51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

LOCALIZATION OF ZINC PROTOPORPHYRIN IX (ZPP) IN PARMA HAM

Wakamatsu*, J., Nishimura T., and Hattori, A.

Division of Bioresource and Product Science, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan

Key Words: Parma ham, Color, Zn protoporphyrin IX, Localization, Autofluorescence

Introduction

The color of traditional dry-cured Parma ham is desirable red despite the fact that nitrite or nitrate is not added. It has been reported that an unidentified myoglobin derivative, which is extractable by 75% acetone, is present in Parma ham (Morita et al., 1996). We have recently shown that the red pigment is Zn protoporphyrin IX (ZPP), in which iron in heme is substituted by zinc (Wakamatsu et al., 2004a). It has been reported that the red pigment is formed by staphylococci (Morita et al., 1996), but the existence of microorganisms inside the products is suspected. We have established a model system in which ZPP is formed by anaerobic incubation of meat and myoglobin in the absence of microorganisms (Wakamatsu et al., 2004b). The results suggested that endogenous enzymes contribute to the formation of ZPP. However, the mechanism of formation and the localization of ZPP in Parma ham have not been elucidated.

Objectives

The objective of this study was to determine the localization of ZPP in Parma ham by observing autofluorescence by near-UV light irradiation in order to clarify the mechanism of ZPP formation.

Methodology

Materials. Whole Parma hams (deboned) were purchased from f.lli Galloni s.p.a..

Purple LED lighting. Five purple LEDs (Peak wavelength: 400 nm, OSSV5111A, OptoSupply) were connected in series at intervals of 10.16 mm, and nine LED series were connected in parallel. The current was regulated by two current regulative diodes (10 mA CRD) in parallel.

Measurement of autofluorescence spectra of Parma ham. Parma ham was cut by a slicer into slices of approximately 2 mm in thickness. The autofluorescence spectra of the lean meat and the subcutaneous fat tissue of Parma ham (approximately 1.5 cm square) were measured by using a spectrofluorophotometer (RF-5300C, Shimadzu Corp.). The excitation wavelength was 400 nm, and fluorescence spectra from 450 nm to 750 nm were analyzed.

Detection of ZPP in Parma ham by purple LED lighting. The slices of Parma hams (approximately 2 mm in thickness) were irradiated by purple LED lighting from two

directions and were photographed by using a digital camera (D70, Nikon Corp.) in a darkroom. The colors of the images were divided in RGB colors by using Adobe Photoshop 6.0 (Adobe Systems Inc.). Green (G) and blue (B) were deleted. The red emission was regarded as localization of ZPP.

Detection of ZPP in Parma ham by fluorescence microscopic observation. Parma ham was cut on a cryostat microtome (CM3000, Leica Microsystems) at -20˚C. The frozen sections (10 μm in thickness) were mounted on slides and thawed at room temperature. The specimens were embedded in a mountant (Aqua-Poly/Mount, Plysciences, Inc.). The embedded specimens were viewed with a fluorescence microscope (BX50-FLA, Olympus Corp.). Using an excitation filter of 400-440 nm, a 565-nm dichroic mirror and a 580-nm barrier filter, the specimens were viewed at room temperature.

Results & Discussion

The autofluorescence spectra of Parma ham are shown in Fig. 1. Emission peaks of lean meat were detected at 473, 593 and 632 nm, and those of subcutaneous fat tissue were detected at 467, 584, 633 and 699 nm. Since the emissions at approximately 590 and 630 nm were consistent with that of ZPP and emission peaks of the autofluorescence spectra of the residue extracted from Parma ham by 75% acetone had disappeared (data not shown), the emission peaks were caused by ZPP. It was shown that ZPP existed in both lean meat and subcutaneous fat tissue. ZPP tended to be more abundant in subcutaneous fat tissue than in lean meat. As shown in Fig. 1, red emission other than that of ZPP was hardly detected. Therefore, it appears that the location of ZPP agreed with that of the red emission.

A sample of Parma ham was cut out from the portion shown by the arrow in Fig. 2A. A cross section of the sample is shown in Fig. 2B. The red emission of Parma ham irradiated by purple LED, that is, the location of ZPP, is shown in Fig. 2B. Although ZPP was distributed widely in Parma ham, it was more abundant in the intermuscular fat and subcutaneous fat than in the lean meat, in agreement with the results shown in Fig. 1. ZPP in the lean meat tended to be more abundant in the inner region than in the outer region, and ZPP in the subcutaneous fat also tended to be more abundant in the inner region than in the outer region. Since the Parma ham pigment, i.e., ZPP, is lipophylic (Møller *et al*., 2003), ZPP might be transferred from lean meat to fat tissue during the processing, resulting in the small amount of ZPP in lean meat adjacent to subcutaneous fat. Further investigation on the transference of ZPP is needed. On the other hand, the intensity of red emission was weak in the superficial portions of semimembranosus (SM) and adductor (AD) muscles; these portions were the cutting plane and were exposed during the processing of ham. Since ZPP was formed under anaerobic conditions in a model system (Wakamatsu *et al*., 2004b), oxygen might inhibit the formation of ZPP. The location of ZPP in other cross-sections was similar to the above-described results (data not shown).

ZPP was also detected by fluorescence microscopy as shown in Fig. 3. Although emission of ZPP was observed within muscle fibers, strong emission in the perimysium was not observed. Even in an enlarged image, ZPP was found to be localized within muscle fibers.

Conclusions

Red autofluorescence of Parma ham induced by near-UV light irradiation was found to be derived mainly from ZPP. Localization of ZPP in Parma ham was clarified by using purple LED lighting and the image processing. Although ZPP was distributed widely in the interior of Parma ham, it was more abundant in fat tissue than in lean meat. It was thought that ZPP transferred from lean meat to fat tissue during the processing. The cutting plane had only a small amount of ZPP. Exposure to oxygen might have inhibited the formation of ZPP in that portion. By microscopic examination, fluorescence of ZPP was observed within muscle fibers, whereas a strong positive reaction in the perimysium was not seen.

References

Møller J.K.S., Adamsen C.E., and Skibsted L.H. (2003) Spectral characterization of red pigment in Italian-type dry-cured ham. Increasing lipophilicity during processing and maturation, European Food Research and Technology, 216, 290–296.

Morita H., Niu J., Sakata R. and Nagata Y. (1996) Red pigment of Parma ham and bacterial influence on its formation, Journal of Food Science, 61, 1021–1023.

Wakamatsu J., Nishimura T. and Hattori A. (2004a) A Zn-porphyrin complex contributes to bright red color in Parma ham, Meat Science, 67, 95–100.

Wakamatsu J., Okui J., Ikeda Y., Nishimura T. and Hattori A. (2004b) Establishment of a model experiment system to elucidate the mechanism by which Zn-protoporphyrin IX is formed in nitrite-free dry-cured ham, Meat Science, 68, 313–317.

Tables and Figures

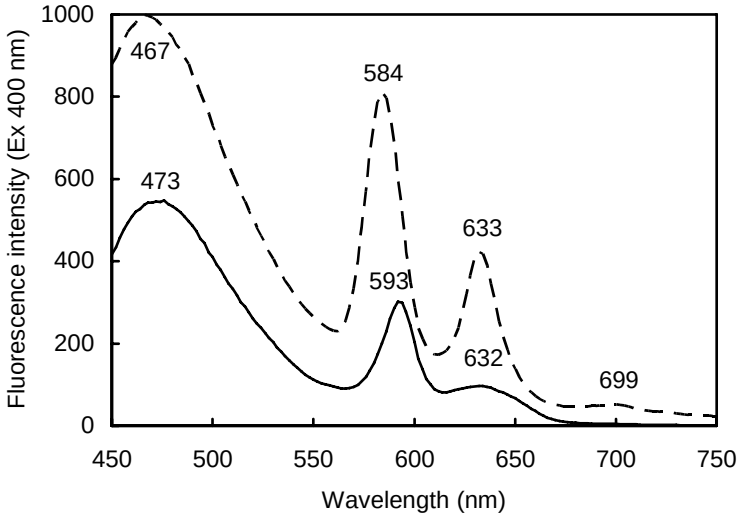

Fig. 1. Autofluorescence spectra (excitation: 400 nm) of lean meat (solid line) and subcutaneous fat tissue (broken line) in Parma ham.

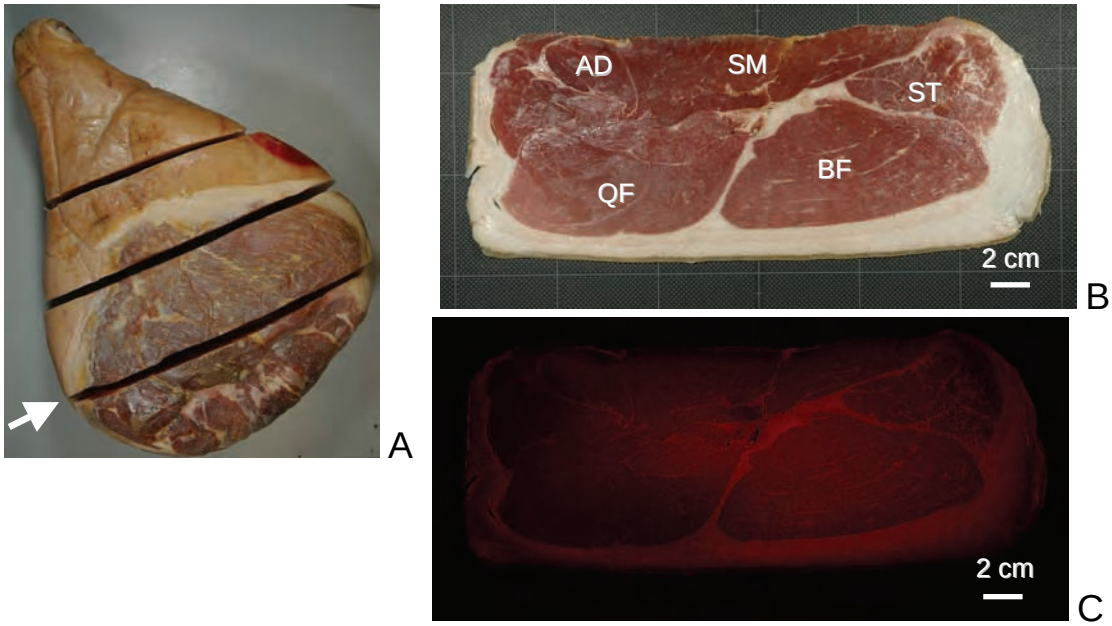

Fig. 2. Sampling from Parma ham (cutting line (A, arrow) and cross section (B)) and localization of ZPP detected by the irradiation using purple LED lighting (C). *Muscles*: AD, adductor; SM, semimembranosus; ST, semitendinosus; QF, quadriceps femoris; BF, biceps femoris.

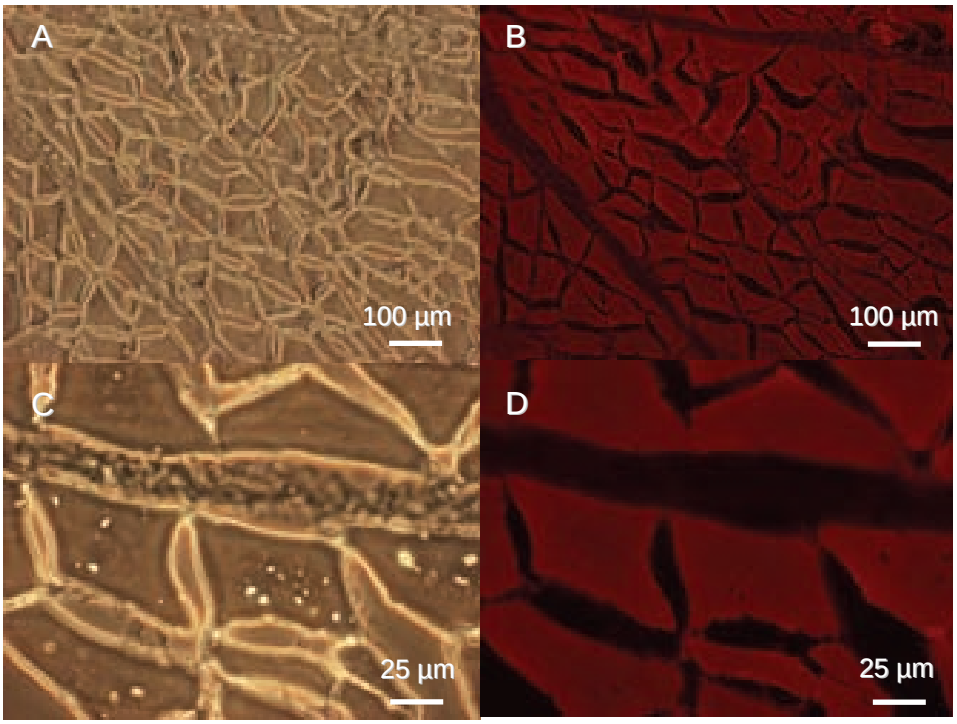

Fig. 3. Detection of ZPP by fluorescent microscopic observation. Phase-contrast views (A and C) and fluorescence views of emission by ZPP (B and D).