RETASTE Conference Abstracts
Vol. 3 RETASTE-APP-490-Poster
Athens, Greece, 27-29 September, 2023

rethink food resources, losses, and waste 2023 retaste

Bioactive Peptides from Chicken Protein Hydrolysate: Evaluation of Toxicity, Efficacy and Application in Muscle Foods

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Abstract

With the tremendous growth in the layer market of the poultry sector, the main concern has been the generation of a high number of spent hens. These spent hens could not be utilized as meat due to their hard texture and lower tenderness due to increased cross-linkages in the collagen network. Hence, spent hens are rendered 'waste' by the poultry industry which may cause environmental pollution if discarded inappropriately. In this work, spent hen meat protein was hydrolyzed using Flavourzyme (Fz) and Alcalase (Ac) at different concentrations (1, 2, 3% for 6 h). The hydrolysate obtained at optimum process conditions was spray- and freeze-dried and subjected to detailed characterization. Results showed that the highest (23.38%) and lowest (10.68%) degree of hydrolysis (DH) was obtained with 3% Ac and 1% Fz, respectively. FTIR spectroscopy clearly revealed changes in the secondary structure of proteins. SDS PAGE profiling of hydrolysates showed that Fz produces low molecular weight peptides (2–75 kDa) as compared to Ac. Particle size and morphology of the dried hydrolysate powders were more prominently affected by the drying method while the enzyme type influenced the functional properties. The solubility, antioxidant activity, and bioaccessibility of spray-dried spent hen hydrolysate powder developed using Fz was found superior to those developed using Ac. Hydrophobic amino acids with rich antioxidant activity were also found to be higher in SD-SPH. SPH subjected to sub-acute toxicity study showed no adverse effects on mice physiology as observed from hematological and blood chemical tests. Further, no gross abnormalities or histological alterations were found pathologically. However, results depicted the oxidative stress markers (lipid peroxidation, superoxide dismutase, and glutathione-s-transferase) were found to be elevated at high doses of SPH (6 g/kg body weight i.e., equivalent to 30 g/day for humans) which indicated induction of oxidative stress on mice organ revealing mild toxicity at this dose. Medium and high doses are 5 and 10 times the base dose (0.6 g/kg b.w. of mice). Such high doses are practically impossible to consume but generally evaluated for toxicity. Therefore, SPH can be used as a nutraceutical or for functional food development up to 3 g/kg b.w. (mice dose) or 15 g/day (equivalent SPH human dose). Further, the efficacy study showed no difference in hematological, histopathological and serum biochemical parameters

when compared with control at tested doses. SPH enhanced lymphocyte proliferation (ex vivo) and immuno-stimulation significantly ($p<0.05$). Mice showed improved spatial learning and anxiolytic behaviour after the experimentation period. Results suggest that SPH contains immune stimulant peptides and its dose of 0.6 g/kg body weight can be used as a nutraceutical or for the development of functional food. Considering the high functionality of SPH, it was further used for formulating protein supplements. A whey-based protein supplement was developed using different SPH concentrations. Based on sensory results, SPH concentration of 10% gave the highest overall acceptability. Optimized SPH concentration was then incorporated into a commercial chicken-based protein supplement (CS). After a comparative evaluation of all three supplements, the developed protein supplement (WS) was found to be nutritionally superior when compared with a standard whey (WW) and incorporated protein supplement (CS). Further, WS showed higher antioxidant potential and bioaccessibility when compared with CS and WW due to the presence of low molecular weight peptides in substantial quantities. Sensory analysis revealed that the overall acceptability of the developed protein supplement (WS) was higher than WW and CS. In conclusion, SPH generated through the green route approach has tremendous applications in the functional or health food industry. The novel supplement generated using SPH may be more acceptable to the consumers and could deliver higher functional benefits than traditionally available protein supplements at marginally higher prices.

Keywords: Meat protein, spacial memory, antioxidant, powder properties, hydrolysis