EFFECTS OF ACIDIFIERS, pH AND MICROORGANISMS ON THE FORMATION OF ZINC PROTOPORPHYRIN IX IN PORK HOMOGENATE

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Abstract – The aim of this study was to elucidate the effects of acidifiers for pH adjustment, pH and microorganisms on the formation of zinc protoporphyrin IX (ZnPP) in pork homogenate. The optimum pH for the formation of ZnPP varied with muscles in the presence of antibiotics. In the presence of antibiotics, the optimum pH for the formation of ZnPP in pork infraspinatus muscle was approximately pH 4.5, but the amount of ZnPP formed varied depending on the acidifier. In the absence of antibiotics, on the other hand, the optimum pH shifted to a higher pH and the amount of ZnPP increased. Several acidifiers remarkably increased the amount of ZnPP formed. The results suggest that the formation of ZnPP is affected by acidifiers, pH and microorganisms and is facilitated by the synergism between acidifiers and microorganisms.

Key Words – Parma ham, zinc protoporphyrin IX (ZnPP), acidifier, microorganism

I. INTRODUCTION

Parma ham, an Italian traditional dry-cured ham, is made only from the leg of a fattened pig and sea salt. Despite a lack of nitrite or nitrate, the color of Parma ham is an extremely stable bright red and is not changed by exposure of the ham to light or heat (Adamsen *et al*., 2004; Morita *et al*., 1996). Wakamatsu *et al*. (2004a) reported that the red pigment extracted from Parma ham included zinc protoporphyrin IX (ZnPP).

To elucidate the mechanism by which ZnPP was formed, Wakamatsu *et al*. (2004b) established a model experimental system in which ZnPP was formed by anaerobic incubation of pork longissimus muscle homogenate in the presence of antibiotics. The formation of ZnPP depended on various factors including pH, temperature, and ionic strength. On the other hand, it has been reported that Staphylococci generated a red myoglobin derivative, i.e., ZnPP (Morita *et al*., 1996) and that a large amount of ZnPP was formed in the absence of antibiotics (Wakamatsu *et al*., 2004b). However, it has not been investigated whether acidifiers for pH adjustment and microorganisms affect the formation of ZnPP.

A starter culture and an acidifier are often used for the manufacturing dry fermented meat products. The elucidation of the optimal conditions for the formation of ZnPP would be useful for manufacturing dry fermented nitrite/nitrate-free meat products with a preferable color. The aim of this study was to elucidate the effects of acidifiers, pH and microorganisms on the formation of ZnPP.

II. MATERIALS AND METHODS

Model experiment

Pork infraspinatus muscle and longissimus muscle were used in this study. Model solutions consisted of 10% of pork homogenates with/without antibiotics (0.07 mg/ml of penicillin G potassium, 0.25 mg/ml of streptomycin sulfate and 0.05 mg/ml of gentamicin sulfate). The model solutions (1.5 g) in test tubes were put into gas-impermeable bags and incubated for 5 days at 25 ºC anaerobically. Twelve acidifiers were used for pH adjustment of pork homogenate (hydrochloric acid, lactic acid, phosphoric acid, citric acid, fumaric acid, succinic acid, maleic acid, malic acid, tartaric acid, gluconic acid, acetic acid and propionic acid).

Extraction of ZnPP and fluorescence analysis

Each model solution (1.5 g) was mixed well with 4.5 ml of ice-cold acetone after incubation, and the mixtures were held on ice in the dark for 30 min. After filtration through filter paper (No. 2, Toyo Roshi Co., Ltd., Tokyo, Japan), the fluorescence intensity (excitation: 420 nm, emission: 590 nm) of the extracts was measured as an index of the amount of ZnPP using a fluorescence spectrophotometer (RF-5300PC, Shimadzu Corp., Kyoto, Japan).

III. RESULTS AND DISCUSSION

In our previous study (Wakamatsu *et al.*, 2004b, 2007), longissimus muscle, in which type IIb fibers are dominant, was used. However, dry fermented meat products, especially sausages, are mainly manufactured from pork shoulder meat. Therefore, we first investigated the effects of different pork skeletal muscles and pH on the formation of ZnPP (Fig. 1). Longissimus muscle showed maximum fluorescence intensity at pH 5.5, similar to that in our previous study (Wakamatsu *et al.*, 2007), while infraspinatus muscle showed maximum fluorescence intensity at pH 4.5. When the mixture of two skeletal muscles was examined, the optimum pH shifted to pH 5.0 and the amount of ZnPP was between the amounts in each of the skeletal muscles. This result suggested that the formation of ZnPP was different among muscles affected by muscle fiber type.

Next, we investigated the effects of acidifiers, pH and microorganisms on the formation of ZnPP by using pork infraspinatus muscle homogenate (Fig. 2). In the presence of antibiotics, the formation of ZnPP showed a maximum at pH 4.5-4.75 and the amount of ZnPP varied depending on the acidifier. Succinic acid (Fig. 2F), tartaric acid (Fig. 2I) and gluconic acid (Fig. 2J) seemed to facilitate the formation of ZnPP. On the other hand, in the absence of antibiotics, the formation of ZnPP was greatly facilitated and optimum pH shifted to a higher pH. Maleic acid (Fig. 2G), malic acid (Fig. 2H), tartaric acid (Fig. 2I) and gluconic acid (Fig. 2J) remarkably increased the amount of ZnPP at the optimum pH. However, acetic acid (Fig. 2K) and propionic acid (Fig. 2L) hardly facilitated the formation of ZnPP at pH 4.5-5.5. Maleic acid, malic acid, tartaric acid and

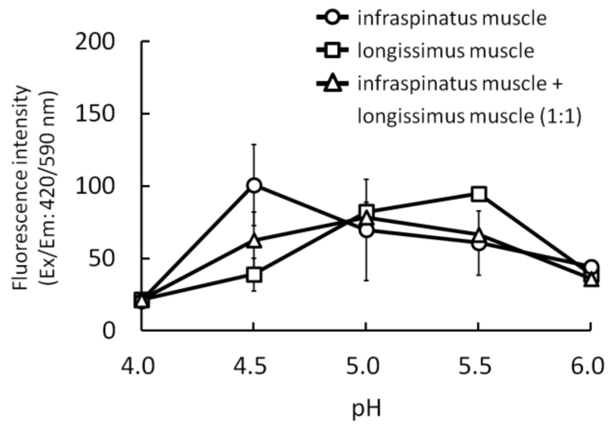

Fig. 1. Effects of pork muscles and pH on the formation of ZnPP in the presence of antibiotics. The fluorescence intensity of acetone extracts of model solutions was measured after incubation. Bars represent the standard deviation of the means (n=2).

gluconic acid might facilitate the generation of ZnPP by microorganisms. Acetic acid and propionic acid might inhibit the ZnPP-generating activity of microorganisms. The results suggested that acidifiers affected the formation of ZnPP regardless of whether microorganisms were involved.

Therefore, an appropriate selection of acidifier, pH and microorganisms in the manufacturing process of dry fermented sausages without nitrite/nitrate would be useful for the formation of ZnPP, resulting in an improvement of color.

IV. CONCLUSION

Acidifiers, pH and microorganisms affected the formation of ZnPP. The results obtained in this study may be useful for manufacturing nitrite/nitrate-free meat products with a preferable color.

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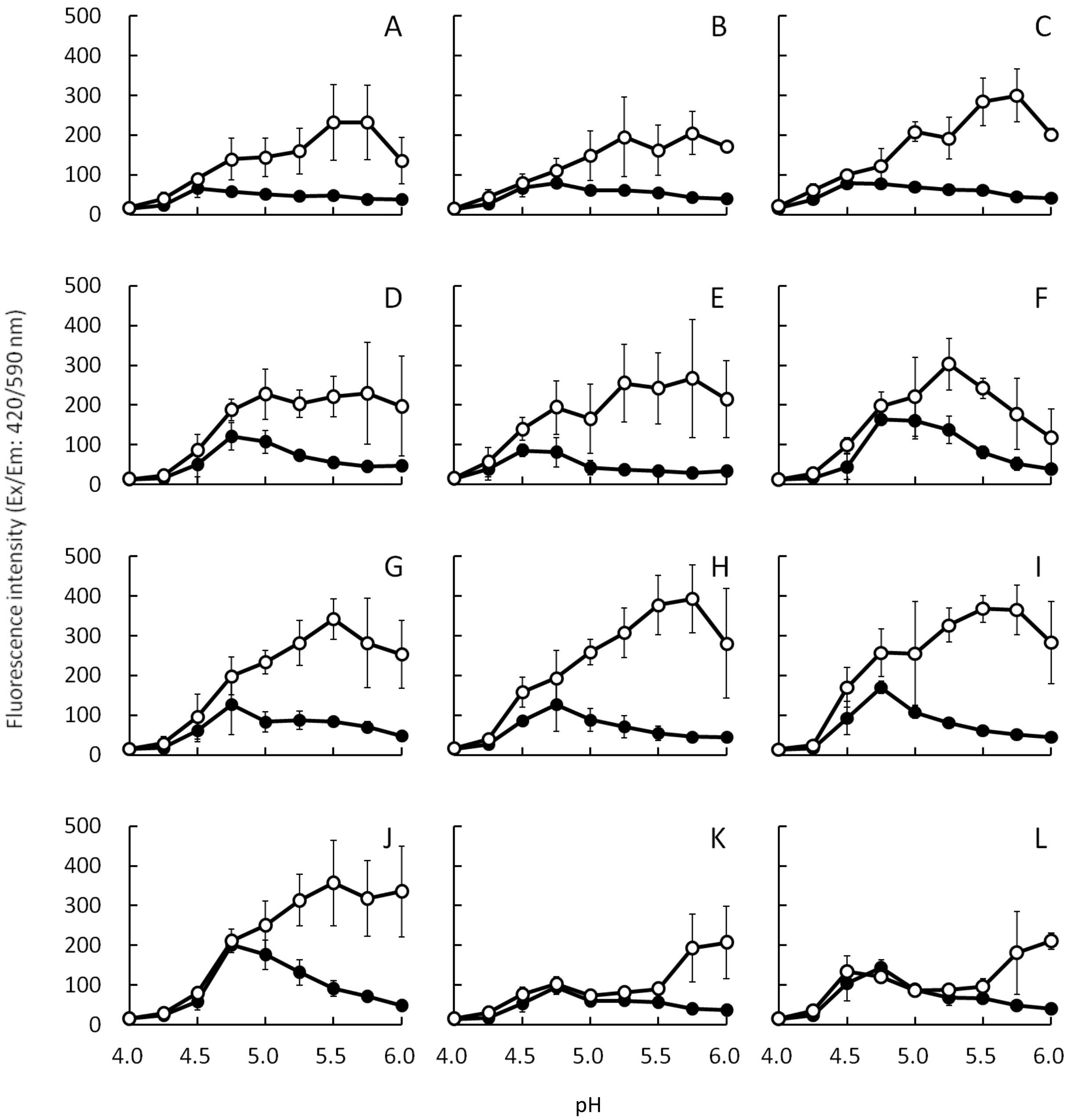

Fig. 2. Effects of acidifiers and pH on formation of ZnPP in the presence (●) and absence (○) of antibiotics. pH adjustment was carried out by using hydrochloric acid (A), lactic acid (B), phosphoric acid (C), citric acid (D), fumaric acid (E), succinic acid (F), maleic acid (G), malic acid (H), tartaric acid (I), gluconic acid (J), acetic acid (K) and propionic acid (L). The fluorescence intensity of acetone extracts of model solutions was measured after incubation. Bars represent the standard deviation of the means (n=3).

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