ENHANCEMENT OF COLOR FORMATION IN MEAT PRODUCTS BY MILK PROTEIN HYDROLYSATES

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Background:

Instances of allergic reaction to milk, particularly in infants, are on the increase with consequent tendency to avoid milk and milk products. As solution to this problem, milk is presently subjected to hydrolysis through the action of enzymes[1]. Protein hydrolysates obtained by the decomposition of milk protein through proteases were used in the present study to prepare sausages with low nitrite content. This was found to quite significantly promote color formation in cooked cured meat.

Objectives:

Although nitrite is widely used in meat products as a color developing agent, carcinogenic nitrosamines may also be produced by this additive. Nitrite has thus come to be used much less in meat processing[2], and numerous nitrite-free products are now presently available. However, except for fermented meat products that redden during a long ripening period, such as a Parma ham[3], these products unfortunately become poor in color and consequently unattractive. Also, without the presence of nitrite, there is the risk of food poisoning, especially that due to botulism. Meat products prepared with hypoallergenic milk were found as a result to have better color and thus should prove safer with no risk of allergic reaction and only slight need for nitrite. In the present study, hydrolysate from skim (defatted-) milk whose main component is casein was examined for its capacity to enhance color formation in meat products.

Methods:

1. Preparation of milk protein hydrolysates: Skim milk, SFC400D (Omu Milk Products Co., Japan), concentrated to 1/4 its original volume with ultra-filtration membrane (cut-off M.W. 50,000) was hydrolysed at 50℃ for 5 hr using 2 commercial proteases, Alkalase and Flavorzyme (Novo Nordisk Co., Ltd.). This was followed by heat application to 90℃ for 20 min to inactivate the enzymes and the milk was then freeze-dried. Ethanol was added at 50% and the system was centrifuged at 5,500 rpm for 20 min. The supernatant was evaporated for removal of the ethanol and lyophilized. The milk protein hydrolysate (MPH) and ethanol extract fraction (EEF) were used for sausage preparation. SFC400D (unhydrolysed) served as the control. HPLC (Shimadzu LC-6A) was conducted using EEF to determine the extent of hydrolysation. The conditions for HPLC were as follows: column; TSK-GEL G2000SWXL, solvent; 45% acetonitrile + 0.1% trifluoroacetic acid, detection; absorbance at 210nm.

2. Preparation of sausage: This preparation was conducted using minced porcine thigh muscle (within 48 hr *postmortem*). SFC400D, MPH or EEF was added at 10% meat sample weight along with 1% NaCl, 17ppm nitrite ($NaNO_2$) and 15% ice. The mixture was vacuumed, stuffed into casing (ϕ 55mm, Krehalon Film) and heated at 75℃ for 20 min. Sausage prepared with 17 or 85ppm $NaNO_2$ without milk protein hydrolysates served as a control. After cooking, a* (redness, Minolta CM-508d) and the color forming ratio (CFR)[4] were measured .

Results and discussion:

1. Enhanced color formation of sausage prepared with milk protein hydrolysate: Fig. 1 shows the values obtained for a*. With 17ppm $NaNO_2$, this parameter was *ca.* 6.0 and increased with MPH in the meat. A value of *ca.* 8.0 with MPH clearly indicated enhanced color formation. This value was not possible at 85ppm $NaNO_2$, as also for the sample to which SFC400D had been added. Fig. 2 shows the absorption spectra for the 75% acetone extract from MPH-added sausage. At maximum absorption at 395nm, nitrosohemochrome formation was evident, thus indicating MPH to also promote color formation. With SFC400D and 17ppm $NaNO_2$, CFR was 27.5%, essentially the same as with the latter alone.

It was evident that CFR did not increase by unhydrolysed skim milk (SFC400D) alone, judging from the result with 17ppm $NaNO_2$ addition. The sample with MPH showed CFR of 45%, or *ca.* 1.6 times that of any of the controls. The addition of MPH is thus cleary shown to enhance color formation.

2. Milk protein hydrolysate analysis by HPLC: Fig. 3 shows the results of this analysis using gel-filtration. In the higher molecular weight region, no absorption peak was observed. The elution time in HPLC indicated nearly all components in EEF to possibly be peptides with molecular weights less than 1,500. Unhydrolysed milk protein in EEF could not be detected by HPLC.

3. Enhanced color formation by EEF: Peptides produced by enzymatic treatment of milk protein are shown by the present results to be responsible for enhanced color formation. Dried material weight recovered by extraction was *ca.* 70% of the enzyme hydrolysate that had moved into the ethanol layer, which was examined as the peptide fraction (M.W.<1,500) by HPLC. CFR of sausage with the EEF is shown to be 64.3% in Fig. 4, this value being 1.4 times (64.3/45.0) that of the MPH-added sample or 2.3 times the CFR of SFC 400D-added sausage.

Conclusions:

The capacity of protein hydrolysate obtained from skim milk to enhance color formation of meat products was investigated. The major component of the powdered preparation was found to have a molecular weight less than 1,500 by HPLC, using a gel-filtration column. A value of a* was higher in pork sausage prepared with 17ppm $NaNO_2$ and 10% MPH than with 85ppm $NaNO_2$. The absorption spectrum of 75% acetone extract from the sausage sample showed increased red colored heme pigment and consequently, MPH brought about enhancement of color formation. CFR of this sausage was *ca.* 1.6 times that with the addition of unhydrolysed milk protein. CFR was greater for sausage to which EEF from MPH had been added. Milk peptides should thus prove useful for enhancing red color in meat products.

Pertinent literature:

1) Itoh, N., The Food Industry (in Japanese), **41**, No.24, 32-38, 1998. 2) Nagata, Y., Shokuhin No Henshoku No Kagaku (in Japanese), Kohrin, Tokyo, p.385-407, 1995. 3) Sakata, R., Niu, J., Morita, H. and Nagata, Y., Proc. 44th Inter. Cong. Meat Sci. Technol., p.988-989, 1998. 4) Sakata, R., Anim. Sci. J., **71**, 1-16, 2000.

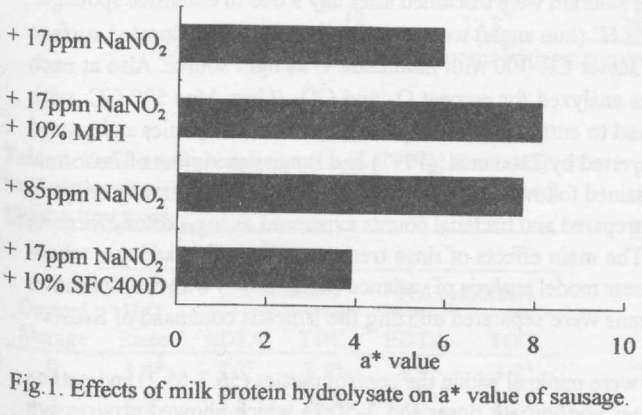

Fig.1. Effects of milk protein hydrolysate on a* value of sausage.

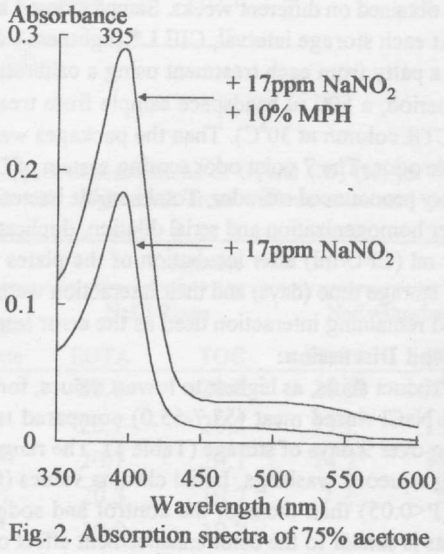

Fig. 2. Absorption spectra of 75% acetone extract from sausage.

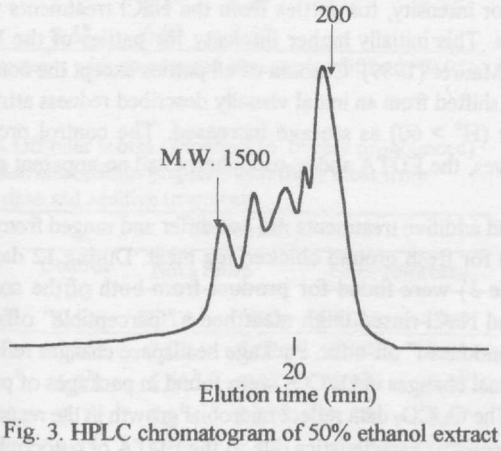

Fig. 3. HPLC chromatogram of 50% ethanol extract fraction from the milk protein hydrolysate.

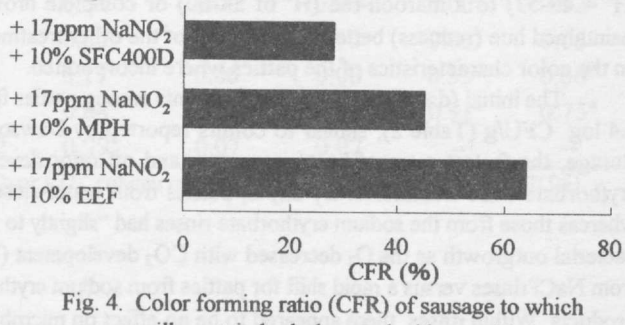

Fig. 4. Color forming ratio (CFR) of sausage to which milk protein hydrolysates had been added.