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Cholesterol Lowering Mechanism of Soy Protein Hydrolysates Prepared with *Meju* Proteases

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Cardiovascular disease (CVD) is the leading cause of death in the developed world. Significant research efforts focusing on the prevention and treatment of this disease have identified elevated plasma cholesterol as a primary risk factor for CVD. There is increasing evidence that consumption of soybean protein in place of animal protein lowers blood cholesterol level. A meta-analysis of controlled clinical studies concluded that substituting soy protein for animal protein significantly lowered total cholesterol, LDL cholesterol, and triglycerides without effecting HDL cholesterol. In 1999, the Food and Drug Administration approved a CVD reduction claim for soybean protein and this has led to increased interest in identification of the responsible moiety. However, the component and molecular mechanisms of the soybean protein responsible for these change has yet been defined.

It has been argued that there is an interruption in intestinal absorption of bile acids and dietary cholesterol when soybean protein is consumed. Lovati et al postulated that hepatic metabolism of low-density lipoprotein (LDL) cholesterol is altered when soybean protein is ingested. Studies in humans and in animal models have suggested that soybean protein might be linked to the direct activation of LDL receptors in liver cells. Other studies in animals have shown that soybean protein consumption changes activities of HMG-CoA reductase and cholesterol 7-hydroxylase.

In this study, we set-up a hypothesis that proteases from traditional soybean fermentation starter (*Meju*) is suitable for making cholesterol lowering soybean protein hydrolysates (SPHs). We have isolated several strains from *Meju* and one promising strain, *Bacillus amyloliquefaciens* FSE-68 was identified on the basis of biophysical tests and 16S rRNA gene sequence. A neutral metallo-protease (NPR68) and an alkaline serine-protease (APR68) were purified by two step purification, first by ammonium sulfate precipitation and then cation exchange chromatography. Six types of SPH were used for this experiment; F1 type SPHs (F1-5, F1-10 and F1-15; hydrolyzed with NPR68 to DH 5, 10 and 15%) and F2 type SPHs (F2-5, F2-10 and F2-15; hydrolyzed with APR68 to DH 5, 10 and 15%).

Many potential mechanisms for cholesterol lowering effect for SPH were suggested, and among the mechanisms three approaches were used to clarify the mechanism and to screen cholesterol lowering SPHs; (1) inhibition of bile acid and/or cholesterol absorption, (2) inhibition of cholesterol synthesis, and (3) stimulation of low-density lipoprotein receptor (LDL-R) transcription. When human hepatic cells were incubated with SPHs, LDL-R transcription was strongly stimulated. However, soybean protein and ethanol extract of soy protein did not show any activity. Among the six types of soy protein hydrolysate, F1-15 showed the strongest effect and addition of F1-15 to human hepatic cells at a concentration from 10 to 200 μg/ml caused a significant dose-dependent rise of LDL-R transcription, which was increased from 120.4% ± 3.7% to 238.0 ± 12.4%. F1-15 was fractionated with GPC and each fraction was tested. The peptide fractions molecular weight between 200 Da and 3,000 Da only showed LDL-R stimulating activity. These finding represent that peptide moiety is likely to be responsible for cholesterol lowering effect of soy protein.