Chapter 5

SUMMARY AND CONCLUSION

The present study in the summarized and conclusive form is illustrated as follows:

Three microorganisms, Aspergillus niger NCIM 548, Aspergillus foetidus NCIM 1027 and Trichoderma viridae NCIM 1195 were procured from National Chemical Laboratory (NCL), Pune and were examined for the production of pectinases by the plate assay method. Aspergillus niger NCIM 548 was selected for the production of crude enzyme (pectinases and cellulases).

Preliminary experiments were performed for the selection of natural substrates for the production of crude enzyme using Aspergillus Niger NCIM 548. The wheat bran, corn bran and kinnow peel in 2:1:2 ratio was selected on the basis of enzyme activity for the production of cellulases and pectinases.

The fermentation conditions were optimized for the production of both cellulase and pectinase under submerged (SmF) and solid state fermentation (SSF) using central composite face centered design (CCD) of Response Surface Methodology (RSM). The optimum conditions for maximum production of pectinase and cellulase were, time; 126 h, pH; 4.6, and carbon source concentration; 65 g/L in SmF and were time; 156 h, pH; 4.80, and moisture content; 65% in SSF.

The production of both pectinase and cellulase were higher in SSF as compared to SmF. The data showed that the production of pectinase and cellulase from SSF was 7.13 and 1.95 times higher respectively than SmF.

The partial purification and the characterization of the pectinase and cellulase was carried out in the second section. The crude pectinase and cellulase produced from A.
*niger* was partially purified using ammonium sulphate precipitation. The purification of pectinase and cellulase was observed to the proportion of 1.65 and 1.3 fold respectively and the enzyme recovery of 74.13% and 58.71% was achieved for pectinase and cellulase respectively.

The effect of pH and temperature on the pectinase and cellulase activity of crude enzyme extract was observed. The maximum activity for the pectinase and cellulase was observed at pH 4.5 and 5.0 respectively. The optimum temperature for maximum activity of pectinase and cellulase was 50°C and 60°C respectively. The effect of pectin and carboxymethyl cellulose (CMC) as substrate on the cellulase and pectinase activity was also observed. The values of Michaelis Menten constant (Km) and maximum velocity (Vmax) for pectinase enzyme, were found to be 16.38mg/ml and 10.71µM/min/ml respectively whereas Km and Vmax for cellulase were 30.31mg/ml and 6.23 µM/min/ml respectively. The small value of the Km for pectinase demonstrates its high affinity for the substrate as compared to the cellulase. The correlation coefficient was 0.962 and 0.936 for pectinase and cellulase respectively, which showed good agreement between the theoretical and experimental data.

The effect of crude and commercial enzyme treatment on pineapple (*Ananas comosus*) juice yield, clarity, and viscosity was studied. The pineapple pulp was treated with the enzyme and juice was further extracted from this enzyme treated pulp. The optimization of the crude and commercial enzyme treatment conditions was carried out using the Response Surface Methodology. The independent variables for the optimization of commercial enzyme treatment were as; temperature ($X_1$), time ($X_2$), concentration of cellulase ($X_3$) and concentration of pectinase ($X_4$) and the response variables were juice yield, viscosity and clarity. The optimized conditions for the commercial enzyme treated pineapple pulp were observed as incubation temperature;
46°C, incubation time; 448min, concentration of cellulase; 13mg/50g pulp and concentration of pectinase; 2.40mg/50g pulp. The values of the responses, juice yield, viscosity, and clarity were 88%, 1.36cP, and 80 %T respectively under these optimized conditions.

The independent variables for the crude enzyme treated pulp were temperature ($X_1$), time ($X_2$) and enzyme concentration ($X_3$). The response variables were juice yield, viscosity and clarity. The optimum conditions for crude enzyme treated pulp were observed as temperature; 47°C, time; 446min and concentration of crude enzyme; 0.14ml/50g pulp. The juice yield, viscosity, and clarity were 85.90%, 1.38cP, and 78 %T respectively under these optimized conditions.

The effect of crude and commercial enzymes for the improvement of juice yield and quality in terms of viscosity and clarity was compared using principal component analysis (PCA). The principal component analysis suggested that the juice viscosity was the parameter to be considered for comparison of crude and commercial enzyme treated samples.

The comparative evaluation revealed that the crude enzyme was competitive to the commercial enzyme for the pineapple pulp treatment to improve juice yield and clarity and thus the use of crude enzyme may be one of the alternatives to reduce the processing cost with respect to the quality.

The pineapple (*Ananas comosus*) mill juice was treated with crude and commercial enzyme for the degumming of the mill juice to improve its quality in terms of filtration rate, clarity, relative viscosity and percentage overrun. The low brix juice, extracted from the core, trimmings and skin of the pineapple, referred as mill juice was concentrated to increase its brix, so that it could be pooled to the pineapple juice. The response surface methodology was successfully used for the optimization of
commercial enzymatic treatment conditions for the degumming of the pineapple mill juice. Under the optimum commercial enzymatic treatment conditions (cellulase 124 mg/100 ml, pectinase 141 mg/100 ml, hemicellulase 78 mg/100 ml, time 455 min and temperature, 46°C), the experimental values of the responses were 0.49 ml/min filtration rate, 86 %T clarity, 1.31 relative viscosity and 61% overrun. The crude enzyme from A. niger NCIM 548 was also used for the degumming of the pineapple mill juice under the same optimized conditions of time and temperature and the values of responses were 0.44 ml/min filtration rate, 81 %T clarity, 1.38 relative viscosity and 66% overrun.

Principal component analysis (PCA) showed that viscosity was the most uncorrelated variable, which can be used to study the variance among samples treated by crude and commercial enzymes. The investigation revealed that the usage of crude enzyme can be effectively tried out for degumming of the pineapple mill juice to make the process highly cost effective.

Economic evaluation of the pineapple juice from the control, commercial enzyme treated and crude enzyme treated pulp was also carried out in this section. The data on cost of production was worked out on the basis of current market price of the raw materials, chemicals and enzymes used and production cost, besides incorporating overhead charges (office expenses, stationary and breakage etc.). The total cost of production of pineapple juice was 119.66, 189.86 and 105.94 (Rs/L) for control, commercial enzyme treated and crude enzyme treated samples respectively. The data revealed that the juice recovery by using crude enzyme treatment method was the most economic process, compared to the control and commercial (purified) enzyme treated sample.