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DETERMINATION OF ZINC PROTOPORPHYRIN IX IN A NEW MODEL SYSTEM WIHTOUT ADDITION OF MYOGLOBIN

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Introduction

Nitrite or nitrate is usually added to meat products as a curing ingredient, and it reacts with myoglobin to yield stable-red nitrosylmyoglobin, MbFe(II)NO. The north Italian traditional dry-cured ham "Parma ham" is made only from the leg of a fattened pig by salting with sea salt, drying, and maturing over period of one year. Although nitrite or nitrate is not added to Parma ham, the color of the ham is an extremely stable bright red and is hardly changed by exposure of the ham to light. Morita *et al*. (1996) reported that the red pigment of Parma ham is a myoglobin derivative that can be easily extracted with 75% acetone, and they suggested that the formation of the red myoglobin derivative in Parma ham is caused by the action of microorganisms. It was later shown that the red pigment extracted with 75% acetone from Parma ham is Zn protoporphyrin IX (ZPP) (Wakamatsu *et al*., 2004a). Wakamatsu *et al*. (2004b) established a model experiment system in which ZPP is formed by incubation of myoglobin, pork homogenate and antibiotics in the absence of oxygen. However, effect of myoglobin added to the model system on the ZPP formation is suspicious, and it is therefore necessary to establish a simpler model system without addition of myoglobin. Studies of ZPP in Parma ham will be useful for manufacturing meat products being desirable color without addition of either nitrite or nitrate.

Objectives

The objectives of this study were to establish a simpler model system in which ZPP is formed without addition of myoglobin and to obtain a clue for elucidating the mechanism of ZPP formation by using the established model.

Methodology

Model experiment: About 20 g of pork loin was homogenized with 30 ml distilled water at 10,000 rpm for 1 min. Model solutions consisted of pork homogenates (final meat concentration of 20%) and antibiotics (final concentrations of 100 units/ml for penicillin G potassium, 0.1 mg/ml for streptomycin sulfate and 0.05 mg/ml for gentamicin sulfate). The solutions were put into gas-impermeable bags and incubated for

5 days at 25 anaerobically. An anaerobic condition was obtained by using an oxygen absorber (A-500HS, I.S.O. Inc., Yokohama, Japan).

Experiments using the model: In experiments on the effect of temperature, model solutions were incubated at 4, 15, 25 and 37 . In experiments on the effect of ion intensity, ion intensities of solutions were adjusted to 0 – 1 M by using sodium chloride. In experiments on the effect of pH, pH values of solutions were adjusted to 3 – 8 by using hydrochloric acid and sodium hydroxide. In experiments on the effect of pork concentration, pork concentrations of solutions were adjusted to 0 – 50% by adding pork homogenate.

Extraction of ZPP: Incubated model solutions (each 1.5 g) were mixed well with 4.5 ml of ice-cold acetone , and the mixtures were held on ice 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 by using a Model RF-53000PC fluorescence spectrophotometer (Shimadzu Co., Kyoto, Japan).

Quantitative analysis of ZPP and heme in the model and Parma ham: Parma ham (10 g) was homogenized with 40 ml distilled water at 10,000 rpm for 1 min. Model solutions (each 1.5 g) (before and after incubation) or Parma ham homogenates (each 1.5 g) were mixed well with 4.5 ml of ice-cold acetone. The mixtures were placed on ice for 30 minutes and were centrifuged (3,000 rpm, 10 min, 4). The supernatants were diluted up to 50 ml with 75% acetone, and fluorescence intensity (Ex/Em: 420/590 nm) was analyzed by using a fluorescent spectrophotometer. The pellets were mixed with hydrochloric acid (final percentage of 0.7%) and acetone (final percentage of 75%). The mixtures were placed on ice for 30 minutes and were centrifuged (3,000 rpm, 10 min, 4). The supernatants were diluted up to 50 ml with 0.7% hydrochloric acid-75% acetone, and absorbance at 383 nm was analyzed by using a Model U-3310 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The concentrations of ZPP and heme were calculated using a standard curve from a standard reagent.

Results & Discussion

Model solutions consisting of only pork homogenate and antibiotics were incubated for 5 days at 25 anaerobically, and the fluorescence intensity (Ex/Em: 420/590 nm) of 75% acetone extracts of model solutions showed that the amount of ZPP was considerably increased after incubation (Fig. 1). This result indicated that ZPP was formed from pork in the absence of myoglobin and microorganisms. Next, we examined the effects of temperature, ion intensity, pH and pork concentration on ZPP formation (Fig. 2). The amount of ZPP increased with increase in incubation temperature. The amount of ZPP showed a maximum at ion intensity of 0.05 M and thereafter decreased. The amount of ZPP showed a maximum at around pH 5.5 and increased with increase in pork concentration. These results indicate that ZPP formation depends on each condition, suggesting that components of pork are involved in ZPP formation. Next, we carried out quantitative analysis of ZPP and heme in the model solution and Parma ham. The concentration of ZPP was analyzed by measuring fluorescence intensity (Ex/Em: 420/590 nm) of 75% acetone extracts, and the concentration of heme was analyzed by measuring absorbance at 383 nm of 0.7% hydrochloric acid-75% acetone extracts. As shown in Table 1, the proportions of ZPP were 7.9% in the model solution after incubation and

6.9% in Parma ham. Thus, the percentage of ZPP formed in the model solution is almost the same as that in Parma ham. This suggests that the model system is useful for determining the mechanism by which ZPP is formed. Next, we carried out quantitative analysis in the model solution before and after incubation. As shown in Fig. 3, the amount of ZPP increased after incubation. Additionally, since the total amount of ZPP and heme increased after incubation, it was speculated that ZPP and heme were newly formed during incubation. ZPP and heme in Parma ham may also be newly formed during processing. Elucidation of the mechanism by which ZPP is formed in Parma ham awaits further studies.

Conclusions

We established a simple model system in which ZPP is formed by incubation of pork and antibiotics anaerobically. ZPP formation depended on various conditions, suggesting that components of pork are involved in ZPP formation. The results also indicated the possibility that ZPP and heme were newly formed during incubation. ZPP equal in proportion to that in Parma ham was formed only from pork in the model established without microorganisms, this model system will be useful for determining the mechanism by which ZPP is formed in Parma ham.

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Tables and Figures

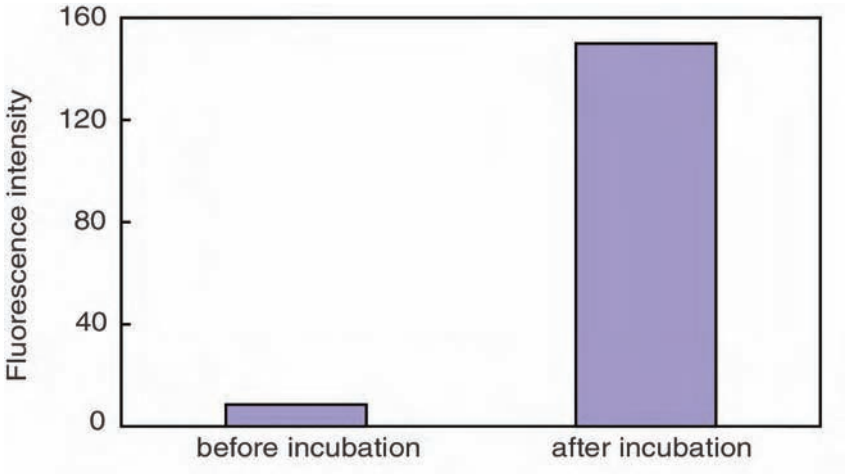

Fig. 1. Fluorescence intensity of ZPP in model solutions before and after incubation (Ex/Em: 420/590 nm).

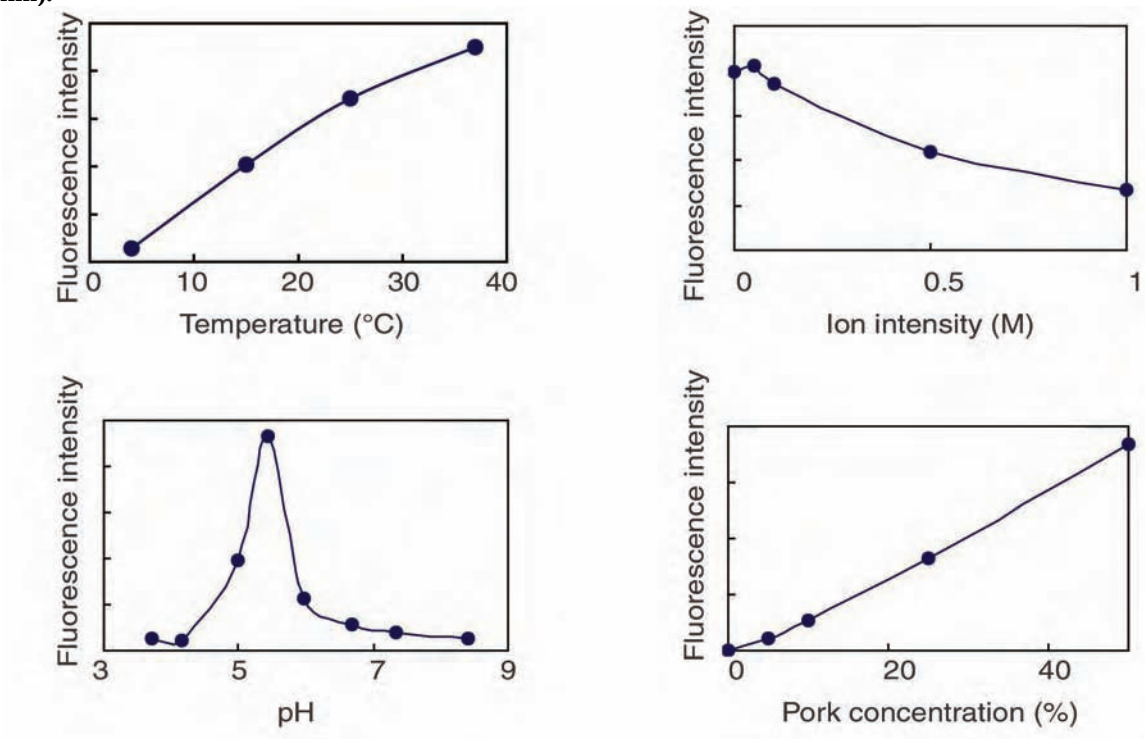

Fig. 2. Effects of incubation temperature, ion intensity, pH and pork concentration onthe formation of ZPP (Ex/Em: 420/590 nm).

Table 1. Concentrations and percentages of ZPP and heme in the model solution after incubation and in Parma ham.

	ZPP	Heme
	(mmol/g)	
Model solution	5.5 ± 0.1	64.7 ± 1.2
	(7.9%)	(92.1%)
Parma ham	10.5 ± 0.2	142.7 ± 1.8
	(6.9%)	(93.1%)

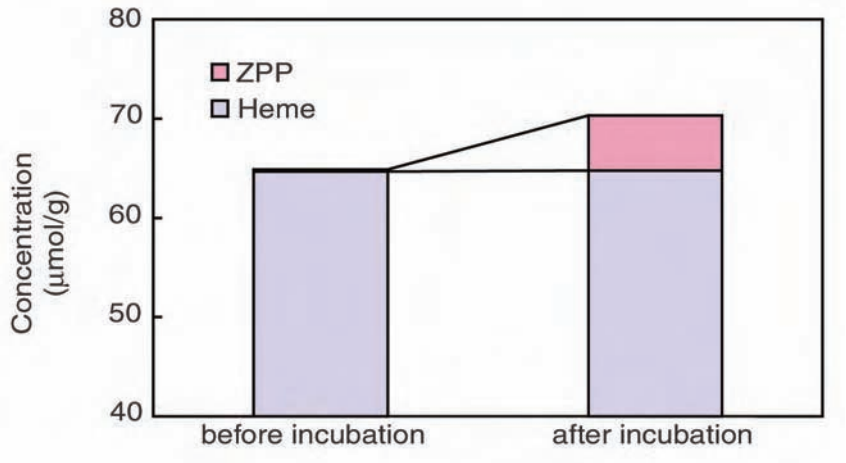

Fig. 3. Concentrationsof ZPP and heme in the model solution before and after incubation.