ZINC PROTOPORPHYRIN IX CONTRIBUTES TO THE BRIGHT RED COLOR IN PARMA HAM

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Background

Myoglobin in meat products to which nitrate and/or nitrite have been added is converted into stable red nitrosylmyoglobin coordinated to nitric oxide, and nitrosylmyoglobin changes into pink-reddish nitrosylhemochromogen after the meat has been heated. The north Italian traditional dry-cured ham "Prosciutto di Parma (Parma ham)" is made from only the leg of a fattened pig by salting with sea salt, drying, and maturing over a period of one year. Despite the fact that nitrite or nitrate has not been added, the color 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 heme pigment was easily extracted with 75% acetone and that it was a new myoglobin derivative unknown in meat and meat products. It has recently been reported that the amount of this lipophylic stable red pigment in Parma ham increases with aging (Parolari et al., 2003).

Objectives

The objective of this study was to identify the stable red pigment in Parma ham in order to obtain information for producing bright red meat products without nitrite and/or nitrate.

Materials and methods

Preparation of the red pigment from Parma ham: Minced Parma ham (5 g) was homogenized in 20 ml distilled water, and the homogenate was centrifuged (3,000 rpm, 5 min, 4 °C) and then filtered through a filter paper (No. 5C Toyo Roshi Co., Ltd., Tokyo, Japan). Three volumes of ice-cooled acetone were added to the filtrate, and the mixture was placed in ice for 15 minutes. The mixture was centrifuged at 3,000 rpm for 5 min at 4 °C. An equal amount of distilled water was added to the supernatant, and the mixture was applied to a disposable C18 column, Sep-Pak® Vac C18 Cartridge (12 cc/ 2g; Waters Co., MA U.S.A.) prewashed with 15 ml of methanol and 15 ml of distilled water. The column was washed with 25 ml 37.5% acetone, and then the red pigment preparation was eluted with 10 ml of 75% acetone. All of the operations were carried out under shading as much as possible.

Absorption and fluorescent analysis: The absorbance spectrum of the red pigment preparation was measured from 350 to 700 nm using a Model U-3210 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The fluorescent and excitation spectra of the prepared red pigment were measured from 500 to 700 nm at 420 nm for excitation and from 300 to 500 nm at 590 nm for emission using a Model 650-60 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan), respectively.

Elemental analysis: Elemental analysis of the red pigment preparation was performed by scanning electron microscopy/energy dispersive X-ray microanalysis (SEM-EDX). The red pigment preparation from Parma ham was dried up using a centrifugal evaporator and was fixed on a metal stub using carbon tape. The sample was coated with carbon and was analyzed with a scanning electron microscope (S-800, Hitachi Ltd., Tokyo, Japan) equipped with an energy dispersive X-ray micro-analyzer (EMAX-2000, Horiba Ltd., Kyoto, Japan) with an accelerating voltage of 20 KeV and a spectral resolution of 10 KeV per channel.

Mass spectrometry analysis: Electrospray ionization high resolution mass spectrometry (ESI-HR-MS) analysis of the red pigment preparation from Parma ham was carried out using a JMS-SX120A (JEOL Ltd., Tokyo, Japan) equipped with an ESI ion source (JEOL MS-ESI 10, JEOL Ltd., Tokyo, Japan). The sample diluted in a chloroform/methanol/acetone (1:1:8 v/v) solvent mixture was infused into the ESI ion source at a flow rate of 1 µl/min. The needle voltage and capillary voltage were 2681 V and -1230 V, respectively. The chamber temperature was set to 105 □. A mixture of PEGs was used as an internal standard.

Results and discussion

The red pigment preparation from Parma ham was purplish-red, and the absorbance spectrum is shown in Fig. 1. A peak at 416 nm in the Soret band and peaks at 544 and 583 nm in the beta band were observed. Since this spectrum pattern was consistent with those previously reported (Morita et al., 1996; Parolari et al., 2003), the unidentified pigment in Parma ham could be prepared. Although metal-free porphyrins generally have four peaks in the beta band, the number of peaks in the beta band of metalloporphyrins decreases to two because of improvement in symmetry of the molecular structure. Therefore, the elemental analysis was carried by using a scanning electron microscope equipped with an energy dispersive X-ray micro-analyzer. As shown in Fig. 2, several peaks originating in zinc were observed, but no peak originating in iron was observed. This may indicate that the red pigment in Parma ham is not an iron-porphyrin complex but a zinc-porphyrin complex. A zinc-porphyrin complex emits fluorescence unlike many metalloporphyrins. Hence, the fluorescent and excitation spectra of the prepared red pigment were measured (Fig. 3). An excitation peak at 423 nm and emission peaks at 589 and 630 nm were observed. Since the fluorescent spectrum was similar to that of Zn protoporphyrin IX, we attempted to identify the red pigment prepared from Parma ham by ESI-HR-MS analysis. As shown in Fig. 4, the highest molecular ion peak was detected at m/z 624. Six main peaks were found when the peak region was expanded (Fig. 4, inset). This peak pattern agrees well with that of Zn protoporphyrin IX ($C_{34}H_{32}N_4O_4Zn$) (Fig. 4, inset), because Zn has five isotopes (m/z = 64, 66, 67, 68 and 70) and the isotopic ratio is characteristic. Iron has four isotopes (m/z =54, 56, 57 and 58) and its isotopic ratio is different from that of Zn. The exact mass of the principal molecular ion computed from the internal standard was 624.1711 and differed by only 0.4 milli- mass units from the monoisotopic mass (624.1715) of Zn protoporphyrin IX. On the other hand, peaks originating in Fe protoporphyrin IX (MW 616.49), Mg protoporphyrin IX (MW 584.95) or Cd protoporphyrin IX (MW 673.05) were not observed.

These results indicate that the unidentified red pigment in Parma ham is not an Fe-porphyrin complex but a Zn-porphyrin complex, i.e., Zn protoporphyrin IX. It was speculated that the Zn protoporphyrin IX in Parma ham was formed by substitution of Fe in heme to Zn. In a study on surface autofluorescent changes of Parma ham during processing, it was found that the amount of a fluorescent component that is likely to correspond to Zn protoporphyrin IX increased from raw to salted meat (3 months) and then hardly increased during the maturing process (Møller et al., 2003). Thus, Zn protoporphyrin IX might be formed during the early period of the manufacturing process, not during the maturing period. Further studies are needed to elucidate the mechanism of Fe-Zn substitution that occurs in Parma ham during the manufacturing process.

Conclusions

The red pigment extracted from Parma ham by water and then 75% acetone was crudely purified by solid phase extraction. The red pigment was purplish red and fluorescent metalloporphyrin. Element analysis using an SEM-EDX revealed that the red pigment preparation contained not iron but zinc. By mass analysis, the red pigment was identified to be Zn protoporphyrin IX. Zn protoporphyrin IX in Parma ham might be formed with heme and zinc contained in pork during the early period of the manufacturing process.

Acknowledgements

We thank Mr. Kenji Watanabe and Dr. Eri Fukushi of the GC-MS and NMR laboratory, Graduate school of Agriculture, Hokkaido University for performing the ESI-HR-MS analysis. We are also grateful to Mr. Toshiaki Ito of the electron microscope laboratory, Graduate school of Agriculture, Hokkaido University for performing the SEM-EDX analysis.

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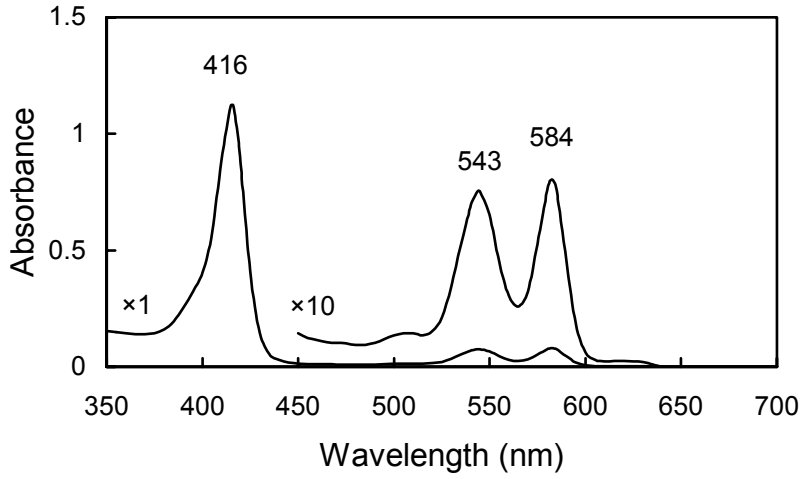

Fig. 1. Absorption spectra of the red pigment preparation from Parma ham. The maximum absorption wavelengths are shown for the preparation. The spectrum from 450 to 700 nm was enlarged 10-fold.

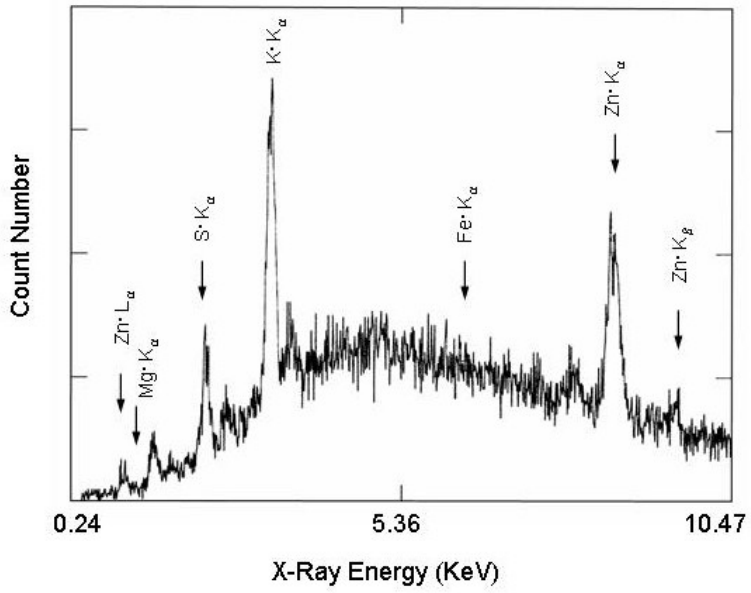

Fig. 2. SEM-EDX X-ray spectrum of the red pigment preparation from Parma ham.

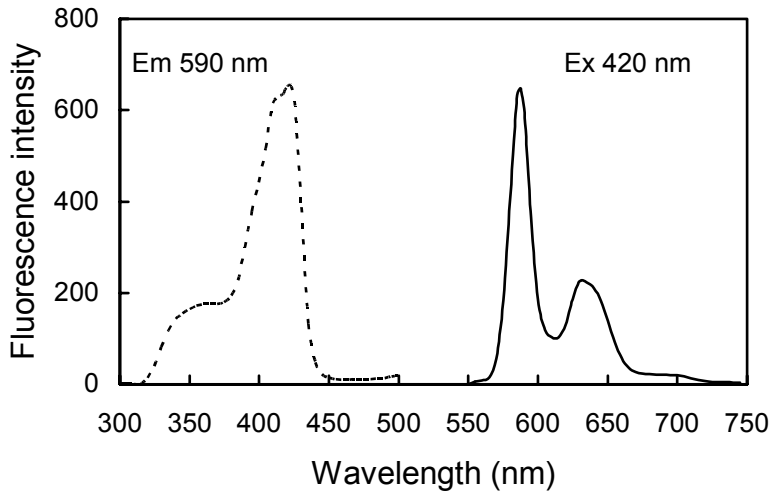

Fig. 3. Fluorescence and fluorescence excitation spectra of the red pigment preparation from Parma ham. Solid line, fluorescence spectrum at 420 nm for excitation; broken line, fluorescence excitation spectra at 590 nm for emission.

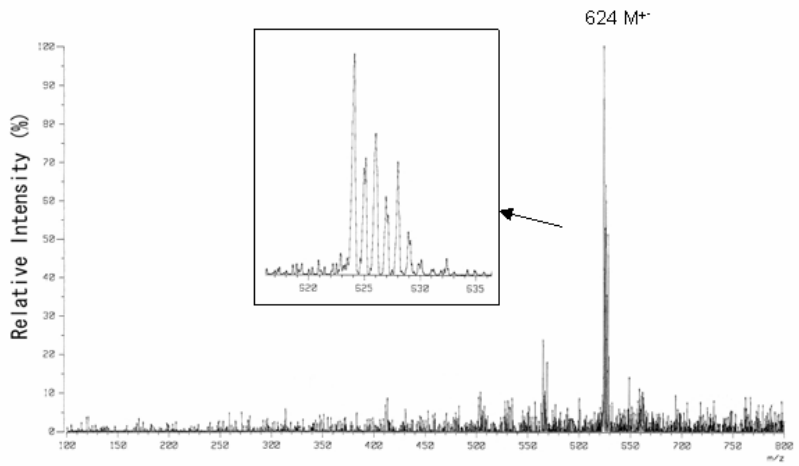

Fig. 4. ESI-HR mass spectrum of the red pigment preparation from Parma ham and magnification (inset).