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IMPROVEMENT OF PROPERTIES OF CONCENTRATED MILK PROTEIN BY TRYPSIN DIGESTION AND ITSUTILIZATION IN MEAT PRODUCTS

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Background:

Milk proteins provide an excellent source of high-quality protein. Protein can be used in nutritional applications such as infant formula, protein-fortified fruit juices, meat products, and other sports or nutritional beverages. Several treatments have been suggested for improving milk proteins. Enzyme hydrolysis[1-3] was shown to be very effective in altering the structure and functions of milk protein. Little is known about the functional properties of concentrated milk protein by enzyme treatment.

Objectives:

Our objective is to study the preparation and properties of hydrolysates by tripsin digestion from concentrated skim milk protein.

Materials and Methods:

Concentrated skim milk protein (CSP) was prepared from defatted skim milk by ultrafiltration (50,000 MW cut-off). Bovine trypsin was obtained from Wako Pure Chemical Industries, Ltd. One liter of CSP solution was digested with 4 ml of 1% trypsin (37 °C, pH 7.5, 3 hr). Enzyme reaction was stopped by heating (80oC, 30 min). Protein species were analyzed by SDS-PAGE following the method of Laemmli[4]. Electrophoreses were performed in 7.5~17.5% polyacrylamide gradient gels. Gels were stained CBB G-250 or Nile red[5]. A fluorescent meter (FR-5000, Shimazu Co.) was used to determine hydrophobicity, and 8-anilide-1-naphthalene-sulfonic acid (ANS) was used as a fluorescence probe. The excitation wavelength was 365 nm, while the wavelength used for measurement was 470 nm. One mg/l of 0.1N quinine sulfate was used as the standard solution[6]. Sausages were prepared from beef. The raw material was minced in a meat grinder. 2% NaCl, 0.3% sodium pyrophosphate and 30% distilled water were added to the ground meat, and milk samples (10%) were added during chopping. The meat sausage was cooked at 80 °C for 30 min and cooled by flowing water immediately after. Gel strength of sausages was measured with a creep meter (Rheoner RE-33005, Yamaden Co.) at 25 °C.

Results and discussions:

CSP had 11.6% protein. Therefore, it is considered that CSP is concentrated about 4 times from original skim milk. SDS-PAGE patterns in Fig.1-(A) reveal that the CSP had a large quantity of casein and a small quantity of BSA, ß-Lg, a-La etc. When CSP was hydrolyzed by trypsin, most bands derived from casein and BSA disappeared, and furthermore about 80% band of ß-Lg disappeared. Two main broad bands with apparent molecular weights of 14,000 and 12,000 were identified. These residual bands may have originated from casein, BSA, ß-Lg, a-La etc. Some fragments were further hydrolyzed to smaller peptides having molecular weights of less than 12,000. Nile red is used as a hydrophobic probe for the fluorescent staining of protein bands in SDS-PAGE[7]. Fig. 1-(B) shows the staining of protein-SDS bands with Nile red. Most of the protein bands of hydrolysate of CSP were stained with Nile red. Daban et al. speculated that Nile red is bound to regions with equivalent hydrophobic characteristics located in uniform structures produced by the association of SDS with proteins [5]. These results suggests that hydrolysate of CSP has these hydrophobic characteristics.

A fluorometric procedure for the denaturation of protein is based upon the interaction in a buffered system with sodium 1-phenyl-naphthylamine-8-sulfonate[6]. The hydrophobicity of proteins may be increased by denaturation, as it might expose the hydrophobic area of molecules. The helical contents of protein decreased strongly with agents of hydrogen bond cleavage such as guanidine hydrochloride. The fluorescence of CSP increased as denaturation proceeded, while the fluorescence of CSP hydrolysate increased gradually (Fig. 2). These results suggest that CSP is partially unfolded by denaturing agents, such as guanigine-HCl, and the secondary structure of native protein is partly denatured by the addition of guanigine-HCl. Sulfhydryl (SH) group and disulfide (S-S)

bonds influence significantly the functional properties of food protein, and play an important role in the formation of relatively rigid structures such as protein gels [8]. S-S bonds of protein can be cleaved by reducing them with a reagent such as ß-mercaptoethanol (ME), which forms mixed disulfide with a cystein side chain. In the presence of a large excess of ME, the mixed disulfides are fully converted into sulfhydryls. The fluorescent intensity of CSP was gradually increased in proportion to the reduction of S-S bonds. The fluorescent intensity of CSP hydrolysate was not changed by the addition of ME (Fig. 3). These results suggest that the tertiary structure of proteins of CSP is changed by the cleavage of disulfide bonds, and exposed the hydrophobic area of molecules. However, it is considered that the tertiary structure of proteins of CSP hydrolysate has already been changed by enzyme treatment.

Gel strength, as measured by shear force determination, was plotted (Fig. 4). Shear force of sausages with CSP was decreased to 88% in comparison with sausage without CSP. Furthermore, shear force of sausage with CSP hydrolysate was decreased to 61% in comparison with sausage without CSP. These results suggest that the addition of CSP hydrolysate to meat products may regulate their shear force. On sensory panel evaluation, sausages with 3~5% hydrolysate of CSP were not rated different for any of the sensory attributes, which suggests that lower levels of hydrolysate might result in a better sensory response. Additions of 3% hydrolysate (commercial level) were not significantly different from each other for any of the sensory parameters. Hydrolysate of CSP proved to be a viable binder alternative for specific emulsion-type meat products by providing textural and sensory attributes.

Conclusions:

To study the improvement of concentrated milk protein (CSP), we have digested it with a trypsin treatment, and examined its functional properties of product and utilization in meat product of it. Polyacrylamide gel electrophoresis showed that high and intermediate molecular weight polypeptide was converted to lower molecular weights by trypsin digestion. The two main broad bands with apparent molecular weights of 14,000 and 12,000 were identified. The polypeptides of CSP hydrolysate had specific ones hydrophobic characteristics. The addition of CSP hydrolysate to meat products might regulate their shear force. Hydrolysate of CSP proved to be a viable binder alternative for specific emulsion-type meat products by providing textural and sensory attributes.

Pertinent literature:

1) Jost, R. and Monti, J.C. J. Dairy Sci., 60, 1387-1393 (1977).

2) Chobert, J.M., Bertrand-Harb, C. and Nicolas, M.G. J. Agric. Food Chem., 36, 883-892 (1988).

3) Turgeon, S.L. and Gauthier, S.F. J. Food Sci., 55, 106-110 (1990).

4) Laemmli, U.K. Nature, 227, 680-685 (1970)

5) Daban, J.R., Bartolome, S. and Montserrat, S. Anal. Biochem., 199, 169-174 (1991)

6) Tomita, S. J. National Chem. Lab. for Industry. 58: 99-107 (1993).

7) Daban, J.R., Montserrat, S. and Bartolome, S. Anal. Biochem., 199, 162-168 (1991)

8) Shimada, K. and Cheftel, J.C. J. Agric. Food Chem., 36, 147-153 (1988).

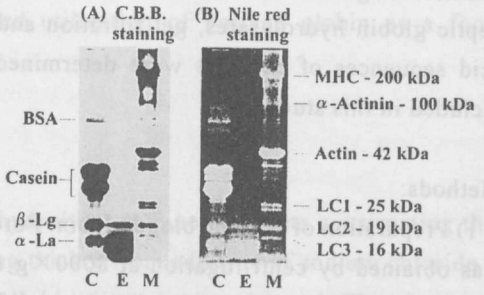

Fig. 1 SDS-PAGE of milk protein samples.

C, concentrated skim milk protein (CMP); E, enzyme treated CMP; M, myofibril (marker); BSA, bovine serum albumin; β-Lg, β-lactoglobulin; α-La, α-lactalbumin; MHC, myosin heavy chain; LC, myosin light chain.

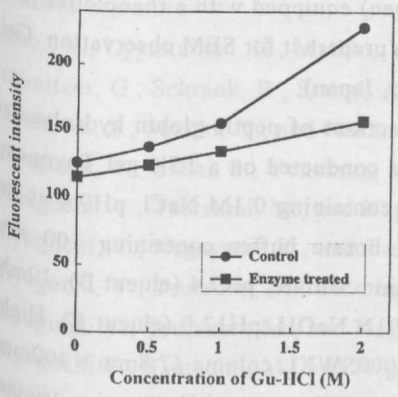

Fig. 2 Effects of Gu-HCl on fluorescent intensity of protein solution (pH 7.0).

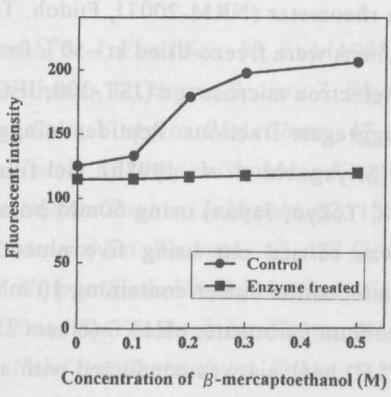

Fig. 3 Effects of β-mercaptoethanol on fluorescent intensity of protein solution (pH 7.0).

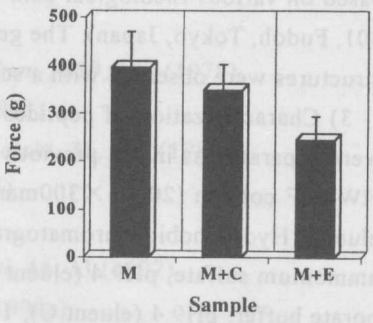

Fig. 4 Effects of milk on breaking force of beef sausage.

M, meat alone; C, control (CSP); E, enzyme treated CSP.