List of Figures

General Introduction:

Fig. G1. The α -retaining double displacement mechanism of α -amylase catalytic reaction system (adapted from Kapeko et al., 1998)

Fig G2. Schematic representation of application of amylase.

Chapter 1:

Fig. 1.1. Phenotypic appearance of the strain *Aspergillus niger RBP7* on Czapek dox agar medium

Fig. 1.2. Zone of hydrolysis produced by *RBP7* on starch containing medium was visualized by iodine

Fig. 1.3. Light microscopic picture of newly isolated Aspergillus niger RBP7

Fig. 1.4. SEM image of the strain RBP7 showing the sporangium containing the conidia

Fig. 1.5. Phylogenetic tree based on 18S r DNA gene sequences of the newly isolated strain *Aspergillus niger RBP7*

Chapter 2:

Fig 2.1. Phenotypic appearance (b, d) of potato peels and details surface structural changes of potato peel was determined under SEM. a) Control potato peels and c) potato peels after the fermentation. Orange circle indicate that the closer view of the potato peels surface structure

Fig 2.2. Growth curve of Aspergillus niger RBP7

Fig 2.3. Effect of substrate concentration on acidophilus amylase production of *Aspergillus niger RBP7*. The substrate concentration of the medium was 1gm (%, w/v) in SSF

Fig 2.4. Effect of incubation pH on acidophilus amylase production of *Aspergillus niger RBP7*. The pH of the medium adjusted at 3.0 in SSF

Fig 2.5. Effect of incubation temperature on acidophilus amylase production by *Aspergillus niger RBP7*. The temperature of the medium adjusted at 40 oC in SSF

Fig 2.6. Effect of inoculums concentration on acidophilus amylase production by *Aspergillus niger RBP7*. The inoculums concentration was adjusted to 0.75 ml (% v/w) in SSF

Fig 2.7. Effect of incubation period on acidophilus amylase production by *Aspergillus niger RBP7*. The incubation period was adjusted to 72 h in SSF

Fig 2.8. Scanning electron microscopic observation of (a) sporulation growth and (b) mycellial growth of RBP7

Fig. 2.9. Production of acidophilus α -amylase by SSF in the presence of different concentration of inorganic substances by *Aspergillus niger RBP7*

Fig. 2.10. Response surface and three-dimensional contour plot (SSF) of the combined effects of a) inoculums concentration (conidia/gds) and medium pH, b) temperature (°C) and medium pH on α -amylase production by *Aspergillus niger RBP7*

Fig. 2.11. Effect of incubation period on acidophilus amylase production by *Aspergillus niger RBP7*. The incubation period was adjusted to 120 h in SmF

Fig. 2.12. Effect of substrate concentration on acidophilus amylase production in SmF by *Aspergillus niger RBP7*. The substrate concentration was adjusted at 4gm (%w/v)

Fig. 2.13. Effect of inoculums concentration on acidophilus amylase production in SmF by *Aspergillus niger RBP7*. The inoculums concentration was adjusted to 1.25 ml (% v/v)

Fig. 2.14. Effect of temperature on acidophilus amylase production in SmF by *Aspergillus niger RBP7*. The temperature was adjusted at 35 oC

Fig. 2.15. Effect of pH on acidophilus amylase production in SmF by *Aspergillus niger RBP7*. The pH was adjusted to 4.0

Fig. 2.16. Response surface and three-dimensional contour plot of the combined effects of a) inoculums concentration (conidia/ml) and medium temperature (°C), b) temperature (°C) and medium pH on α -amylase production by *Aspergillus niger RBP7* in SmF

Chapter 3:

Fig. 3 .1. PAGE analysis of each fraction during purification: Z, Zymogram; P, Purified enzyme; C, Crude enzyme; M, Molecular weight marker (kD) after column chromatography

Fig. 3.2. Lineweaver–Burk (a) and Michaelis–Mentens (b) plots of enzyme kinetics of purified α - amylase at optimum pH and temperature

Fig. 3 .3. Percentage loss of starch iodine colour complex in presence of starch as substrate after enzymatic degradation by amylase from *Aspergillus niger RBP7*. Sample were withdrawn at 0, 5, 10, 15, 20, 25, 30 min and processed for formation blue colour in presence of iodine

Fig. 3 .4. Optical rotation of the products formed by the action of the purified amylase from *Aspergillus niger RBP7* in presence of solid sodium carbonate

Fig. 3 .5. Effect of enzyme inhibitors on amylase activity of acidophilic amylase from *Aspergillus niger RBP7.* 1. Control, 2. P- chloromercuro benzoate (10-2M), 3. Iodoacetamide (10-2M). Amylase activity was measured at pH-3.0 and temperature 40 oC.

Fig. 3.6. Maximum activity of acidophilus amylase RBP7 at pH- 3.0

Fig. 3 .7. The stability of purified α - amylase of *Aspergillus niger RBP7* at pHs 2-6 for 60-300 min of incubation

Fig. 3.8. Maximum activity of acidophilus amylase RBP7 at temperature 45 oC

Fig. 3.9. The stability of purified α - amylase of *Aspergillus niger RBP7* at temperatures 20-80 oC for 60-120 min of incubation

Fig. 3.10. Effect of storage time and light sensitivity on stability of α - amylase RBP7

Chapter 4:

Fig. 4.1. Hydrolytic capability of different α -amylase. a) Time course study of the enzymatic hydrolysis of raw food stuffs and production of reducing sugars. b) Paper chromatography analysis of different reducing sugars by α -amylase after hydrolysis of raw foods stuffs

Fig. 4.2. Cell viability test at different concentration of purified amylase

Fig. 4. 3. Morphological character of Vero cell after 48h a) control and b) treated

Chapter 5:

Fig. 5.1. Phylogenetic tree of 36 α -amylase protein sequences from different *Aspergillus niger* strains, constructed with 100 bootstrap values

Fig. 5.2. Phylogenetic tree of 36 α -amylase protein sequences from different *Aspergillus nigers* trains, constructed with 100 bootstrap values

Fig. 5.3. Topology structure of three x-ray crystallographic structures. Arrow indicating the active site residues and green bullet indicated the calcium (Ca2+) binding residues.

Fig. 5.4. (A) Residues responsible for catalytic site formation on three X-ray crystallographic structures of α -amylase. (B) Multiple sequence alignment showing substitution of His (H) by Glu (E) at the alignment position 825 for all the acid α – amylase