THERMAL STABILITY OF HEME PIGMENTS IN PORK

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Abstract – To reveal the relationship between cooked pork color and heme pigments, color (L*, a*, b*), extractable heme pigments and total protein content and redox state of myoglobin were determined at 3℃ increments over the 37 to 73℃ range. Additionally, thermal stability, differential scanning calorimetry and the fluorescent characteristic of purified Mb isolated from pork were measured to provide insight into myoglobin properties in pork. Purified Mb aggregated from 78℃ while pork Mb solubility decreased from 55℃. Fluorescent evaluation suggested that surface conformational change occurred in purified Mb over 55℃. The Mb precipitation in pork may be related to the surface structural change. The thermal kinetics of Mb and Hb in pork were similar, and water solubility of these heme pigments decreased significantly from 55℃ to 73℃. Cytochrome c showed high thermal stability and remained in reduced state after cooking at 76℃. Water soluble protein content decreased from 43℃ to 67℃. Ferric Mb aggregated at a lower temperature than ferrous Mb. L* and b* values showed greater correlation with the content of water soluble proteins than heme pigments kinetics. The value of a* was correlated with water soluble ferrous Mb but not with water soluble ferric Mb content.

Key Words – denaturation, co-precipitation, color defect

I. INTRODUCTION

Heme pigments such as myoglobin (Mb) are responsible for meat color, with the degree of oxidation of Mb and the kind of gas ligand bonded to Mb both serving to partly affect color. The thermal color change of meat is considered an important characteristic for many consumers because brown discoloration provides a noticeable indication of doneness. Therefore, residual red (pink) color defect in cooked meat is perceived by consumers as a sign of an undercooked product which is unsafe to eat. The pink color in cooked meat products has been noted in lighter pigmented species such as poultry and pork. High pH, ligand gasses and existence of heat stable heme pigment, cytochrome c (Cyt c) have been identified as factors responsible for the defect by numerous investigations. Marksberry [1] reported that some patties developed a cooked, well-done appearance at temperatures much lower than expected. Hague *et al*. [2] termed this condition premature browning (PMB) and presented additional evidence that internal color was not always a reliable indicator of doneness. Generally, the browning with cooking occurs owing to forming of water-insoluble brown pigment, ferrihemichrome. The development of PMB was thought to depend on the redox state of Mb, ionic strength, protein concentration and pH [3]. Such color defects can cause consumers to misinterpret the degree of cooking. In the present study, the color change in meat was investigated with a focus on Mb, the major pigment in meat. There are few reports of thermal profiling of hemoglobin and Cyt c in cooked pork while these heme pigments have a part in color [4].

In this study, the thermal kinetics of heme pigments (Mb, hemoglobin: Hb and Cyt c), Mb derivatives and water-soluble proteins in pork were analyzed in order to demonstrate the role of these parameters in determining pork color change with cooking. Additionally, heat aggregation stability, differential scanning calorimetry and the fluorescent characteristic of Mb isolated from pork were measured for the purpose of gaining an insight into Mb properties in pork.

II. MATERIALS AND METHODS

Pork was coarse ground through a 3.0 mm plate, and vacuum packaged in 20 g aliquots. Initial pH was determined by homogenizing pork (5 g) with distilled water (20 ml) and determining pH using a pH meter. All tests were conducted using fresh pork leg, except the investigation into the effect of redox state on water solubility of heme pigments,

for which sliced pork leg pack stored at 4℃ for 1 week to oxidize the samples was used.

After the pork patties were cooked in a circulating water bath, three bags were removed from the bath at 3℃ increments over the 37℃ to 73℃ range, and cooled immediately in an ice bath. Cooked samples were pounded in a mortar and the color values (L*, a* and b*) were evaluated. Observations were made at each of these three points on the samples. Heme pigments from each whole sample were extracted using the procedure of Waga *et al*. [4]. Samples were blended with four volumes of cold 40mM phosphate buffer (pH 6.8) for 1 min in a homogenizer. Homogenates were centrifuged at 10,000 ×g for 10 min at 4 ℃ and the supernatant was filtered through 0.45 μm mesh.

The protein concentration of the extract was measured by protein assay kit (BioRad, USA).

The concentrations of Mb, Hb and Cyt c in the extract were determined by the method of Waga *et al*. [4]. After addition of $Na_2S_2O_4$, 10 ml CO gas was blown into the extract solution. CO treated extract was measured spectrophotometrically and concentrations of several heme pigments (%, w/v) were determined from the following equations:

$$[Mb] = 0.0125A_{538} - 0.3544A_{568} + 0.4913A_{578}$$
$$[Hb] = -0.0418A_{538} + 0.4756A_{568} - 0.3744A_{578}$$
$$[Cyt\ c] = 0.1863A_{538} - 0.1141A_{568} - 0.1083A_{578}$$

The metmyoglobin (MMb) ratio was calculated using the method of Krzywicki [5] with a modification: A_{572} and A_{524} were used after the elimination of the absorbance derived from Cyt c since the absorbance had a large effect on the MMb calculation at high temperature in this study.

Purified Mb was prepared using the method by Ishioroshi *et al*. [6] with some modifications. Pork samples were blended with an equivalent amount of cold distilled water for 1 min in a homogenizer. The homogenates were centrifuged at 10,000 ×g, and the supernatant was fractioned by ammonium sulfate 75-90%. Purified Mb solution was obtained from the ammonium sulfate fraction using hydrophobic interaction chromatography.

The purified Mb was heated over 37℃ to 76℃ at a rate of 3℃/min. Heat-aggregation temperature and redox state were determined by the absorbance at 525, 572 and 750 nm using a spectrophotometer (UV-2700, Shimazu, Japan). The redox state of Mb was evaluated as MMb ratio. The conformational change in Mb with heating was evaluated by differential scanning calorimeter (DSC) and fluorescence spectroscopy method [7]. In DSC evaluations, the heating rate was 5℃/min to a final temperature of 90℃.

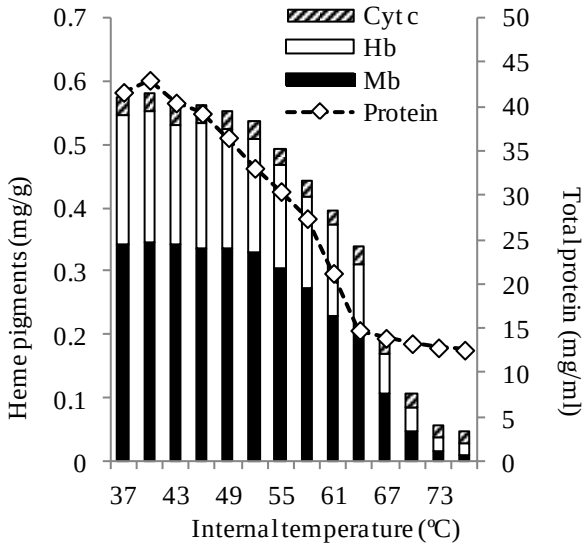
Differences were considered significant at $P<0.05$. These experiments were conducted twice.

III. RESULTS AND DISCUSSION

Initial pHs of pork were nearly equal among all samples, pH 5.68 ± 0.03. Mancini *et al*. [3] mentioned that pH influenced myoglobin denaturation. In this study, the pH effects on color were not considered. The pork color change to tan/white was visually observed from at 58-61℃. In instrumental color evaluation, L*, b* and C* increased with cooking temperature up to 64℃. The value of redness, a* decreased from 46℃ to 61℃ and rose slightly over the temperature. Hue angle increased from 37℃ to 64℃ and slightly decreased until 73℃.

The concentration of water soluble heme pigments and protein in pork at several temperatures are shown in Fig. 1. Increasing endpoint temperature will increase the percentage of myoglobin denaturation [8].

Figure 1. Thermal kinetics of heme pigments and water soluble proteins



Water soluble protein content decreased from 43℃ to 67℃ and a slight change was observed

over the temperature. The amount of total heme pigments decreased significantly from 55℃ to 73℃, and the maximum decrement was observed at 67℃. The values of L*, b* and C* were more correlated with the content of water soluble proteins than the kinetics of heme pigments. These results agreed with an earlier report indicating that oxy-Mb and MMb contents effect on a* significantly, but not on L* and b* values [9, 10].

The thermal kinetics of Mb and Hb were similar, and decreased significantly from 55℃ to 73℃. Cyt c showed high thermal stability, and a noticeable decrease was not observed at all temperatures. Cyt c remained in reduced state (absorption peaks was observed at 520 and 550 nm) after cooking at 76℃. Reduced state Cyt c shows pink color, and has been suggested as one cause of the pink defect [11]. However, a pink color was not observed in this study. It is possible that the occurrence of pink color was not only by the presence of pink pigment. Holownia *et al*. [12] reported the influence of some ingredients (sodium chloride, tri-polyphosphate, sodium erythorbate and sodium nitrate) on cooked chicken meat color, and only sodium nitrate produced pink color by the production of nitrosyl Mb. Pink color resulting from Cyt c could be related to other non-color proteins since the effects of water soluble proteins on cooked pork color was observed.

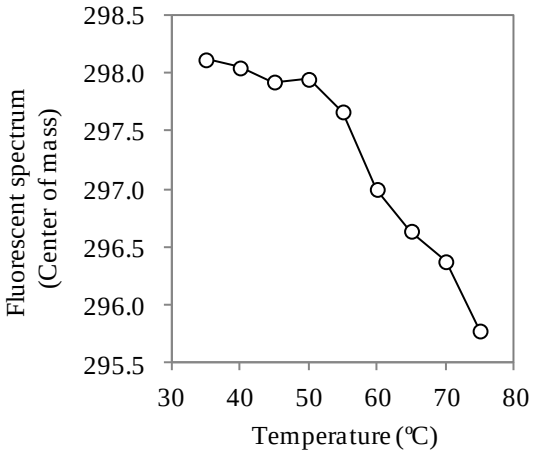
Hunt and Zenger [13] showed that pork color turns tan/white when cooked at 77℃ for 1 sec. To achieve a well-done appearance in ground beef patties, approximately 80% of myoglobin must be heat denatured [14]. In this study, denaturation ratio of heme pigments reached 80% at 70℃. But the thermal color change had almost stopped over 73℃, and at this point, heme pigments denatured ratio reached 91%.

Thermal stability of Mb was affected by its redox state. Hunt *et al*. [15] indicated that MMb (ferric state) denatured more easily than deoxy-Mb (ferrous state). As expected, ferric Mb aggregated at lower temperature than ferrous Mb in this study. Only ferric Mb aggregated over the range of 37℃ to 52℃. Thus, the redox state of Mb was thought to be important for aggregation and surface hydrophobicity. Ryan *et al*. [14] suggested that Mb denaturation ratio affected the visual/instrumental meat color. However, the redness was correlated not with water soluble ferric Mb content, but with ferrous Mb content. Therefore, it is inferred that the redness of meat depends not on the solubility of Mb but on the redox state of Mb or Hb. These data clearly indicated that development of cooked meat color was interrelated with redox state of Mb. The results also suggest that easily-oxidizable conditions result in a higher rate of PMB occurring. Furthermore, this suggestion agrees with an earlier report in which it was noted that PMB occurred with higher frequency in aerobic packaging than anaerobic packaging [16].

Purified porcine Mb started to aggregate from 78℃, which is higher than that of meat. Zhu *et al*. [17] indicated that purified MMb denatured from 60℃ using 50 mM buffers. The difference among these temperatures was observed owing to experimental conditions: oxy-form Mb was used; experimental solutions were prepared with low conductivity, >50 mS/m in this study. Geileskey *et al*. [18] had reported higher denaturation temperatures with isolated Mb solutions than that of meat.

The center of mass of fluorescent spectrum irreversibly changed from 55℃ (Fig. 2). Fluorescent change was observed owing to the change of fluorescent amino acids on protein surface with structural change such as folding or unfolding. Thus, surface conformational change was thought to have occurred in Mb over 55℃.

Figure 2. Thermal change of Mb fluorescent spectrum



The endothermic conformational change was observed at 72.5℃ in DSC evaluation. These results suggested that thermal denaturation of Mb

likely occurs through a process involving several stages.

IV. CONCLUSION

Mb in pork lost its water solubility at a lower temperature than in isolated Mb. Evaluations of DSC, aggregation temperature and fluorescent spectrum suggest that thermal structural change in Mb occurs through a series of steps. The surface structural change of Mb, observed over 55ºC, may provoke its surface hydrophobicity change. This conformational change of Mb could induce a co-precipitation.

The redness of pork did not correlate with water solubility of ferric Mb whereas it depended on the ferrous Mb content.

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