

## ACCELERATING EFFECT OF WHEY PROTEIN HYDROLYSATE ON COLOR FORMATION IN MEAT PRODUCTS

Ryoichi Sakata, Hidetoshi Morita, Takeshi Norimatsu, Toshio Oshida, Keiko Horiguchi\*  
Noriyuki Itoh\*\*, Sadayuki Nagata\*\*, Takahide Okayama\*\*\* and Michio Muguruma\*\*\*\*

School of Veterinary Medicine, Azabu University, Sagamihara-shi 229-8501, Japan

\*Meiwagakuen Junior College, Maebashi-shi 371-0034, Japan

\*\*Omu Milk Products Co., Ltd., Omuta-shi 836-0895, Japan

\*\*\* Faculty of Agriculture, Kobe University, Nada-ku, Kobe-shi 657-8501, Japan

\*\*\*\*Faculty of Agriculture, Miyazaki University, Miyazaki-shi 889-2192, Japan

**Keywords:** color formation, myoglobin, nitrite, peptides, pork sausage, whey protein hydrolysate

### Background

In the previous study on the avoidance of milk allergy, enhanced color formation was observed subsequent to the addition of milk protein hydrolysate to meat containing nitrite at low level<sup>1,2)</sup>. Milk proteins are mainly casein and whey protein, and the hydrolysate of the latter may be expected to enhance color formation.

### Objective

This study was conducted to clarify the accelerating effect on color formation by the hydrolysate of whey protein concentrate prepared with enzyme (WPC80 hydrolysate) and assess this hydrolysate for potential use as a color accelerating agent for processed meat products.

### Methods

**1. Preparation of whey protein hydrolysate:** Distilled water was added to WPC80 (concentrated powder containing 80% whey protein, AMPC Inc. Co., Ltd.) at 10%, and the system was heated to 65 °C for 30 min for pasteurization. Commercial protease (Flavourzyme, Novo Nordisk Co., Ltd.) was added at 0.207% followed by hydrolysis at 50 °C for 16 hr. The reaction mixture was heated to inactivate the enzyme at 90 °C for 20 min and centrifuged at 10,000 × g for 15 min. The supernatant was freeze-dried and used as the WPC80 hydrolysate. Protein decomposition in the hydrolysate was assessed by HPLC (Shimadzu LC-6A), using a gel-filtration column. Column; TSK-GEL G2000SWXL, solvent; 45% acetonitrile + 0.1% trifluoroacetic acid (TFA), flow rate; 0.5 ml/min, detection; absorbance at 215 nm.

**2. Preparation of pork sausage:** The sausage was prepared using minced porcine thigh muscle (within 48 hr *postmortem*). WPC80 or WPC80 hydrolysate (5%), or 0.1% sodium ascorbate (NaAsc) was added to the meat along with 2% NaCl, 100ppm NaNO<sub>2</sub> and 15% ice. The mixture was vacuumed, stuffed into casing (φ 55 mm, Krehalon Film) and heated at 75 °C for 45 min. Color forming ratio (CFR)<sup>3)</sup> was then determined.

**3. Preparation of heated Mb model solution:** Myoglobin (Mb) model solution was obtained using 0.1% Mb and WPC80 hydrolysate at 5% with 0.1M acetate buffer (pH 5.5). While N<sub>2</sub> gas was being bubbled through the solution contained in a screw-capped glass tube (10 ml vol.), NaNO<sub>2</sub> was added at 100 ppm and the system was heated at 75 °C for 60 min. The tube was then rapidly cooled to 0 °C and CFR was measured. Instead of WPC80 hydrolysate, WPC80 or sodium erythorbate (NaEry) was added to the Mb model solution at 5% or 0.1%, respectively and CFR was measured. Using the nitrosyl heme pigment extract from heated Mb solution, residual nitrite was determined by the method of Mirna and Schütz<sup>4)</sup>.

### Results and discussion

**1. Color formation of sausage:** Fig.1 shows values obtained for CFR of the sausage sample. CFR in the WPC80 hydrolysate was essentially the same as with NaAsc addition. In WPC80-added sausage, CFR was less than that for other sausages, indicating virtually no color accelerating effect by the whey protein concentrate. Peptides produced through enzymatic degradation would thus appear to enhance meat product color formation.

2. **Protein hydrolysate analysis by HPLC:** In the chromatogram of WPC80 degraded for 16 hr, the main peak appeared in the lower molecular weight region (Fig.2). HPLC elution time indicated nearly all components in the WPC80 hydrolysate to possibly be peptides with molecular weights below 1,000. The HPLC chromatogram obtained at greater degradation time remained essentially the same as that at 16 hr (data not shown). Color analysis of the sausage samples and other experiments were thus conducted with WPC80 hydrolysate degraded for 16 hr.

3. **Color formation of heated Mb model solution:** Fig. 3 indicates CFR values obtained for the Mb model solution. NaEry was used as positive control, since the acceleration of color formation should occur as noted with NaAsc<sup>51</sup>. With the NaEry-added Mb solution, CFR was higher at 5 min heating and maximal value was attained at 10 min. WPC80 hydrolysate CFR increased slowly during heat application, but at 60 min, its value was basically the same as in the case of NaEry. With WPC80, slow increase in CFR was observed as with the hydrolysate, and throughout heating, the value was lower than any other. Fig. 4 shows CFR and residual nitrite content for the WPC hydrolysate-added sample. CFR increased with added WPC hydrolysate concentration and at the same time, residual nitrite decreased. The WPC hydrolysate would thus appear capable of enhancing nitric oxide production required for color formation.

**Conclusions**

The results of this study clearly indicate the whey protein hydrolysate to accelerate color formation and consequently, this hydrolysate should prove useful for enhancing color formation in meat products.

**Pertinent literature**

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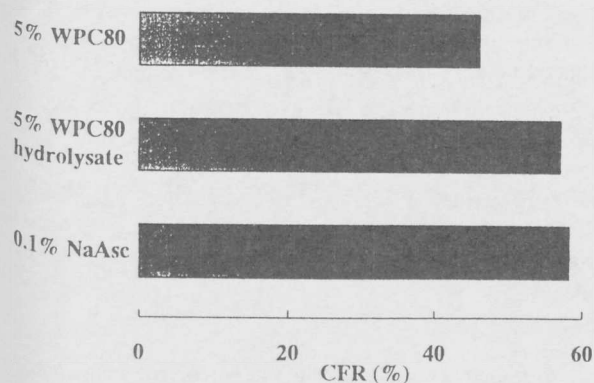


Fig.1. Color forming ratio (CFR) for the sample sausages.

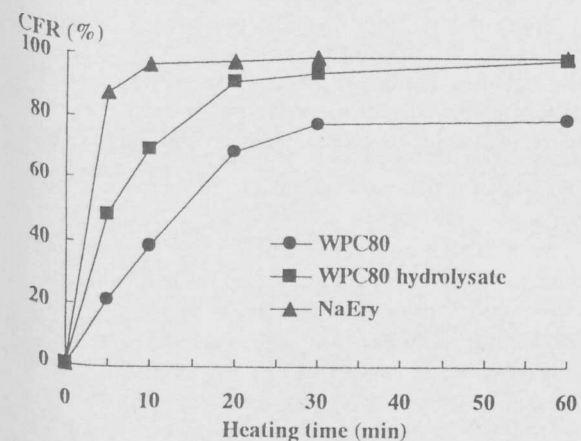


Fig.3. Color forming ratio (CFR) of Mb model solution following WPC80, WPC80 hydrolysate or sodium erythorbate (NaEry) addition and heating at 75°C.

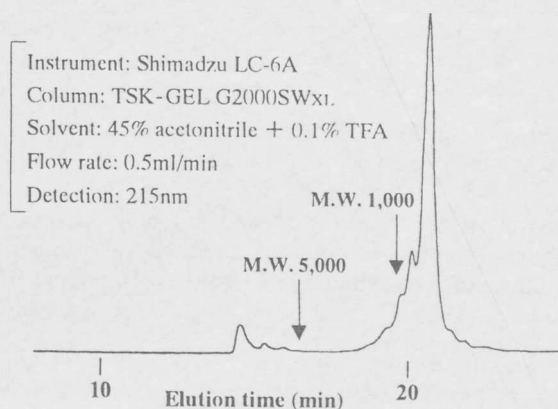


Fig.2. HPLC chromatogram of the WPC80 hydrolysate.

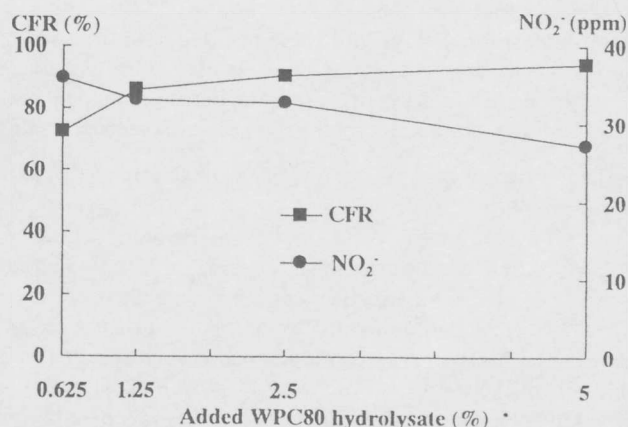


Fig.4. Color forming ratio (CFR) and residual nitrite content of Mb model solution following WPC80 hydrolysate addition and heating at 75°C for 60 min.