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Isolation, purification and characterization of the protease of *Pseudomonas fluorescens* ISH and its role in deterioration of Iraqi soft cheese

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Abstract

The presence of psychrotrophic *Pseudomonas fluorescens* was investigated in samples of soft cheese that produced in the dairy plant of college of Agriculture - University of Baghdad.

Depending on the cultural, morphological and biochemical tests, 24 isolates was diagnosed as proteolytic bacteria. The diagnosis was confirmed by Vitek 2 compact system.

The More efficient isolates were selected to quantitative investigation of the protease production, and the local isolate *P. fluorescens* ISH had been selected based on their productivity of the enzyme comparing with other isolates (117.8 units/mg protein) , and thus it was used in the current study to produce the enzyme by submerged cultures.

The optimum conditions for the protease production were the use of Minimal salts medium with 1% skim milk and pH 8 at a 15^{O}C for 96 hours and the inoculum size 1 x 10^{7} using a shaking incubator at 125rpm of speed.

The enzyme was purified by ammonium sulphate precipitation with saturation rate 30-80%, and ion exchange chromatography on DEAE-Sepharose column and gel filtration on Sephacryl S-200, with 18.6 fold and 32.8% recovery.

The results of the enzyme characterization showed that the estimated molecular weight was 47.2 kDa by gel filtration and 47.8 kDa by SDS-PAGE, with 2.6% Carbohydrate. The optimum pH of activity was 8.0 and optimum pH of stability was 7.5. The optimum temperature for the activity

was 40^{O}C, and the enzyme was retained its original activity after incubation for 10 minutes at temperatures ranging from 30-40 ^{O}C.

The enzyme showed a significant affinity toward the casein as a substrate, with activity of 215 units/ml, compared with 86, 43 and 24 units/ml for gelatin, collagen and BSA respectively.

The Km of the enzyme was 1.15 mg/ml (0.48 mM), while the value of Vmax and Kcat was 111.2 mM/min and 614.12 min^{-1} respectively. The activation energy was 8.5 kcal/mole toward casein.

the protease was metalloprotease because it was not affected by pepstatin A, PMSF, aprotinin, soybean inhibitor (SBTI) and E 64, but lost its activity completely when using the chelating agent EDTA, and the using of 1,10-Phenanthroline inhibited the enzyme completely, was evidence that its belonging to zinc metalloprotease. The activity was affected by the existence of DTT; evidence that the enzyme containing disulphid bonds. And the results of testing the effect of detergents on the activity showed that the enzyme was not lipoprotein. And the enzyme showed high stability in presence of DMSO.

Use of calcium ions led to increase the activity, while the cobalt and manganese did not affect, and there was a decrease in the activity when the enzyme was treated with mercury, copper, silver, zinc and nickel.

Hydrophobic amino acid formed 37.6% of the total amino acids, and the low molecular weight amino acids formed 20%, while the percentage of Proline 14.4% and Cysteine 6.8%.

The protease showed a high affinity toward the β-casein, the activity was 99 , 139 , 185, 206 , 232 and 239 units/ml after 5 , 10, 15 , 20 , 25 and 30 minutes respectively , compared with its activity towards αs-casein which was 91 , 108, 115 , 129, 136 and 145 units/ml, and towards k-casein , which was 66 , 71, 79 , 91, 102 and 117 units/ml after 5 , 10, 15 , 20 , 25 and 30 minutes, respectively.

The percentage of soluble nitrogen of total protein, and the percentage of non-protein nitrogen of total protein were increased in the cheese manufactured from the milk that treated with protease, and the cheese manufactured from milk that incubated in cold storage for 24 hours then it was pasteurized in 63^{O}C for 30 minutes. And the percentages were decreased when using the temperature of 75^{O}C for 10 minutes in pasteurization.

The sensory evaluation results showed superiority of cheese transactions which manufactured from milk that pasteurized at 75^{O}C for 10

minutes, compared with cheese manufactured from milk that pasteurized in 63^{O}C for 30 minutes.