Description of research outcome
Prepared by: Dr. Arijit Nath
Scholarship type: Hungarian State Scholarships for Short-term or Long-term Study visits

Background art: Food protein-derived bioactive peptides with unique biological values have been catapulted to large numbers of consumers. Soybean is cultivated around the world due to its unique nutritional and economic importance. Therefore, it may believe that there is a wide opportunity to prepare bioactive peptides from soybean protein. Soybean protein-derived peptides play an important role in regulation of metabolic pathways and have a significant contribution in modulation of metabolic syndrome. A cluster of biochemical, physiological and clinical abnormalities is associated with metabolic syndrome that leads to several health hazards and may even cause death.

Objective: The aim of proposed research project is production of bioactive peptides from soybean milk protein through microbial membrane bioreactor.

Method: Two different *Lactobacillus* species, such as *Lactobacillus plantarum* and *Lactobacillus casei* was used for hydrolysis of proteins in soybean milk. In first step, individual microbe was inolucated to sterile soybean milk for hydrolysis of protein. Batch mode ferementation was performed for 48 h at temperature 35 ^{o}C. Microbial growth with time was observed by 600 nm with a fluorescent plate reader (BMG Labtech, Ortenberg, Germany). Samples were collected every 2 h intervals and centrifuged for 10 min at temperature 5 ^{o}C by a laboratory centrifuge (Hermle, Gosheim, Germany).

In later exercise, an in-house developed membrane bioreactor, i.e., a bioreactor with an external membrane separation unit, was adopted for preparation of antioxidant, anti-angiotensin and antibacterial peptides with molecular weight lower than 5 kDa from microbial hydrolysis of soybean milk protein. An advanced-controlled bioreactor (Solida Biotech, München, Germany) was fitted with a cross-flow single-channel tubular membrane module, made of stainless steel (SS316). Microbial hydrolysis of soybean milk protein was performed in bioreactor with selected Lactobacillus strain for 48 h at temperature 35 ^{o}C. 1.5 L of fermentation broth was used in bioreactor and volume reduction ratio (VCR) was maintained 2. Hypoallergenic, antioxidant, anti-angiotensin and antibacterial peptides were passed through the porous channel of ultrafiltration membrane as permeate. In the membrane separation unit, effect of operational trans-membrane pressure (TMP) and retentate flow rate (Q_R) were studied. The effect of static turbulence promoter in filtration process was investigated. TMPs of 2 bar and 3 bar were studied with constant Q_R of 100 L/h. Furthermore, Q_R of 100 L/h and 200 L/h were studied with constant TMP of 3 bar.

To understand the molecular weight of soybean proteins and soybean-based peptides in membrane permeate, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In membrane permeate, the antioxidant capacity, anti-angiotensin activity, antibacterial activity and

allergenic activity were measured. The antioxidant capacity was measured by the ferric reducing ability of plasma (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays. The anti-angiotensin enzyme activity was measured by the recombinant angiotensin converting enzyme and substrate (Abz-FRK(Dnp)-P). The antibacterial activity was measured against *Bacillus cereus* by disc-diffusion method. The allergenic activity of peptides in membrane soybean milk protein was measured by ELISA method.

Results: Peptides with molecular weight less than 5 kDa were produced by mentioned membrane bioreactor. Permeate flux was reduced with increase of the filtration time due to the formation of concentration polarization layer on membrane surface. Permeate flux was increased with increase of the TMP; however, permeate flux was not significantly changed with Q_R. Positive effect of static turbulant promoter was noted on permeate flux. Peptides with hydrophobic amino acids offer better reducing activity towards ferric ions and suppress their pro-oxidant activity than native proteins. Membrane permeate offered anti-angiotensin activity. The mechanism of anti-angiotensin activity was non-competitive. Membrane permeate offered antibacterial activity against *Bacillus cereus.* Furthermore, allergenicity was reduced more than 99% in membrane permeate compared to unhydrolyzed soybean milk. No significant differences of mentioned biochemical activities were noted for two different Lactobacillus species.

Conclusion: In the past two decades, the attention of food and feed industries has been shifted. An attention has been placed on significant income with new perspective along with the marketing of regular food staffs. Therefore, the production of hypoallergenic peptides with unique functional value may receive an attention in food industries. However, the present research was performed in a laboratory-scale setup, it may believe that the results will provide an initial idea of the industrial scale production of bioactive peptides from soybean milk.