STUDIES ON ACCELERATION OF RAS CHEESE RIPENING USING PROTEOLYTIC ENZYMES

By

ADEL MAHMOUD MOHAMED KHOLIF

B.Sc. Agric. Sc. (Dairying), Al-Azhar University, 1999

M. Sc. Agric. Sc.(Dairy Science and Technology)

Ain Shams University, 2010

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ABSTRACT

Adel Mahmoud Mohamed Kholif: Studies on Acceleration of Ras Cheese Ripening Using Proteolytic Enzymes. Unpublished Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2017.

Milk clotting enzyme (MCE) from Fruit Seeds of *Solanum elaeagnifolium* plant, which has the capacity of forming milk curds was obtained by fractional precipitation with ammonium sulphate. The extracted enzyme was purified by using sequential chromatographic technique of the most active fraction on Sephadex G 200 to 4.21 folds with 4 % recovery.

Molecular weight of the enzyme was found to be 28 kDa, and its optimum temperature was 40 °C. The enzyme activity was stable at 40 to 60 °C with incubation times from 10-30 min. The enzyme showed the pH optima of 5.9, and quite stable in broad pH range of 4.0 to 6.5.

Effect of calcium chloride ($CaCl_2$) and sodium chloride (NaCl) at a concentration of 20 mM and 2 % gave the highest relative activity of the purified MCE about 1.31 and 1.10 fold, respectively. At a concentration of 10 mM and 1 % the enzyme gave the highest relative activity to proteolytic about 1.08 and 1.05 fold respectively.

The metal ions at 1 mM Zn^{2+}, Ba^{2+}, EDTA, Mg^{2+}, Mn^{2+} were activators, whereas Fe^{2+}, Mg^{3+}, Ni^{2+} were inhibitors of the purified MCE. The Cu^{2+} at 1 mM was most effective stimulator, 5 mM Ba^{2+} was activator, whereas Zn^{2+}, EDTA, Mg^{2+}, Cu^{2+}, Mn^{2+}, Fe^{2+}, Mg^{3+}, Ni^{2+} at 5 mM were inhibitors. The Mg^{3+}, Ni^{2+} at 5mM were the most inhibitors of the purified MCE and showed a typical hyperbolic velocity saturation curve with Km value of 0.0399 % with skim milk as a substrate.

Upon storage of the purified enzyme for 15 days at refrigerator temperature and room temperature, it retained 90.39 % and 75.34 % of MCA respectively.

Thus the obtained MCE was chosen to be used in a further study as enzyme for accelerating Ras cheese ripening made from buffalo's milk.

Three Ras cheese treatments were made from buffalo's milk. All cheese treatments made with traditional cheese starters (*Lactococcus lactis* spp *lactis* and *Lactococcus lactis* spp *cremoris*, 1:1). The first was a control cheese made with microbial rennet (Chy-Max. Chr Hansen, Hoersholm, Danmark). The other two treatments, T1 was made by using the veal rennet and T2 made by using the purified coagulant selected from the part I**.** All cheeses were stored at 12-14 ºC for 120 days for ripening, and were examined periodically for some chemical, textural profile analysis. and organoleptical properties.

The obtained results indicated that Ras cheese T2 contained significantly lower ($p < 0.01$) moisture than in the other cheese treatments, Also increased acidity ratio than in the other cheese treatments, and contained increased in soluble nitrogen (SN), non protein nitrogen (NPN), soluble tyrosine and soluble tryptophan ratio than in the other cheese treatments. Regarding the rate of accumulation of total volatile fatty acids (TVFA), it was increased with the increase of the ripening period in all Ras cheese treatments.

In all treatments, cheese acceptability increased during the first period (30 days) of ripening as well as at the other ripening periods (60, 90 and 120 days). However, the improvement was slow in T1, while it was more faster in T2. Cheese samples of T2 gained the highest score at 3 month of ripening, while the other treatments reached the same degree of ripening at 4 months.

It could be concluded that, good characterizes Ras cheese can be produced from heat treated buffalo's milk using the purified coagulant from *S. elaeagnifolium* fruit seeds with addition of traditional starter.

Key words: Milk clotting activity, proteolytic activity, fruit seeds plants, *Solanum elaeagnifolum*, calf rennet, microbial rennet, purification of milk clotting enzyme, Ras cheese, cheese ripening.

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