-sections of non-inoculated group sausages ripened using condition C was stronger compared to ripening under conditions A and B. These results suggest that the 7-day resting period at 1°C may suppress the growth of GB(A)-1 or the formation of ZnPP in meat, resulting in the low amount of ZnPP in the dry sausage by GB(A)-1.

Key words: Fermented dry sausage, Color, Zinc protoporphyrin IX, *Lactococcus lactis*