Summary of Long-term Bilateral research project report

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Presently, there is a growing interest about the use of food protein-derived peptides against chronic human diseases and for maintenance of general well-being. Circulatory hormone angiotensin, an important effector of the renin-angiotensin system influences in metabolic abnormality, closely related to oxidative stress and inflammation. The contribution of food protein derived peptides to protect health from oxidative stress as well as control angiotensin converting inhibitory activity are noteworthy.

In my research stay in Hungary, I emphasized to prepare low-molecular weight of peptides with antioxidant capacity, and anti-angiotensin converting enzyme activity and hypoallergenic property from soybean milk through enzymatic route. In the investigation, two different enzymes, such as trypsin (serin protease) and papain (cysteine protease) were used. Enzymatic hydrolysis of soybean milk proteins with cysteine protease papain and trypsin was carried out by measuring 0.008g/L, 0.016 g/L, 0.032 g/L, and 0.064 g/L of enzyme in soybean milk which represents the enzyme and substrate ratios ranging from 0.029:100–0.229:100.

The degree of hydrolysis was measured by tricarboxylic acid (5% (v/v)) soluble nitrogen content. The degree of hydrolysis of soybean milk proteins was found to increase with increase of enzyme to substrate (soybean milk protein) ratio. The degree of hydrolysis was 3.6 ± 0.15, 5.75 ± 0.90, 11.09 ± 0.60 and 17.48 ± 0.61 when ratio of papain and soybean milk protein was 0.029:100, 0.057:100, 0.114:100, and 0.229: 100. It was observed that a partial hydrolysis was occurred at higher ratio of trypsin and soybean milk protein, i.e. 0.229: 100. Hydrolysis potentiality of papain and trypsin was measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis. It was observed that papain is more efficient than trypsin to hydrolyze the soybean milk protein. When papain was applied to hydrolyze the soybean milk protein, the large molecular weight protein subunits are converted to low molecular weight peptides with an increase in enzyme to substrate ratio. As example, 75 kDa protein subunit (β-conglycinin, α') was observed to be completely hydrolysed in papain hydrolysed soybean milk samples, when the enzyme to substrate ratio was increased from 0.029:100 to 0.229:100 indicating the formation of low molecular weight peptides.

Allergenicity of native soybean milk and enzyme-treated soybean milk was carried out by immunoblotting assay. Immune-reactive soybean milk proteins were identified with clinically proved soybean milk positive pooled human serum and peroxidase-labeled anti-human

Immunoglobulin E. Allergenicity was reduced when papain to soybean milk protein ratio was increased from 0.029:100 to 0.229:100. Unfortunately, allergenicity was not reduced when trypsin was used for hydrolysis of soybean milk protein.

Therefore, papain treated soybean milk was considered to understand the change of antioxidant capacity and anti-angiotensin converting enzyme activity. Antioxidant capacity of papain hydrolyzed milk was carried out by ferric reducing ability of plasma (FRAP) assay and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay. Papain prefers to cleave at (hydrophobic amino acid)-(Arg or Lys or Glu or His or Gly or Tyr) in protein structure. With the increase of the concentration of papain, more amounts of peptide bonds in soybean milk proteins were hydrolyzed and hydrophobic amino acids were exposed. Antioxidant capacity of papain treated soybean milk had a direct linear correlation with the degree of hydrolysis. Therefore, papain-treated soybean milk with higher degree of hydrolysis of protein offered higher antioxidant capacity.

Inhibition of angiotensin converting enzyme activity of native soybean milk and papain treated soybean milk was measured considering substrate Abz-FRK(Dnp)-P and recombinant angiotensin-converting enzyme. Inhibition of angiotensin converting enzyme activity in an unhydrolyzed soybean milk protein was 76 % and when soybean milk protein was treated with papain percentage inhibition was increased. Percentage of inhibition of angiotensin converting enzyme was increased to 80% to 92% when ratio of papain and soybean milk protein was increased from 0.029:100 to 0.229:100. It can be justified by the fact that interaction between active side of angiotensin converting enzyme and native soybean milk proteins might not be facilitated, because steric hindrance might present. Low molecular weight peptides, produced due to hydrolysis of soybean milk protein have a higher charge-to-mass ratio than larger peptides. Low molecular weight of peptides (acts as inhibitor) have a strong binding affinity to the angiotensin I converting enzyme and changes the confirmational structure of enzyme, which is not suitable for reaction between enzyme (angiotensin converting enzyme) and substrate. Likewise, the type and mechanism of inhibition were determined using Lineweaver–Burk plot. The result showed that the type of inhibition is non-competitive inhibition. The kinetic parameter K_m value of 18.2 μM was recorded, and it remained unchanged while the value of *Vmax* was varied.

From the experimental results, it may conclude that papain treatment to soybean milk protein can reduce the allergenicity and increase the biological activities, such as antioxidant capacity and angiotensin converting enzyme inhibitory activity. Therefore, it may believe that results of present investigation will reduce the limitations of soybean milk consumption and will receive an attention in food and nutrition context.