

Title: THERMORESISTANCE OF PROTEOLYTIC ENZYMES PRODUCED BY PSYCHROTROPHIC BACTERIA ISOLATED FROM BUFFALO RAW MILK REFRIGERATED

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Abstract:

Psychrotrophic bacteria may be present in raw milk, producing hydrolytic enzymes. These enzymes, unlike bacteria, are not eliminated by the heat treatment process, remaining milk and causing change in quality of the final dairy product. Thus, this study aimed to evaluate the heat resistance and the milk clotting ability of proteolytic enzymes of 21 bacteria isolated from raw milk from buffalo cooled. Bacterial isolates were grown on 50 ml of sterile skim milk and incubated at 30 ° C in a shaker at 200 rpm. A volume of 15 ml was centrifuged at 1,900 x g for 10 min and the supernatant was separated. An aliquot of 1 ml of bacterial crude extract was treated 63.5°C temperature for 30 min, and another 1 ml aliquot remained without heat treatment for the same period. After the treatments in both samples they add 110 µL of 1% sodium azide, to ensure the inactivation of bacterial cells. The crude extract of heat-treated and non-treated were mixed with bovine milk pasteurization in tubes to achieve a final activity of 5 U / ml. Proteolytic enzyme was considered positive from the milk clotting times in the display 6, 18, 24, 48, 72, 96 and 120 hours at temperatures of 7 and 30 ° C. As a negative control, only milk was incubated with addition of 110 µL of 1% sodium azide; for the positive control was used the enzyme chymosin. Of the 21 bacterial evaluated five enzymes that produce the fully clotted bovine milk after five days of observation at both incubation temperatures. Proteolysis produced by two of these isolates coagulated milk in the first 6 hours to a temperature of 30 ° C. It may be noted that the (s) enzyme (s) produced (s) for these isolates is heat resistant, remaining active and effectively hydrolyzing using milk proteins even after the pasteurization treatment (63.5 ° C). This heat resistance presented by these enzymes occurs by reorganization capacity of protein tertiary structure that is damaged during pasteurization, making it active. The results demonstrate that the bacterial isolates of raw milk of buffalo have extracellular enzyme production, that are heat resistant. This may cause to hydrolyze proteins even after the thermal treatments, and can thereby reduce the final quality of dairy products.

Keywords: psychrotrophic bacteria, proteolysis, heat resistance, coagulation.

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