ACCELERATING EFFECT OF MILK WHEY PEPTIDES ON COLOR FORMATION IN MEAT PRODUCTS

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Background

The attractive red color is a prime characteristic of meat products, and it is one of the important qualities which affects consumers' purchase decisions. Recently, both casein and whey protein have been shown to have an accelerating effect on color formation when used in meat products. In this previous study; enhanced color formation was observed subsequent to the addition of milk protein hydrolysate to meat containing low levels of nitrite.

Objectives

This study was conducted to clarify the accelerating effect on color formation of hydrolysate of whey protein concentrate (80%, WPC80) prepared with prepared with an enzyme (WPC80 hydrolysate), and to assess this hydrolysate for its potential use as a color accelerating agent for processed meat products.

Methods

1. Preparation of milk whey peptides: WPC80 was dissolved at 10%, then hydrolyzed at 50°C for 16 hrs, using 0.1% Flavourzyme (Novo Nordisk Co., Ltd.). The reaction mixture was heated to inactivate the enzyme at 90°C for 20 min and centrifuged at 5,000 xg for 10 min. The supernatant was freeze-dried and used as the WPC80 hydrolysate. Protein degradation in the hydrolysate was assessed by high performance liquid chromatography (HPLC), using a gel filtration column.

2. Preparation of pork sausage: The sausage was prepared using minced porcine meat. WPC80 or WPC80 hydrolysate (5%) was added to the meat along with 2.5% NaCl, 10 or 20 or 50 ppm nitrite and 15% ice. The mixture was stuffed into casing and heated at 75°C for 30 min. After cooking, the a* value (redness) was measured by chromameter (Minolta CM1000).

3. Preparation of heated Mb model solution and determination of reducing ability: Myoglobin (Mb) model solution was obtained using 0.1 or 0.2% Mb and WPC80 hydrolysate with 0.1M acetate buffer (pH5.5). N_2 gas was bubbled throughout the solution contained in a screw-capped glass tube while nitrite was added at 50 or 100 ppm and the system was heated at 75°C for 60 min. The tube was then rapidly cooled to 0°C and the color formation ratio (CFR) and heat denaturation of Mb were measured. Reducing ability was measured by the ferricyanide reducing method.

4. Isolation of WPC80 hydrolysate by column chromatography: WPC80 hydrolysate was fractionated by gel filtration column (Superdex 30pg, 1.6Å~95cm). The active fractions having a high CFR were collected and concentrated, and the fraction was put on a column (DE53, 2.5Å~28cm). The amino acids of each peptide were analyzed by HPLC with a Wakosil PTC column after hydrolysis with 6N HCl at 110°C for 24 hrs under a vacuum.

Results and discussion

1. Protein hydrolysate analysis by HPLC: In the gel filtration chromatogram of WPC80 degraded for 16 hrs, the peaks appeared in the lower molecular weight region. The HPLC elution time indicated that all components in the WPC80 hydrolysate may be peptides with molecular weights of 1,000~5,000 and below 1,000.

2. Color formation of sausages: Figure 1 shows the a* values of the sausage samples. The a* values of WPC80 hydrolysate were higher than both those of WPC80 alone and those of the control. The a* values of WPC80 hydrolysate with 10 ppm nitrite were nearly the same as those of the control with 50 ppm nitrite. Milk whey peptides through enzymatic degradation would thus appear to enhance color formation in meat products.

3. Color formation of heated Mb model solution: Figure 2 presents the CFR values obtained for the model solution. The CFR increases with increasing concentrations of WPC80 or WPC80 hydrolysate added to the Mb solution. The CFR values obtained with WPC80 hydrolysate were higher than those with WPC80. This indicates the same tendency that we found in the case of the color formation in sausages. The heat denaturation of Mb in the presence of WPC80 hydrolysate is higher than that in the presence of WPC80. Reducing ability increases with an increase in the addition of the sample (Fig. 3). The reducing ability of WPC80 hydrolysate is higher than that of WPC80. These results suggest that both the heat denaturation and the reducing ability of Mb are involved in color formation in meat products. That is, the Mb changes into denatured MetMb, and when the denatured MetMb subsequently combines with nitric oxide the denatured MetMb is reduced and nitrosohemochrome forms.

4. Isolation of color formation promoted peptides: WPC80 hydrolysate was isolated by gel filtration chromatography (Superdex 30pg). The CFR values, heat denaturation of Mb and reducing ability in fraction Nos. 5-6 of the gel filtration chromatography were high. Fraction No. 5 was further purified by anion exchange chromatography (Fig. 4). The CFR values, heat denaturation of Mb and reducing ability were low in non-adsorbed fractions (Nos. 1, 2 and 3) and high in the adsorbed fraction (No. 8), but reducing ability was low in the latter. Amino acid analysis was conducted on gel filtration chromatography fraction No. 5 and on anion exchange chromatography fraction No. 8 after 6N HCl hydrolysis. Gel filtration chromatography fraction No. 5 showed a comparatively high presence of Asn, Gln, Pro, Lys and Tyr, while anion exchange chromatography fraction No. 8 did not show a high concentration of Lys and Tyr, but did show higher concentrations of Asn, Gln and Pro. These results suggest that acidic peptides, which included a comparatively high number of amino acids of Asn and Gln, are involved] in color formation in meat products.

Conclusions

The results of this study clearly indicate that milk whey peptides accelerate color formation in meat products. The peptides related to color formation in meat products showed apparent molecular weights of 1,000~3,000 and acidic peptides including Asn and Gln. The possible action of milk whey peptides appears to accelerate heat denaturation of Mb and reducing ability, thus enhancing the color formation of cooked cured meat products. We thus expect milk whey peptides to prove useful for enhancing red color in meat products.

Pertinent literature

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(3) Sakata R, Morita H, Norimatsu T, Itoh N, Nagata S, Okayama T, Muguruma M. The Japanese Journal of Swine Science: Vol. 38, No. 3: 115-124, 2001.

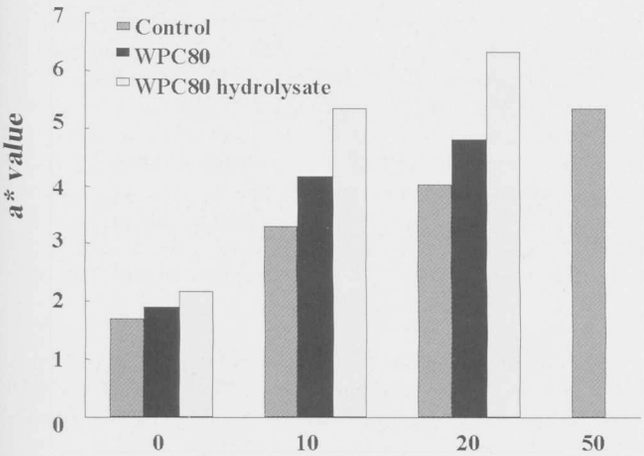

Fig.1 The a* value of sample sauseges

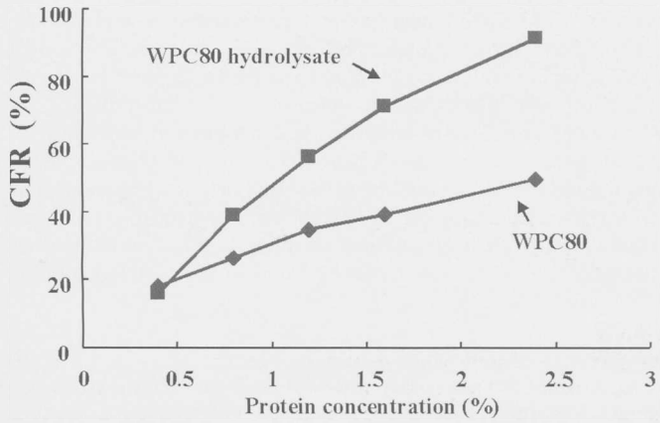

Fig.2 Color formation ratio (CFR) of Mb model solution following WPC80 or WPC80 hydrolysate addition and heating 75°C for 60 min

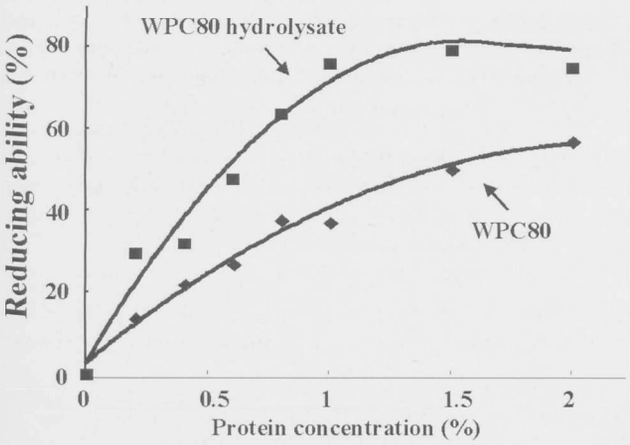

Fig.3 Reducing ability of the addition of WPC80 or WPC80 hydrolysate and heating at 75°C for 60 min

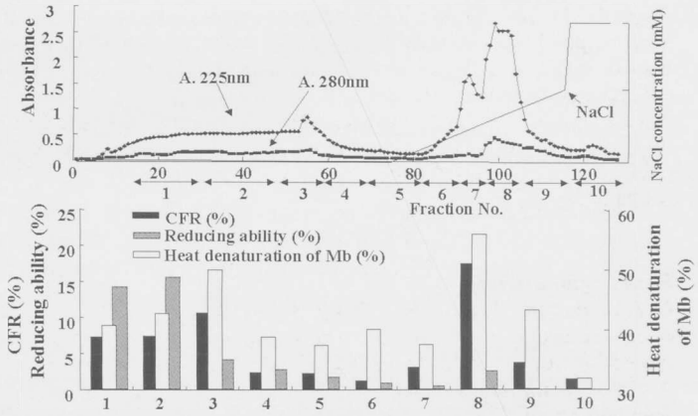

Fig.4 Anion exchange chromatogram of gel filtrate fraction No.5, and these fractions were measured for CFR, reducing ability and heat denaturation of Mb