Isolation and characterization of bioactive peptides from hydrolysates of whey protein from Iraqi buffalo milk and tested its antioxidant activity

A Thesis Submitted to

The College of Agriculture -University of Basrah

In Partial Fulfillment of the Requirements for the Master Degree

In Agriculture Science in Food Sciences

By

Orass Tariq Yasseen El-Ibresam

B.Sc. Agriculture Sciences Food Sciences

2000 A.D.

Supervised by

Prof. Dr. Ali khudhair Jaber AL-Rikabi

2021 A.D. **1443 A.H.**

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Summary

The study included the exploitation of Iraqi buffalo milk whey produced by the cheese industry by the enzymatic method to obtain a protein concentrate and using it in the preparation of bioactive peptides and testing their antioxidant activity, and the results of the current study reached the following:

1- Separation of whey proteins by ultrafiltration with a pore size of 10 KDa then concentrate them in a rotary evaporator and dry them by lyophilization method, obtaining a whey protein concentrate, studying the physicochemical properties of whey before and after ultrafiltration and for the lyophilized whey protein concentrate. The results showed a high the percentage of protein in the lyophilized whey protein concentrate was 85.57%, and the percentage of lactose, salt and moisture was low.

2- Using Pepsin, Alcalase and Papain enzymes in the process of hydrolysis the center of lyophilized whey proteins and following up the degree of hydrolysis for four hours to determine the best time for hydrolysis. Alcalase enzyme showed the highest hydrolysis degree of 39.62%, followed by Pepsin 29.40% and Papian 37.27% enzyme at 240 minutes and calculating the rate peptide chain length.

3- Antioxidant activity tests were carried out on lyophilized proteolytes to determine the most effective hydrolysate, which was able to bind ferrous ion, capacity for reducing power, DPPH radical capture, and hydrogen peroxide. Also, the activity of Alcalase hydrolysate was higher than Pepsin and Papain 79.07%, 82.48%, 157.17%, and 89.56%, respectively, at the highest concentration of 25 mg/ml.

4- Separation, purification and characterization of Alcalase peptides using gel filtration technique and five peaks were obtained. The peptides of the first and

second peaks showed high antioxidant activity compared to the rest of the peaks, which amounted to 84.31% and 81.23%, respectively. The content of the two peaks peptides was estimated of the amino acids, it was found that they contain most of the amino acids in different proportions. The molecular weight of the two peaks was estimated using the electrophoresis technique, where bundles appeared at the first peak whose weights ranged from (9-83) KDa. and bundles at the second peak whose weights ranged from (7-19) KDa.

5- The antioxidant activity tests of Alcalase1 and Alcalase2 were conducted, where the first peak showed a higher activity in the ability to bind ferrous ion and the capacity of reducing power, and it reached 82.75% and 141.35%, respectively, at a concentration of 3 mg/ml, while the second peak was superior in ability to DPPH root capture and hydrogen peroxide capture were recorded as 90.13% and 71.62% at the same concentration.

6- The stability of the antioxidant activity of Alcalase1 and Alcalase2 was tested by DPPH root scavenging method at a concentration of 3 mg/ml. The first and second peaks recorded the highest stability at pH 7, reaching 82.19% and 86.08%, respectively, and at a temperature of 60°C it was 85.16%. and 87.41% and at a saline concentration of NaCl 4% recorded 80.79% and 83.91%, respectively.

7- The peroxide number was estimated for the beef tablets treated with different concentrations of the hydrolyzed prepared enzyme Alcalase and the lowest value for the peroxide number at the concentration of 400 mg / 100 g of beef was about 3.877 mEq / kg oil on the 15 day compared with the control sample, which amounted to 13.460 mEq\ kg oil.