Purification of Hypocholesterolemic Peptides and Antihyperlipidemic Effect of Fermented *Chlorella* Hydrolysate Extracted by High Hydrostatic Pressure-Assisted Atmospheric Pressure Plasma

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Abstract

Statins are a class of clinical drug that often used to treat hyperlipidemia to inhibit the initial rate-limiting enzyme in cholesterol synthesis, HMGR (3-Hydroxy-3-methylglutaryl-coenzyme a reductase, HMGR). However, they may cause side effects such as muscle-related diseases. Therefore, the purpose of this study was to disrupt the walls of *Chlorella vulgaris* using HPP (High hydrostatic pressure processing, HPP) combined with APPJ (Atmospheric pressure plasma jet, APPJ) to prepare statin-like peptides. The potential HMGR-inhibiting active peptides were prepare by fermentation and hydrolysis, and analysised by *in vitro* and *in vivo* tests. *Chlorella* treated with HHP (600 MPa, 3 min) before APPJ (10 kV, 15 min) treatment (HA) showed the highest cell wall disruption 11.78 mg/g of soluble protein, total phenolic compounds content is 1011.78 μg GAEs/g and cell wall disruption ratio in 68.8%. HA was added with lactic acid bacteria, then fermented at 42 °C for 0-24 hrs, found that the pH values decreased from 7.08 to 4.84; and the lactic acid bacteria content increased from 2.0×10^5 to 1.2×10^8 CFU/mL. Considering the optimal pH value was required for enzyme Protease N for further hydrolysis, fermentation time of 8 hrs was selected. HAFs were hydrolyzed with Protease N under high hydrostatic pressure (100 MPa) for 0, 3, 6 and 24 hrs (HAFH-0~24). The HAFH-24 had the best hydrolysis effects among all the groups, containing soluble protein, peptides and free amino acids with amount of 342.59, 473.52 and 407.36 mg/g respectively. In addition, the hydrolyzate of HAFH-24 group had the best retention effect in terms of peptide contents after (HAFH24-PP) gastrointestinal simulation (503.18 mg/g) and HMGR inhibitory ability (77.03%). HAFH24-PP was further separated by gel permeation chromatography into 6 fractions. The fraction E (molecular weight range about 210-290 Da) had the highest IER (Inhibitory efficiency ratio) of 18580 %/mg/mL, which means fractions E had the highest HMGR inhibitory ability per unit of peptide. On the other hand, preliminary result of animal experiments showed that *Chlorella* hydrolysates were effective for the reduced TG (Triglyceride, TG) content in mice liver range from 14 to 49%. Further experiments will separate and purify the fraction E of the HAFH24-PP, and identify peptides with the sequence that would regulate cholesterol activity by inhibiting HMGR. Then, we will predict the interaction of the peptide sequence with HMGR. Finally, the blood and feces from mice, will be analyzed for the hydrolysate effect on regulating cholesterol lead by *in vitro* test.