

Table of Contents

a. Declaration	II
b. Certificate of H.O.D, External Expert	III
c. Certificate of Supervisor	IV
d. Acknowledgement	V
e. List of table and figure	X- XII
f. Abstract	Page-1
Ch 1: Introduction	Page 3-6
Ch 2: Review of Literature	Page 8-23
2.1 Enzymes	
2.2 Enzyme Technology	
2.3 Pectinases	
2.4 Pectinase production	
2.4.1 Pectic substrates	
2.4.2 Microbial Pectinases	
2.5 Optimization of pectinase production using renewable agricultural and industrial waste as substrate from <i>Aspergillus niger</i>	
2.6 Partial Purification	
2.7 Electrophoretic separation of cell fraction by SDS-PAGE	
2.8 Juice clarification	
2.9 Juice extraction	

3.1 Materials

3.1.1 Microorganisms

3.1.2 Substrate sources

3.1.3 Materials used for analysis

3.2 Methods

3.2.1 Preparation of spore suspension

3.2.2 Substrate preparation

3.2.3 Production medium

3.2.3.1 Selection of relevant variables and experimental ranges

3.2.3.2 Experimental design and statistical analysis

3.2.3.3 Shake flask Studies

3.2.3 Downstream processing

3.2.4 Enzyme assay

3.2.5 Protein Assay

3.2.6. Partial Purification

3.2.6.1 Ethanol precipitation

3.2.6.2 Ammonium sulphate purification

3.2.6 Analysis of the source material

3.2.7.1 Determination of moisture

3.2.7.2 Estimation of pectin content

3.2.8 Analysis of sample

3.2.8.1 Estimation of Protein by Lowry's Method

3.2.8.2 Total soluble solid (TSS)

3.2.8.3 pH

3.2.8.4 Enzyme assay

3.2.8.5 Determination of molecular weight by SDS PAGE

- 3.2.8.6 Diagrammatic representation of SDS Page
- 3.2.9 Analysis of the enzyme treated product
 - 3.2.9.1 Juice clarification
 - 3.2.9.2 Juice extraction
 - 3.2.9.3 Total soluble solid (TSS)
 - 3.2.9.4 Titratable acidity
 - 3.2.9.5 Estimation of Ascorbic Acid(Vitamin C)
 - 3.2.9.6 Determination of reducing sugar
 - 3.2.9.7 CIE Lab parameters
 - 3.2.9.8 Juice yield
- 3.2.10 Statistical analysis

Ch 4: Results and Discussion

Page 47-72

- 4.1 Analysis of the substrate source
 - 4.1.1 Pectin content
 - 4.1.2 Moisture content
- 4.2 Results of experimental design and statistical analysis
- 4.3 Analysis of enzyme extract
 - 4.3.1 Enzyme assay
 - 4.3.2 Effect of partial purification of optimized enzyme
 - 4.3.2.1 Crude enzyme treated with organic solvents
 - 4.3.2.2 Crude enzyme treated with salt precipitation
 - 4.3.3 Changes in TSS and pH of the enzyme extract
 - 4.3.4 Protein assay

4.3.5 Results of SDS PAGE:

4.4 Results of the analysis of the enzyme treated product

4.4.1 Juice clarification

4.4.2 Change in acidity, ° Brix and reducing sugar of the enzyme treated clarified juice

4.4.3 Juice extraction

4.4.4 Changes in ascorbic acid of the enzyme treated juice

4.4.5 Changes in color of the enzyme treated juice

Ch 5: Conclusion and Summary

Page 74-75

Ch 6: Bibliography

Page 77-81

LIST OF TABLES

Table No.	Title	Page No.
2.1	Some commercial enzymes and source microorganisms	12
4.1	Analysis of source material	48
4.2	Optimization of culture conditions for production of crude enzyme	49
4.3	Pectinase activity of crude and partially purified enzyme	50
4.4	Changes in protein content, TSS and pH of the crude and partially purified enzyme	51
4.5	Absorbance of the enzyme treated juice at time 30 min and 40 min	53
4.6	Changes in acidity of enzyme treated juice at 30 min and 60 min	57
4.7	Changes in Degree Brix of enzyme treated juice in 30 and 40 min	58
4.8	Changes in Reducing sugar of enzyme treated juice with time 30 and 40 min	58
4.9	Changes in Physico chemical properties and yield of juice after enzyme treatment	65
4.10	Changes in ascorbic acid	70
4.11	Changes in color using Hunter Color spectrophotometer	70

LIST OF FIGURES

Figure No.	Title	Page No.
3.1	Steps for SDS PAGE	39
3.2	Flow Chart for Clarification of Juice	40
3.3	Extraction of Enzyme treated juice and yields	41
4.1	Steps for preparation of Enzyme extract	47
4.2	Pectinase activity of Crude and partially purified enzyme	50
4.3	The purity of crude and partially purified enzymes were assessed by SDS PAGE analysis	51
4.4	Diagrammatic representation of different stages of SDS PAGE	52
4.5	Graph for absorbance value of different types of enzyme in 30 and 60 min of banana juice	54
4.6	Graph for absorbance value of different type of enzyme in time 30 and 60 min of watermelon juice	55
4.7	Graph for absorbance value and different concentration of enzyme in 30 and 60 min of fruit juices	56
4.8	Changes in acidity of different types of enzyme in 30 and 60 min	59
4.9	Changes in Degree Brix in different types of enzyme of banana juice time 30 and 60 min	60
4.10	Changes in Degree Brix with different types of enzyme in watermelon juice with time 30 and 60 min	61
4.11	Changes in Reducing Sugar with different types of enzyme in banana juice in 30 and 60 min	62
4.12	Changes in Reducing Sugar with different types of enzyme in watermelon juice in 30 and 60 min	63
4.13	Change in absorbance of different types of enzyme in banana juice in 30 and 40 min	66

4.14	Changes in reducing sugar with different types of enzyme in 30 and 40min	66
4.15	Changes in increase in amount of juice in different types of enzyme	67
4.16	Changes in acidity and degree Brix of different types of enzymes treated extract in 30 and 40 min	68
4.17	Changes in increase with different types of enzyme in 30 and 40 min	69
4.18	Color variation in watermelon juice before enzyme treatment	71
4.19	Color variation in watermelon juice after enzyme treatment.	71
4.20	Color variation in banana juice before enzyme treatment	72
4.21	Color variation in banana juice after enzyme treatment	72