

Production of Bioactive Peptides from Ovotransferrin using Two Enzyme Combinations and Determining the Functional Properties of Its Hydrolysates

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Peptides from food sources possess numerous bioactivities which make them useful in improving human health and preventing diseases. Although many studies related to egg protein hydrolysis are available, less work has been carried out on the production of bioactive peptides from ovotransferrin using two-step enzyme hydrolysis. Objectives of the research were to produce bioactive peptides from ovotransferrin using two enzyme combinations and to determine the functional properties of its hydrolysates. Lipolyzed ovotransferrin was prepared with 20 mg/mL concentration and treated with protease (3hr at 55°C) (Pro), papain (3hr at 37°C) (Pap), elastase (24hr at 37°C) (Ela), and α -chymotrypsin (3hr at 37°C) (Chy) as the first enzyme treatment. Hydrolysate of first step hydrolysis was treated with the above enzymes in different combinations and incubated for 0-24 hours at the optimal temperatures. 15% SDS - PAGE was used to select the best incubation time for each treatment. Best combinations were used to test for antioxidant, metal chelating, and antimicrobial activities using *E. coli* and in Total Plate Count. Protease + Papain (ProPap), Protease + α -chymotrypsin (ProChy), α -chymotrypsin + Protease (ChyPro), α -chymotrypsin + Papain (ChyPap), Elastase + α -chymotrypsin (ElaChy), Elastase + Papain (ElaPap), Elastase + Protease (ElaPro) treatments with immediate enzyme addition and heat treatment for 100°C for 15 minutes, Papain + Protease (PapPro), Papain + α -chymotrypsin (PapChy) incubated for 3h followed with heat treatment were selected as the best. Hydrolysates produced with ChyPap treatment showed some level of metal chelating activity (4.11±0.28%) and Ela (-2.88±0.16%), ElaChy (-7.80±0.28%), and ElaPap (-6.38±0.14) showed metal releasing activities. ChyPap (0.59±0.08 MDA mg/kg) treatment followed by ProChy (0.74±0.07 MDA mg/kg), ProPap (0.78±0.04 MDA mg/kg) and ChyPap treatments showed strongest antioxidant activity than ovotransferrin (0.98±0.07 MDA mg/kg) and hydrolysates produced with single enzyme treatments (p<0.05). However, none of the hydrolysates showed any antimicrobial activities against locally isolated *E. coli* and in Total Plate Count. Accordingly, hydrolysates produced with two enzyme combination treatments showed strong antioxidant and some metal chelating activities which are important in food processing industries.

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