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Red Myoglobin Derivative Formed in Parma Ham Processing without Nitrite or Nitrate Addition

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Background

Parma ham is a traditional meat product of Parma, Italy. The characteristic red pigment of cured meat products is usually produced through the addition of nitrite and/or nitrate as a curing ingredient. Parma ham, however, assumes a stable crimson (red) color even without such treatment. The reason for this has yet to be clearly determined. Lactobacilli, pediococci and staphylococci are available on the market as starters of fermented sausage, and their positive effects on color formation have been demonstrated and explained as due to nitrate reduction or decline in pH. But there is no report maintaining or proving that they are directly the cause for the reddening of meat products. The conversion of brown metmyoglobin (MetMb) to red myoglobin (Mb) derivatives by bacteria in medium[1-4] and in meat[3] has been reported. The physico-chemical properties of red pigment formed in Parma ham and contribution of bacteria to reddening, however, are matters yet to be elucidated.

Objective

Red pigment formed in Parma ham processed without nitrite or nitrate was compared with that of cured ham. Bacteria in Parma ham were isolated, assessed for their ability to convert MetMb to red Mb derivatives in bacterial medium and identified. Model fermented sausages were prepared using screened strains and the spectra of the acetone extract were measured. Purified red pigment from Parma ham was analyzed by infra-red (IR) spectroscopy and HPLC.

Methods

Two types of raw ham used in this study : Parma ham possessing crimson color even without nitrite or nitrate treatment, and Tipo Parma meaning Parma-type raw cured ham.

Isolation of bacteria from Parma ham : One gram Parma ham was mixed with 9.0 mℓ sterile saline (0.85 %). One-hundred μℓ of each dilution were plated on MRS agar (Oxoid). Bacteria were isolated from the agar plate following incubation at 30 °C for 5 days.

Assessment of capacity for MetMb conversion to red Mb derivatives : The bacteria were examined for their ability to generate red Mb deivative from MetMb in MRS broth as previously described[2,3]. MRS broth was supplemented with 2.0 mg MetMb per mℓ.

Identification of bacteria isolated from Parma ham : API STAPH test strips (API Analytab Products) and N-ID TEST SP-18 (Nissui Pharmaceutical Co., Ltd.) were used to identify the selected bacteria.

Preparation of sausages : All sausages were prepared from 100 g minced pork containing sodium chloride (3.0 %). Control-1 sausage was treated with sodium nitrite (0.01 %) and sodium ascorbate (0.05 %). To control-2 sausage was added only sodium chloride. All other model sausages were inoculated with screened bacteria (bacterial pellet from 200 mℓ-culture)[4]. Each sample was stuffed in Krehalon film casing (Kureha Chem.) and incubated at 20 °C for 30 days.

The stability of red pigment from Parma ham and Tipo Parma : Vacuum-packed sliced samples (7 mm in thickness) of Parma ham and Tipo Parma were heated to 75 °C for 15 min. Water- and acetone-extracts from Parma ham and Tipo Parma were kept in light (250 lux) or darkness at 4 or 20 °C, respectively, for discoloration tests.

Purification of red Mb derivative[5] : From each sample (40 g), red Mb derivative was extracted using 75 % acetone method[6]. The acetone was evaporated, leaving approximately 40 mℓ of aqueous suspension. This suspension was extracted with two 40-mℓ portions of ethyl acetate and then the ethyl acetate was removed, leaving a red oil. The oil was washed four times with 10-mℓ portions of toluene. The block solid thus produced was collected by centrifugation, dissolved in dimethyl sulfoxide (DMSO) and used as the purified Mb derivative from the samples.

Spectral analysis : Spectral scans of water extracts from Parma ham, Tipo Parma and model fermented sausages were obtained using a Hitachi 228 spectrophotometer. Visible absorption spectra of 75 % acetone extracts from Parma ham, Tipo Parma and model sausages were measured by the method of Okayama and Nagata[6]. Spectral analysis of cultures, whose color was noted change from brown to red, was conducted as previously described by Morita et al.[3]. IR spectra from 4,000 to 600 cm^{-1} were recorded on a IR-810 spectrometer (Japan Spectroscopic Co., Ltd.). Each purified Mb derivative solution in the amount of several drops was placed on a potassium bromide plate to produce a solid state film for IR analysis.

High-performance liquid chromatography (HPLC) : Purified red pigments were fractionated with HPLC (Nihonbunko 880-PU) on a column (Silica 60G, 4.6 mm I.D.× 250 mm). Elution was conducted with ethyl acetate-carbon tetrachloride-DMSO (5:3:2, v/v) at a rate of 0.5 mℓ/min and absorbance was read at 417 nm.

Results and discussion

Analysis of water- and acetone-soluble pigments in Parma ham and Tipo Parma : Red pigment in Parma ham was water-soluble but not completely extractable from the sample with water. From Tipo Parma, red pigment could barely be extracted with water, according to Sakata and Nagata[7], nitrosoheme pigment formed in cured meat is generally difficult to extract with water in spite of its high solubility. Red pigment present in both of the samples could quite easily be extracted with 75 % acetone. The visible spectral pattern for the acetone-extracted pigment in Parma ham showed absorption maxima at 417, 546 and 584 nm[3]. The acetone extract from Tipo Parma could be detected at four absorption peaks, 395, 476, 535 and 563 nm, this being the typical pattern of MbNO [8,9]. Spectral patterns of the acetone extracts from Parma ham and Tipo Parma differed significantly, as shown in Fig. 1. It thus follows the red pigment in Parma ham differs from reduced Mb, MbO_2, MetMb and MbNO, all Mb derivatives present in meat and meat products.

Isolation of bacteria from Parma ham and assessment of bacterial capacity for MetMb conversion to red Mb derivatives : Bacterial counts of Parma ham ranged from $10^4 \sim 10^5$ CFU/g in six trials. For assessment of ability to generate red Mb derivatives, 471 isolates were examined. Most of the isolates turned from brown to red, and the ten isolates (SH-1 ～SH-10) with the most pronounced color conversion were selected.

Identification of selected bacteria : The selected bacteria SH-1～SH-10 would be identified as Staphylococcus epidermidis, Staphylococcus warneri and Staphylococcus lentus.

Analysis of the acetone extracts of pigment from model fermented sausages : Model sausages SH-1 and SH-10 were prepared by inoculation with S. lentus SH-1 or SH-10, respectively. In control-1, MbNO was formed with a bacterial count of 10^6 CFU/g. In control-2, the bacterial count was 10^6 CFU/g and the spectral pattern of the acetone extract showed absorption maxima at 417, 546 and 584 nm. These findings are consistent with the previous study[3], in which bacteria derived from pork generated the red Mb derivative and peaks appeared at 417, 546 and 584 nm. The model sausages SH-1 and SH-10 took on a more desirable red color than control-2. Each acetone extract from the two samples showed essentially the same spectral pattern as Parma ham. Bacterial counts were $10^5 \sim 10^8$ CFU/g for all the sausages.

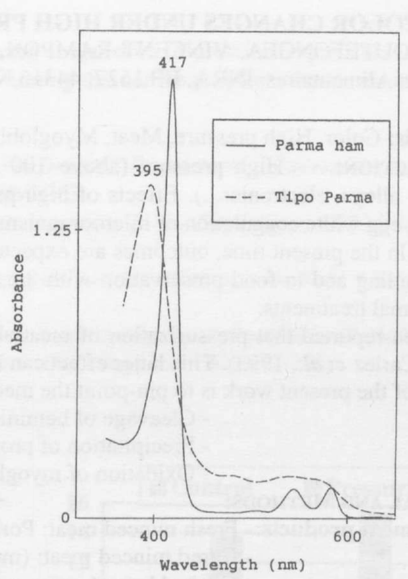

Fig. 1. Absorption spectra of heme pigments extracted with 75 % acetone from Parma ham and Tipo Parma.

Stability of red pigments from Parma ham and Tipo Parma : Both red pigments formed in Parma ham and Tipo Parma were stable toward heat treatment (75 °C, 15 min). The red pigment in cooked Tipo Parma is nitrosohemochrome. Stability of the pigment in acetone extract from Parma ham was compared with that of Tipo Parma. As shown in Fig. 2, the pigment of Tipo Parma underwent discoloration within 12 hr even if kept in the dark at 4 °C, whereas that of Parma ham continued to remain quite stable for more than 3 weeks under the same conditions. The stable red pigment of Parma ham should prove useful for meat processing and as a food grade red colorant. Developing a stable red color without the need for any color fixing agent in meat products is also important from the standpoint of food hygiene.

IR and HPLC analysis : The IR spectra of nitrosoheme pigment formed in Tipo Parma and control-1 sausage showed a strong band in the nitrosyl stretch region (1,670 cm^{-1}) via IR, as also observed by Killday et al.[5]. The IR spectra of the heme pigments from Parma ham and model sausages SH-1 and SH-10 also had a similar strong stretch near the 1,670-cm^{-1} region. But study should be conducted to determine wether their heme pigments include nitric oxide. By HPLC under the above conditions, three fragments were separated. The main component was present in the second fraction (98 %). The structural characterization of the red Mb derivative formed in Parma ham and model fermented sausages is presently being conducted by NMR and mass-spectral analysis.

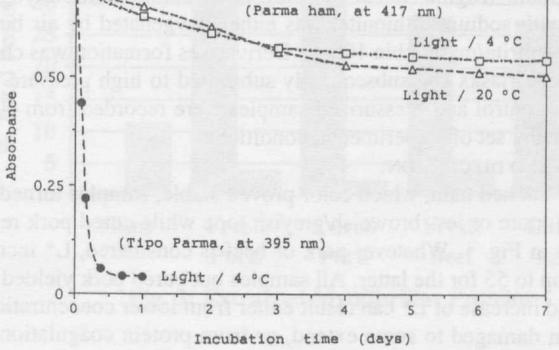

Fig. 2. Stability of red pigments in 75 % acetone extracts from Parma ham and Tipo Parma.

Conclusions

Red pigment formed in Parma ham and model fermented sausages was regarded, based on the present findings, as the same Mb derivative spectrophotometrically. Red color formation of Parma ham may possibly be induced by the action of bacteria (staphylococci). The red color of the acetone extract was maintained stable, thus showing red pigment to be a new Mb derivative, so far unknown in meat and meat products.

Pertinent literature

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