5. SUMMARY

Production of the enzyme β-D-galactosidase (β-D-galactosidase galactohydrolase, E.C.3.2.1.23) from *Kluyveromyces marxianus* has been investigated under different incubation periods and different temperatures. Besides, that evaluation of the produced enzyme to obtain their optimum conditions. Also, the immobilization of crude β-galactosidase can be achieved in many ways, which their techniques are based on the structure of enzyme and the nature of support. β-D-galactosidase enzyme from *K. marxianus* has been immobilized on different supports, i.e. sand, chitin, Ca-alginate gel beads and concanavalin A-sepharose (Con A-S) and different techniques including adsorption, matrix entrapment and covalent binding. Also, kinetic parameters for soluble and immobilized forms of lactase enzyme were determined. Besides, that application of immobilized enzyme for continuous conversion of lactose, this point is very important in an industrial applications.

The obtained results indicated that the supernatant β-galactosidase activity increased continuously as the incubation period increased up to 9 days, where the highest activity was achieved. The high level activity of β-galactosidase was 5.36 unit/ml after 9 days incubation at 30°C. Thus, the optimum temperature for incubation was found to be 30°C where the supernatant of growth medium showed the higher enzyme activity.

Evaluation of the produced β-galactosidase to obtain their optimum conditions were estimated. The optimum temperature

was 55°C, and reaction activity reached 1456.9 mg glucose/L (72.85 mg/ml/min). While, the effect pH values on the native β-galactosidase activity indicated that the optimum pH was 6.2 and the reaction activity equaled 73.68 mg/ml/min. On the other hand, the effect of enzyme concentration on the relative activity by using lactose as standard was studied. The obtained results indicated that the activity of the crude preparation of β-galactosidase enzyme reached its maximum 82.62 mg/ml/min.

The effect of substrate concentration on the rate of lactose hydrolysis was studied. The results showed that the reaction velocity to convert lactose to glucose was increased up to 3.5 g/L and the maximum reaction velocity (V_{max}) of 845.64 and the Michaels constant was 1.30 g/L.

Effect of immobilization techniques with different supports on the relative activity of the produced β-galactosidase were determined. The obtained data indicated that the enzyme was bounded with all the evaluated different supports but with different retention activities. The retention activities were 67.10, 75.00, 70.83 and 85.00% for Ca-alginate gel beads, sand, chitin and Con A-sepharose enzyme complex, respectively. While, the optimum factors influence the immobilized β-galactosidase within Ca-alginate, stability and reuse of immobilized preparations on other different supports were studied.

The optimum pH value of immobilized form was 6.4 revealed that the maximum amount of glucose concentration was 215.2 mg glucose/ml. On the other hand, Ca-alginate β-galactosidase complex had optimum temperature of 50°C with maximum activity of 24.83 mg glucose/ml/min/25 beads.

The rate of the reaction velocity of immobilized enzyme with different substrate concentrations was measured.

The maximum reaction velocity (V_{max}) was found to be 734.84 at a substrate concentration of 4.0 g/L for the immobilized β-galactosidase with Ca-alginate gel beads. Michaeles-Menten constant (K_m) for immobilized form was determined to be 1.5 g/L and compared with native enzyme (1.3 g/L).

Stability of the immobilized β-galactosidase on Ca-alginate gel beads, sand, chitin and concanavalin A-separose were evaluated. On the basis of relative activity, it could be observed that the immobilized β-galactosidase on Ca-alginate, sand and chitin lost 43.8, 42.5 and 40.0% after 96 hr, respectively. But, the percentage loss of relative activity of immobilized form on Con A-sepharose complex reached 7.4% compared with original activity.

The rate of enzymatic hydrolysis of lactose at different incubation periods by using soluble and immobilized forms on different supports were studied. The maximum amounted to 1321.2 mg glucose/ml for native enzyme after 150 min. On the other hand, the high levels of enzymatic rate of lactose (20%) were 410.6, 678.1, 437.1 and 1125.8 mg glucose/ml for immobilized forms on Ca-alginate, sand, chitin and Con A-sepharose after 120, 60, 150 and 150 min, respectively.