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1 Short communication

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3 Direct demonstration of the presence of zinc in the acetone-extractable red pigment
4 from Parma ham

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24 Abstract

25

26 We studied the presence of zinc in the red pigment extracted from Parma ham by
27 scanning electron microscopy/energy dispersive X-ray microanalysis. The large peak of
28 about 8.6 KeV and the small peaks at about 1.1 KeV and 9.5 KeV were derived from K_{α} ,
29 $L_{\alpha 1}$ and K_{β} X-ray of zinc, respectively. Thus, the results suggested that zinc existed in
30 the red pigment extracted from Parma ham. In contrast, the K_{α} X-ray peak of iron (6.4
31 KeV) was not detected and the K_{α} X-ray peak of magnesium (1.3 KeV) was hardly
32 detected. These results revealed that the red pigment contained not iron but zinc. In
33 addition to the results of mass analysis in previous studies, the red pigment was
34 demonstrated to be zinc protoporphyrin IX.

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37 Keywords: Parma ham; zinc; zinc protoporphyrin IX; scanning electron
38 microscopy/energy dispersive X-ray microanalysis

39 1. Introduction

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41 Parma ham, Italian traditional dry-cured ham, is manufactured without the
42 addition of a curing agent such as nitrite or nitrate and the colour is not brought
43 about by nitrosylmyoglobin. Wakamatsu, Nishimura and Hattori (2004)
44 reported that the fluorescent red pigment extracted with 75% acetone from Parma
45 ham was identified as zinc protoporphyrin IX by its fluorescent property and
46 chromatographic behaviour and by results of electrical ion spray mass (ESI-MS)
47 analysis.

48 It has also been shown in model systems that anaerobic conditions are suitable
49 for the formation of zinc protoporphyrin IX and that endogenous enzymes as well
50 as microorganisms may be involved in the formation of zinc protoporphyrin IX
51 (Wakamatsu, Okui, Ikeda, Nishimura, & Hattori, 2004). The distribution of zinc
52 protoporphyrin IX in Parma ham was shown by using autofluorescence
53 (Wakamatsu, Odagiri, Nishimura, & Hattori, 2006). Adamsen, Møller, Laursen,
54 Olsen & Skibsted (2006) found by fluorescent analysis that the red pigment was
55 present not only in Parma ham but also in Iberian ham but that the pigment
56 content in meat products cured with nitrite was very low. The content of zinc
57 protoporphyrin IX increases throughout the processing and maturation of Parma
58 ham (Adamsen, Møller, Parolari, Gabba & Skibsted, 2006). Møller, Adamsen,
59 Catharino, Skibsted, & Eberlin (2007) recently showed by ESI-MS and TOF-MS
60 analysis that zinc protoporphyrin IX was present not only in Parma ham but also
61 in Iberian ham.

62 Although these results obtained by mass spectrum analysis are consistent
63 with zinc protoporphyrin IX, the presence of zinc in the red pigment has not been
64 demonstrated directly (Wakamatsu, Nishimura and Hattori, 2004; Møller,
65 Adamsen, Catharino, Skibsted, & Eberlin, 2007). In this study, we demonstrated
66 the presence of zinc in the red pigment extracted and purified from Parma ham by
67 scanning electron microscopy/energy dispersive X-ray microanalysis (SEM-EDX).

68 2. Materials and methods

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70 2.1. Materials and chemicals

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72 Parma ham was purchased from f.lli Galloni s.p.a. (Italy). Acetone was
73 purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

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75 2.2. Extraction and purification of Parma ham pigment

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77 The extraction and purification of the pigment were carried out according to
78 the method described by Wakamatsu, Nishimura & Hattori (2004). Minced
79 Parma ham was homogenized in distilled water. After centrifugation, the
80 supernatant was filtered through a filter paper. Three vol. of cold acetone was
81 added to the filtrate and kept on ice for 15 min. After centrifugation, the
82 supernatant was diluted 1:1 with distilled water. The mixture was applied to a
83 disposable C18 column, Sep-Pak® Vac C18 Cartridge (12 cc/ 2g; Waters Co., MA
84 U.S.A.) prewashed with methanol and distilled water. The column was washed
85 with 37.5% acetone, and then the red pigment preparation was eluted with 75%
86 acetone.

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88 2.3. Elemental analysis by scanning electron microscopy/energy dispersive X-ray
89 microanalysis (SEM-EDX)

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91 Elemental analysis of the red pigment preparation was performed by scanning
92 electron microscopy/energy dispersive X-ray microanalysis (SEM-EDX). The red
93 pigment preparation from Parma ham was dried up using a centrifugal
94 evaporator (CVE-2000, Tokyo Rikakiki Co., Ltd., Tokyo, Japan) and was fixed on
95 an aluminium stub using carbon tape. The sample was coated with carbon and
96 was analyzed with a scanning electron microscope (S-800, Hitachi Ltd., Tokyo,
97 Japan) equipped with an energy dispersive X-ray micro-analyzer (EMAX-2000,
98 Horiba Ltd., Kyoto, Japan). The sample was observed with an accelerating
99 voltage of 20 KeV and a spectral resolution of 10 KeV per channel during 100 sec.

100 3. Results and discussion

101

102 We examined the presence of zinc in the red pigment preparation from Parma
103 ham by using SEM-EDX analysis. SEM-EDX analysis makes it possible to carry
104 out elemental analysis of a selected infinitesimal area under SEM observation.
105 The spectrophotometric and fluorescent behaviours of the red pigment
106 preparation were the same as those previously reported (Wakamatsu, Nishimura
107 and Hattori, 2004; Møller, Adamsen, Catharino, Skibsted, & Eberlin, 2007) (data
108 not shown). An SEM image of the red pigment preparation and the fluorescent
109 X-ray spectrum of the square area are shown in Fig. 1. SEM-EDX analysis of the
110 square area in the SEM image was carried out (Fig. 1A). Zinc has several energy
111 values of fluorescent X-ray, and the main energy values are 8.630 KeV of K_{α} , 9.570
112 KeV of K_{β} , 1.012 KeV of $L_{\alpha 1}$ and 1.034 KeV of $L_{\beta 2}$. Large peaks were observed at
113 about 2.3, 3.3 and 8.6 KeV in the fluorescent X-ray spectrum of the red pigment
114 (Fig. 1B). The peak of about 8.6 KeV seems to be derived from K_{α} X-ray of zinc.
115 Furthermore, the small peaks at about 1.1 KeV and 9.5 KeV seem to be derived
116 from $L_{\alpha 1}$ and K_{β} X-ray of zinc, respectively. Thus, the results suggested that zinc
117 existed in the red pigment extracted from Parma ham. In contrast, the K_{α} X-ray
118 peak of iron (6.4 KeV) was not detected and the K_{α} X-ray peak of magnesium (1.3
119 KeV) was hardly detected. This means that the red pigment was neither an iron
120 nor magnesium complex. The peaks at about 3.3 KeV and about 2.3 KeV seem to
121 be derived from K_{α} X-rays of potassium and sulphate, respectively. Both are
122 thought to be contaminations from Parma ham. Accordingly, our results

123 indicated that the red pigment shown as metalloporphyrin spectrophotometrically
124 was a zinc-porphyrin complex.

125 Although the red pigment purified from Parma ham is nearly identical to zinc
126 protoporphyrin IX presupposing the presence of zinc in previous studies using
127 mass spectral analysis (Wakamatsu, Nishimura and Hattori, 2004; Møller,
128 Adamsen, Catharino, Skibsted, & Eberlin, 2007), the presence of zinc in the red
129 pigment has not been demonstrated directly. In this study, the presence of zinc
130 demonstrated that zinc protoporphyrin IX (Fig. 2) exists in traditional dry-cured
131 hams such as Parma ham and Iberian ham without addition of nitrite or nitrate.

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134 4. Conclusions

135 SEM-EDX analysis revealed that the red pigment of Parma ham purified by
136 solid-phase extraction contained not iron but zinc. The results of this study
137 together with the results of mass spectral analysis in previous studies show that
138 the red pigment is Zn protoporphyrin IX.

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159 Figure legends

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161

162 Fig. 1. SEM image (A) and SEM-EDX X-ray spectrum (B) of the red pigment
163 preparation from Parma ham. SEM-EDX analysis of the square area of the SEM
164 image was carried out. The sample was observed with an accelerating voltage of
165 20 KeV and a spectral resolution of 10 KeV per channel during 100 sec.

166

167 Fig. 2. Structure of zinc protoporphyrin IX.

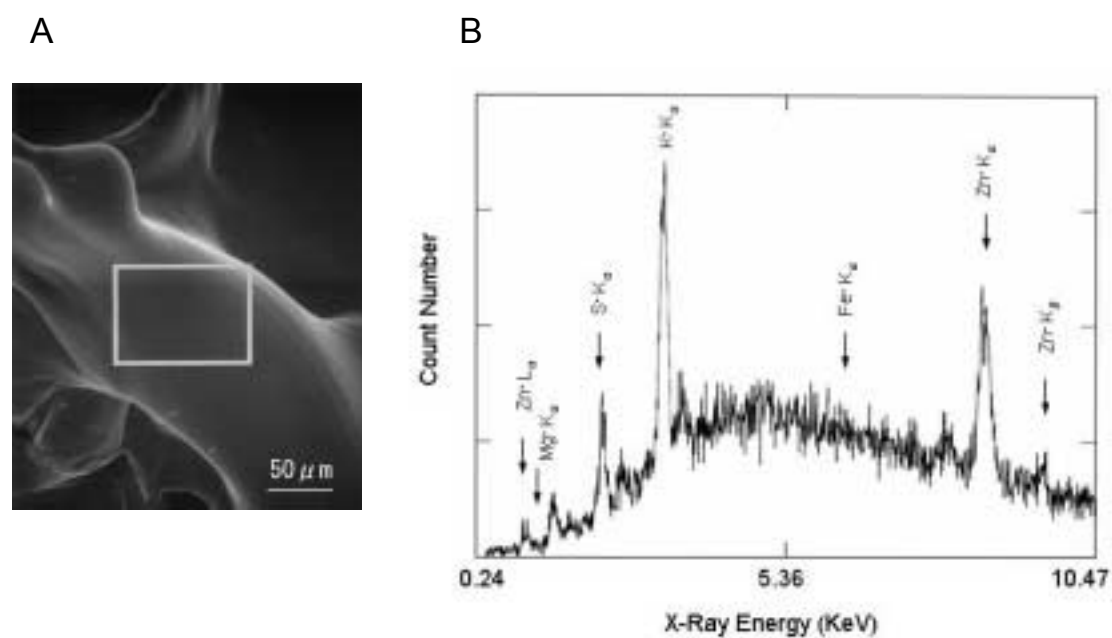


Fig. 1. SEM image (A) and SEM-EDX X-ray spectrum (B) of the red pigment preparation from Parma ham. SEM-EDX analysis of the square area of the SEM image was carried out. The sample was observed with an accelerating voltage of 20 KeV and a spectral resolution of 10 KeV per channel during 100 sec.

Figure 2

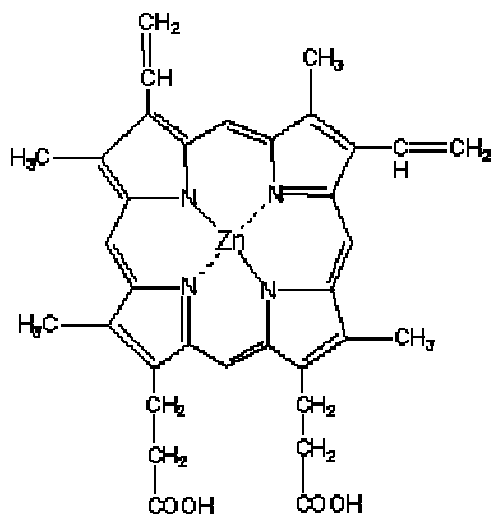


Fig. 2 Structure of zinc protoporphyrin IX