

**PRODUCTION OF BIOACTIVE PEPTIDES FROM  
OVOTRANSFERRIN USING TWO ENZYME  
COMBINATIONS AND DETERMINING THE  
FUNCTIONAL PROPERTIES OF ITS  
HYDROLYSATES**

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by

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## ABSTRACT

Peptides from food sources possess numerous bioactivities, which make them useful in improving human health. Although many studies related to egg protein hydrolysis are present, less work has been carried out with the production of bioactive peptides from ovotransferrin using a two-step enzyme combination. The research objectives were to produce bioactive peptides from ovotransferrin using two enzyme combinations and determine the functional properties of its hydrolysates. Lipolyzed ovotransferrin solution (20 mg/mL) was treated with protease (3hr/55oC), papain (3hr/37oC), elastase (24hr37oC), and  $\alpha$ -chymotrypsin (3hr/37oC) as the first treatment identified previously. Produced hydrolysates were treated with the above enzymes in different combinations and incubated for 0-24 hours at the optimal pH and temperatures, followed by heat inactivation at 100oC for 15 min. 15% SDS-PAGE was used to select the best conditions and analyze antioxidant, metal chelating, and antimicrobial activities. Protease + Papain (ProPap), Protease +  $\alpha$ -chymotrypsin (ProChy),  $\alpha$ -chymotrypsin + Protease (ChyPro),  $\alpha$ -chymotrypsin + Papain (ChyPap), Elastase +  $\alpha$ -chymotrypsin (ElaChy), Elastase + Papain (ElaPap), Elastase + Protease (ElaPro) treatments as 0h (since all band patterns in the gel were same), Papain + Protease (PapPro), Papain +  $\alpha$ -chymotrypsin (PapChy) treatments for 3h were selected as the best conditions. Hydrolysates produced with ChyPap treatment showed some level of metal chelating activity ( $4.11\pm 0.28\%$ ), and Ela ( $-2.88\pm 0.16\%$ ), ElaChy ( $-7.80\pm 0.28\%$ ), and ElaChy ( $6.38\pm 0.14$ ) showed metal releasing activities. ChyPap ( $0.59\pm 0.08$  MDA mg/kg) treatment followed by ProChy ( $0.74\pm 0.07$  MDA mg/kg), ProPap ( $0.78\pm 0.04$  MDA mg/kg) and ChyPap treatments showed strongest antioxidant activity than ovotransferrin ( $0.98\pm 0.07$  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 agar. Accordingly, hydrolysates produced with two enzyme combinations as ChyPap, ProChy, and ProPap showed strong antioxidant and some metal chelating activities.

**Keywords:** Enzyme combination, Hydrolysates, Ovotransferrin, TBAR