Studies on Physicochemical Characteristics of Red Pigments in Meat Products

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The attractive red color is a prime characteristic in meat products, and it is one of the important qualities with which consumers make their purchase decisions. The color and its intensity depend on the quantity and characteristics of the red pigments in the meat. Color formation in cured meat products involves, basically, reaction of endogenous pigments in muscle, essentially myoglobin (Mb), with nitric oxide (NO) from added nitrite. The nitrosated and typical red pigment, nitrosomyoglobin (NOMb) is called "cured meat pigment", and it is converted to nitrosohemechrome by the cooking process. This latter pigment has a pinkish color, which is known to be stable with cooking. These red pigments exist naturally in cured meat and meat products. The scheme of these reactions of Mb, in color formation, is shown in Fig. 1.

Our previous research was undertaken to determine the effective endogenous factor essential for the color formation in meat products\textsuperscript{20,26,27}, and taken also into consideration to clarify its mode of action\textsuperscript{28,29,31,32}. The endogenous factor was separated from a low molecular weight sarcoplasm (LMS) fraction of porcine skeletal muscle by gel filtration, ion exchange and paper chromatography. The fraction with strong color forming ability was found to have a molecular weight of 200~550 by gel filtration. The neutral subfraction was active in color formation, and separated into 3 ninhydrin spots. It would thus appear that a peptide may be essential for the color formation as a part of the endogenous factor in the LMS fraction. The action of the endogenous factor, especially that of the LMS fraction, to promote color formation appears to occur through acceleration of heat denaturation of heme pigments, causing them to readily undergo nitrosation. Recently, peptides obtained from degradation of both casein, as a main component, and whey protein were shown to have a promoting effect on color formation, when they were used in meat products\textsuperscript{44}. During these studies, NOMb was found to have unique characteristics, which could not be observed in other Mb derivatives. Also we noticed that the NOMb characteristics were not well-known in the meat research field. In this review, highlights of our reports are described and also some related papers of this title are introduced.

1. Water-extractability of native nitrosoheme pigments from cured meat

The red pigments in meat products are generally extracted and then measured spectrophotometrically for analysis. A widely used method for this is Hornsey's acetone procedure\textsuperscript{10}. Okayama and Nagata\textsuperscript{19} showed that cooked cured meat pigments, i. e., nitrosohemochrome, could be extracted with 75% acetone by this procedure with a slight modification.
The absorbance of the extract was measured with much greater sensitivity at 395 nm and expressed as color forming ability (CFA). In our first research, an examination was made of the following: 1) extractability with 75% acetone of NOMb prepared using myoglobin, 2) extractability of native nitrosoheme pigment (NOHP) from commercial raw ham and cured meat with water, using 75% acetone, and 3) the effects of endogenous factors on the extractability.

NOMb was prepared from a reaction medium containing Mb, NaNO₂ and sodium ascorbate (NaAsc) in a buffer solution (pH 5.5) according to the procedure of Lee and Cassens with a slight modification. The maximum formation of NOMb was determined by monitoring the optical density. The NOMb spectrum with maximal absorbance at 547 and 578 nm was observed during incubation of the reaction medium (Fig. 2) and the absorbance at 547 nm rapidly attained a maximum (Fig. 3). Mb in the reaction medium after 20 min incubation was judged completely nitrosated on the basis of a calculation using the mMolar extinction coefficient of NOMb at 547 nm [13.3 (Fox and Thomson)]. Figure 4 shows the absorption spectra of 75% acetone extracts from the NOMb reaction medium incubated for 60 min and extracted with 75% acetone. No significant difference in the absorption spectra of the 75% acetone extracts could be observed between NOMb and heat denatured NOMb (nitrosohemochrome), and they had the same optical density at 395 nm, one of the absorption maxima in Soret band.

Table 1 shows the extractability of native NOHP...
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Fig. 2. Absorption spectra of the NOMb formed from reaction of Mb with nitrite in the presence of ascorbate during incubation for 0 min (-----) and 20 min (----) at room temperature. The reaction medium contained 0.4 mM Mb, 50 mM NaNO₂ and 50 mM sodium ascorbate in M/35 Veronal-acetate buffer (pH 5.5). Wave scanning was carried out after the solutions were diluted 1:10 with the buffer. (Sakata and Nagata, 1983)23)

Fig. 3. Formation of NOMb from a reaction medium containing Mb, nitrite and ascorbate at pH 5.5. The change with time in absorbance during incubation at room temperature is plotted. At intervals, 1 ml of sample was diluted to 10 ml with buffer solution and the absorbance was swiftly monitored at 547 nm. (Sakata and Nagata, 1983)23)

Fig. 4. Absorption spectra of the 75% acetone extracts from the reaction medium. The reaction medium contained NOMb (-----) or nitrosohemochrome (——). To record the spectra from 350 to 450 nm, each extract was diluted 1:10 with 75% acetone. (Sakata and Nagata, 1983)23)

from commercial raw hams. The NOHP was extracted with water and the degree of extraction was determined using 75% acetone. Since raw hams in practice are processed at lower temperature (<20°C, in our country), most of their heme pigments were considered to be in the native state. However, the extractability of native NOHP was generally low and varied. The reason for the low extractability was considered to be that the denaturation of the heme pigments occurred during meat processing and/or that native heme pigments could not be sufficiently extracted by the present procedure. For confirmation of these possibilities, water extraction was carried out on the porcine meat cured with NaNO₂, NaAsc and NaCl. As evident from Table 2, the extractability of native NOHP was low as was also the case in raw ham (cf. Table 1). No significant difference was observed in extractability between cured meat sample with or without NaCl. Most of the heme pigments could be easily extracted with water from uncured raw meat. However, extracting native NOHP from cured meat was found to be difficult in spite of its high solubility in water. These findings are evidence that the extractability of NOHP is low in raw hams. In Fig. 5, the absorbance of the NOMb
Table 1. Extractability of native NOHP from raw hams

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extracted NOHP (A)</th>
<th>Total NOHP (B)</th>
<th>Extractability (A/B, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.094</td>
<td>0.646</td>
<td>14.6</td>
</tr>
<tr>
<td>2</td>
<td>0.221</td>
<td>0.596</td>
<td>37.1</td>
</tr>
<tr>
<td>3</td>
<td>0.068</td>
<td>0.457</td>
<td>14.9</td>
</tr>
<tr>
<td>4</td>
<td>0.072</td>
<td>0.606</td>
<td>11.9</td>
</tr>
<tr>
<td>5</td>
<td>0.068</td>
<td>0.661</td>
<td>10.3</td>
</tr>
<tr>
<td>6</td>
<td>0.514</td>
<td>0.689</td>
<td>74.6</td>
</tr>
<tr>
<td>7</td>
<td>0.064</td>
<td>0.781</td>
<td>8.2</td>
</tr>
</tbody>
</table>

(Sakata and Nagata, 1986)²⁵³

Table 2. Extractability of native NOHP from cured meats

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extracted NOHP (A)</th>
<th>Total NOHP (B)</th>
<th>Extractability (A/B, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.071</td>
<td>0.306</td>
<td>23.2</td>
</tr>
<tr>
<td>B</td>
<td>0.071</td>
<td>0.302</td>
<td>23.5</td>
</tr>
<tr>
<td>C</td>
<td>0.060</td>
<td>0.430</td>
<td>15.1</td>
</tr>
<tr>
<td>D</td>
<td>0.180</td>
<td>0.496</td>
<td>36.4</td>
</tr>
<tr>
<td>E</td>
<td>0.047</td>
<td>0.184</td>
<td>25.5</td>
</tr>
<tr>
<td>F</td>
<td>0.098</td>
<td>0.442</td>
<td>22.2</td>
</tr>
</tbody>
</table>

Mean ± SD  24.4 ± 6.9

(Sakata and Nagata, 1986)²⁵³

water extract from myofibrils cured at pH 5.5, following treatment with 75% acetone was measured at 395 nm. The total NOMb gradually increased with curing time, but the absorbance of extracted NOMb with water was essentially low.

Bendall and Wismer-Pedersen⁴ reported that myofibrillar proteins in pale, soft and exudative (PSE) porcine muscle were tightly surrounded by denatured sarcoplasmic proteins. Scopes⁴⁵ noted that denatured sarcoplasmic proteins in PSE muscle lowered the extractability of myofibrillar proteins by binding with them. In our previous paper²¹,²⁴, the decline in color formation of PSE muscle may possibly have resulted from an interaction between heme pigments and myofibrils in muscle postmortem under the conditions of low pH and relatively high temperature. These physicochemical characteristics of muscle proteins under PSE conditions are not considered to have any direct relation to the phenomenon observed in cured meat in this paper, since no denaturation occurred. However, it may be assumed that myofibrils react with heme pigments, one class of sarcoplasmic proteins, under certain conditions. Such an interaction may result in virtually no loss of NOHP from cured meat during water soaking in the course of meat processing.

2. Extractability and stability toward an oxidizing agent of myoglobin derivatives

Even though NOMb per se is highly water-soluble, its extractability from meat with water was found to decrease significantly following the formation of the pigment in cured meat, as described above. This may explain why little NOMb dissolves from cured meat during “desalting” (immersion in water to remove excess salt after curing), with the consequent retention of the characteristic and attractive red color of meat products. A study was made to clarify the reason for interactions between pigment and myofibrillar proteins²⁵. There is an indication that NOMb may have specific physicochemical characteristics, in contrast to other Mb derivatives. Thus, to clarify the
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Fig. 6. Reflectance spectra of minced meat with and without K$_3$Fe(CN)$_6$. (Sakata et al., 1996)$^{41}$

![Reflectance spectra of minced meat](image)

Fig. 7. Reflectance spectra of cured meat with and without K$_3$Fe(CN)$_6$. (Sakata et al., 1996)$^{41}$

![Reflectance spectra of cured meat](image)

mechanism for the insolubilization of NOMb formed in meat during curing, the following experiments were carried out using meat and Mb in model systems: 1) extractability with water of Mb derivatives present together with NOMb in cured meat, 2) effects of ferricyanide [K$_3$Fe(CN)$_6$] as an oxidizing agent on heme pigment stability$^{40,41}$.

Figure 6 shows reflectance spectra following treatment of minced meat with and without ferricyanide. The control sample showed the typical spectral pattern of O$_2$Mb, which exhibited reflectance minima at 545 and 575 nm. Each spectrum changed to that of metmyoglobin (MetMb) on adding K$_3$Fe(CN)$_6$. In the case of cured meat treated with the same concentration of K$_3$Fe(CN)$_6$, the reflectance spectra with ferricyanide showed patterns similar to those shown in Fig. 6 (Fig. 7). Discoloration of the cured meat became evident with the color changing from red to brown-yellow. MetMb content was determined from the scattering coefficient of reflectance at 525 and 575 nm$^{47}$ and Mb was confirmed in all cases to be oxidized in meat treated with ferricyanide, as shown in these Figs. The cured meat was prepared by adding nitrite, NaCl and NaAsc. The color forming ratio (CFR) was determined by the method of the authors$^{22}$, being expressed as % NOMb to total Mb. CFR of cured meat was 62.7%, but only 28.0% for meat from which cured pigments had been extracted with water.


Fig. 8. Absorption spectrum of heme pigments extracted from a residue after washing the K$_3$Fe(CN)$_6$-added cured meat with water. (Sakata et al., 1996)$^{41}$ Heme pigments were extracted with 75% acetone–0.7% HCl. (Sakata and Nagata, 1992)$^{33}$

![Absorption spectrum of heme pigments](image)
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(remaining NOMb kept in the meat residue washed with water). All the Mb derivatives, though not NOMb in the cured meat, could be extracted with water. The resulting meat residue, after washing the K$_3$Fe(CN)$_6$ - treated cured meat, was extracted with 75% acetone - 0.7% HCl to determine total heme pigment$^{33}$. Figure 8 shows the absorption spectrum of heme pigments extracted with acetone-HCl. The spectrum showed the characteristics of heme pigments with a peak at 383 nm of Soret band. The absorbance corresponded to the amount of pigments remaining in the cured meat after being washed with water. These results confirm that the remaining NOMb could not be extracted even when converted to MetMb by the oxidizing agent. NOMb thus appears to react strongly with endogenous muscle components, especially myofibrils, in cured meat, as previously reported$^{18,25}$.

NOMb and O$_2$Mb was prepared in a model solution and K$_3$Fe(CN)$_6$ (0 ~ 0.5%) was added to both. One reaction mixture was incubated at pH 5.5 and the other, at pH 7.0, followed by measurement of absorption spectra. O$_2$Mb formation was determined from its mMolar extinction coefficient at $\lambda_{583}$nm (15.1$^{2}$). In spite of aerobic condition in this experiment, Mb was completely nitrosated, but the oxygenation of Mb did not proceed to 100%. In a 90% solution of O$_2$Mb at each pH, all O$_2$Mb was rapidly oxidized to MetMb by 0.5% ferricyanide (Fig. 9). On the other hand, this treatment failed to oxidize NOMb completely (Fig. 10). These results clearly show that NOMb formation occurs more easily than that of O$_2$Mb, and NOMb is also more stable toward oxidizing agents than O$_2$Mb. Some reports claim that cured meat pigments per se easily show discoloration$^9$ and that the pigments are more unstable than those of cooked cured meat$^{11}$. But this does not agree with our findings. The physicochemical stability of the heme pigments in meat should be accurately assessed in future studies. Myofibrillar action may be related to Mb derivatives, which may possibly be the cause of the insolubility and stability of NOMb in cured meat.

3. Stability of oxymyoglobin and nitrosomyoglobin during freezing

The stability of Mb derivatives during freezing was assessed and compared with a model system containing O$_2$Mb and NOMb$^{42,43}$. The MetMb production ratio (%) in a discolored commercial frozen beef (vacuum-packed) sirloin was based on reflectance spectra$^{40}$ and the buffer extraction method$^{48}$. Sliced fresh beef (round) was vacuum packed and frozen (-25 ~ -30°C) and then examined for color changes during 1 month of storage. The discolored sample showed 43% MetMb production. No discoloration of the freshly sliced rounds was noted, even when repeatedly frozen and thawed. Sirloin that had been minced, frozen and thawed showed 56% MetMb. The model solution, a reaction mixture (pH 5.5) consisting of Mb and ascorbic acid (Asc), was prepared, evacuated and oxygenated to encourage the production of O$_2$Mb as much as possible. NaNO$_2$ was added to the reaction mixture to convert MetMb to NOMb. The solution was assessed for color changes and reducing ability (RA). Frozen storage was under anaerobic and aerobic conditions, respectively, for O$_2$Mb and NOMb. The initial O$_2$Mb production ratio was 86% and was followed by a drop to 53% after 1 week of freezing and then to 32% after refreezing for 1 month (Fig. 11). RA decreased with the duration and number of times of refreezing. Up to 100% NOMb was produced under aerobic conditions at 20°C within 24 hours. Although NOMb was oxidized to some extent by freezing, NOMb remained relatively stable after refreezing and storage for 6 months (Fig. 12). No residual nitrite could be detected in the NOMb solution. It is evident from the results obtained that O$_2$Mb is fairly stable in meat cuts but unstable in a model solution as compared with NOMb. Its stability changes, however, under various freezing conditions, e.g. storage period. There are several methods of minimizing this problem, so that the Mb oxidation in the meat is greatly reduced or slowed down, for example, by means of the packaging technology used (e.g. vacuum packing seal and gas packing). Factors in discoloration have been reported as being meat pH, temperature, pO$_2$, lipid oxidation, ageing and the microbial MetMb-reducing enzyme system$^5$. Discoloration can be controlled partly by using films which are very permeable to oxygen and gas packaging with plenty of oxygen.
Fig. 9. Effects of K₃Fe(CN)₆ on O₂Mb in the model solution. (Sakata et al., 1996)⁴¹)

Fig. 10. Effects of K₃Fe(CN)₆ on NOMb in the model solution. (Sakata et al., 1996)⁴¹)
The discolored imported beef samples thus were not handled very properly.

Pork loin meat was cured under aerobic and anaerobic conditions. The color formation was promoted in anaerobically cured meat and the residual nitrite was reduced (Fig. 13). The anaerobically cured meat was frozen at \(-20^\circ\) and thawed monthly, and the color and residual nitrite were analyzed for the uncooked and cooked cured sample until 6 months of freezing. The aerobic samples had comparatively poor cured color and they were not frozen. CFR did not decrease during freezing (Fig. 14). With heating, the CFR decreased slightly with the storage period of the cured meat. This tendency was in agreement with the nitrite content which also decreased with frozen storage (Fig. 15). In the meat cooked after curing and then frozen, CFR (○, Fig. 15) and nitrite content (■) were lower after the 3-month period of storage compared with the case of frozen cured meat which was then cooked. The results indicate that cured meat pigment appeared, essentially, to be stable toward freezing in the experiments of cured meat, as well as Mb model system.

A study was made of pork stored at ca. \(-19^\circ\) and the oxidation was followed for 12 months of storage based on thiobarbituric acid (TBA) data\(^{34}\). Processing sausages with this meat revealed that color formation was lowered\(^{38}\). Recently, in manufacturing meat products such as ham and bacon, the following method is widely used in Japan: cured meat is frozen and stored for a few months prior to smoking and
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Fig. 13. Effect of aerobic and anaerobic curing on the CFR (A) and residual nitrite content (B) of cured meat. (Sakata et al., 1995)

Fig. 14. Effect of freezing period on CFR and residual nitrite content of anaerobically cured meat. (Sakata et al., 1995)


Fig. 15. Effect of freezing period on CFR and residual nitrite content of anaerobically cured and cooked meat. (Sakata et al., 1995)

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cooking. By this method, the meat companies can store the meat product materials for a long time, and are able to control easily the quality, quantity and time to produce the products. This processing method is empirically recognized to show no deteriorative effect on the color formation of meat products. This freezing method can be fully supported technically and theoretically by the data of the characteristic stability of NOHP in these experiments.

4. **Inhibitory action of nitrite on decrease in heme pigment content in meat with NaCl**

The addition of NaCl has been reported to cause discoloration of meat during storage\(^1,5,12,48\). However, little information is available on the change in heme pigment (HP) content in NaCl-treated meat. In this study, an examination was made of the effects on HP content in meat of not only NaCl but also NaNO\(_2\) and NaAsc, which are generally applied in combination with NaCl as curing agents. HP content decreased with increase in NaCl concentration and the decrease was about 50% and 80% at NaCl concentrations of 2% and 10%, respectively (Fig. 16). This phenomenon could be observed in aerobic storage condition of the salted meat. Under an anaerobic state (i.e. vacuum-packed), HP did not change compared to the control, and NaCl had no effect and HP content remained constant during storage. Thus, the reason for the HP decrease may possibly be an accumulation of peroxide, which can degrade HP. When previously mixed with NaNO\(_2\) or NaAsc, NaCl (2%) prevented HP destruction (Fig. 17). This can be also concluded to be one of the characteristics of NOMb produced in the cured meat.

5. **Red pigment produced in Parma ham without adding nitrite**

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 red color even without such treatment, only a limited quantity of sodium chloride (salt from Mediterranean sea) is added. Parma ham has been suspected to be contaminated with nitrate from the seasoned marine-salt. However, the total amount of nitrate from the salt was

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Fig. 18. Absorption spectra of heme pigments extracted with 75% acetone from Parma ham and a raw cured ham, Tipo Parma. (Morita et al., 1996)

The red pigment of Parma ham was water soluble, but it was not completely extracted with water. The residue, which had a weak-pink color, was extracted with 75% acetone. From the Tipo Parma (a Parma-type dry-cured ham produced with nitrite addition), the red pigment could barely be extracted with water. The red pigment in both samples could quite easily be extracted with 75% acetone. The visible spectral pattern for the acetone-extracted pigment in Parma ham showed maxima at 417, 546 and 584 nm. The acetone extract from Tipo Parma could be detected at four absorption peaks, mainly at 395 nm, this being the typical pattern of NOMb. Spectral patterns of acetone extracts from Tipo Parma and Parma ham differed significantly (Fig. 18). The pigment of Tipo Parma was NOMb formed by the reaction of Mb with NO. Therefore, the typical electron spin resonance (ESR) signal of NOMb was virtually shown from Tipo Parma (Fig. 19 [A]), the same as noted by another report. With Parma ham, a different ESR pattern emerged (Fig. 19 [B]). Thus, the red pigment in Parma ham differed from reduced Mb, O2Mb, MetMb and NOMb normally present in meat and meat products. Bacterial counts of Parma ham ranged from 10^4 to 10^5 CFU/g. 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 with the most pronounced color conversion were selected. The selected bacteria would be identified as Staphylococcus epidermidis, Staphylococcus warneri and Staphylococcus lentus. Both red pigments formed in Parma ham and Tipo Parma were stable toward heat treatment (75°C, 60 min), because the red pigment extracted with 75% acetone from cooked Parma ham revealed the same absorption spectrum as that of raw Parma ham. Stability of the pigment in acetone extract from Parma ham was compared with that of Tipo Parma. As shown in Fig. 20, 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 3 weeks more under the same conditions, thus showing red pigment to be a new Mb derivative, so far unknown in meat and meat products. The stable red pigment of Parma ham should prove useful for meat processing as a food grade red colorant. Developing a stable red color without the need for any color forming agent in meat products is also important from the standpoint of food hygiene. The structural characterization of the red pigment in Parma ham is being studied by ESR and...
chromatographic analysis. The mechanism of the red pigment formation must be clarified, when the isolation of red pigments could be completed.

6. Utilization of nitrosohemoglobin as a colorant of processed meat products

The red color of meat products is generally determined by nitrite added as a color forming agent in curing, except in some special products, such as Parma ham. Its use, however, has tended to decrease out of concern for possible carcinogenic nitrosamine production in processed meat products. This in turn has led to problems of decrease in color formation, color
stability, discoloration and yellowing. The red cell fraction of slaughtered animal blood presently has few uses and thus possible applications in foodstuffs are being sought. Animal blood hemoglobin (Hb) is used as a natural colorant in meat products, but its preparation is difficult. We prepared Hb from cattle blood and examined the possibility of its use for accelerating cured meat coloration. Using cattle blood Hb powder, the best conditions for producing nitrosohemoglobin (NOHb) from Hb in regard to NaNO2- NaAsc concentration, pH, time and temperature were determined. The nitrosation of Hb in a Hb reaction mixture proceeded rapidly and the CFR was high and stable (Fig. 21).

In the following study, the purified NOHb fraction was prepared from cattle blood, and optimum reaction conditions for nitrosation of Hb were determined. More than 80% of the total Hb in a reaction mixture (pH 4.5) of 25 mM NaNO2-25 mM NaAsc was rapidly nitrosated at 2 and 20°C. Added nitrite in a NOHb mixture virtually disappeared and no aerobic bacteria could be detected after 3 days of storage. When 0.5% or 1.0% of the NOHb reaction mixture was added to porcine loin meat with non-meat protein ingredient solution, NaCl, NaNO2 and NaAsc, nitrosoheme pigment formation was greater than that of the control (without addition of NOHb) meat product, and added NOHb showed quantitative effects on the color formation of sausage. TBA values were quite low and showed only slight variation, indicating lipid oxidation did not occur after 2 weeks of storage when NOHP prepared from cattle blood was added to
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sausage. Thus optimum conditions for the nitrosation of Hb from cattle blood were determined and native NOHb containing little nitrite was prepared. Shahidi and Pegg46) examined the encapsulation of dried powder of nitrosohemochrome prepared from cattle blood or hemin and found these pigments to be stable and have potential applications. The powder of NOHb solution should be obtained by a spray drying method and the possibility of long-term stabilization should be confirmed as basis for application to meat product processing.

Conclusion

By this investigation of the characteristics of red pigments, especially NOMb in meat products, an ordinary technique in meat processing could be supported. We explained also the extraction method of colored pigments with organic medium accompanying with the methods of calculation and expressing the color forming ability in meat products. Now we are investigating the structural property of NOMb [the coordination of NO complex of iron (II) myoglobin] produced in meat and by bacteria in medium using ESR17). Essentially, we have studied in our research how to reduce nitrite level and how to find a substitute in respect to color formation. For this concept, to clarify the characteristics of red colored pigments of Parma ham and to use the nitrosated Hb should be also available, based on the possibility of producing meat products without nitrite, and improvement in meat processing technology can hopefully be developed.

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