

**PECTINASE PRODUCTION AND OPTIMISATION FOR FRUIT JUICE
CLARIFICATION: EVALUATION OF FUNGAL ISOLATES FERMENTED ON
SELECTED AGRO-WASTES**

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CLARIFICATION: EVALUATION OF FUNGAL ISOLATES FERMENTED ON
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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN
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DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND
TECHNOLOGY, COVENANT UNIVERSITY, OTA, NIGERIA.**

OCTOBER, 2021

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota.

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Signature and Date

CERTIFICATION

We certify that the thesis titled **“PECTINASE PRODUCTION AND OPTIMISATION FOR FRUIT JUICE CLARIFICATION: EVALUATION OF FUNGAL ISOLATES FERMENTED ON SELECTED AGRO-WASTES”** is an original research work carried out by **AMETEFE, GEORGE DZORGBENYA (16PCP01325)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Shalom N. Chinedu and Prof. Emeka E. J. Iweala. We have examined and found the work acceptable for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry.

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DEDICATION

I dedicated this thesis to God, for His faithfulness, and to my parents, Dr. and Dr. (Mrs.) Gilbert Tory Doe Ametefe, for their continuous encouragement and funding of my education. God bless you greatly.

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LIST OF ABBREVIATIONS

2D plot	2 Dimensional plot
3D plot	3 Dimensional plot
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BBD	Box-Behnken design
BLAST	Basic Local Alignment Search Tool
Ca ²⁺	Calcium cation
CaCl ₂	Calcium chloride
CCD	Central Composite Design
Co ²⁺	Cobalt cation
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DNSA	Dinitro salicylic acid reagent
EDTA	Ethylenediaminetetraacetic acid
EMI	Electromagnetic Induction
Endo PG	Endo polygalacturonases
Exo PG	Exo polygalacturonases
Fe ²⁺	Ferrous cation
FeSO ₄	Iron sulphate
GRAS	Generally Regarded as Safe
HCl	Hydrogen chloride
K ₂ HPO ₄	Dipotassium phosphate

KCl	Potassium chloride
KI	Potassium iodide
KNO ₃	Potassium nitrate
Mg ²⁺	Magnesium cation
MgCl ₂	Magnesium chloride
MgSO ₄	Magnesium sulfate
Mn ²⁺	Manganese cation
Na ⁺	Sodium cation
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Na ₂ SO ₄	Sodium sulphate
NCBI	National Center for Biotechnology Information
NH ₄ (SO ₄) ₂	Ammonium sulphate
OFAT	One Factor at a Time
OVAT	One Variable at a Time
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
PE	Pectin esterase
PG	Polygalacturonases
PGA	Polygalacturonic acid
PGL	Pectate lyases
PL	Pectin Lyases
PME	Pectin Methyl Esterases

PMG	Polymethylgalacturonases
RSM	Response Surface Method
SDS	Sodium dodecyl sulfate
SMF	Submerged fermentation
SSF	Solid state fermentation
Taq	<i>Thermus aquaticus</i>
TE	Tris-ethylenediaminetetraacetic
UV-Vis spectrophotometer	Ultraviolet Visible spectrophotometer
V_{\max}	Maximum Velocity
Zn^{2+}	Zinc cation

ABSTRACT

Pectinases are a group of enzymes that degrade pectin into simpler units. The enzymes have several industrial uses such as clarification of fruit juices. In this study, five pectinolytic fungi isolated from fruit wastes in dumpsites at Oshodi, Lagos State, were screened and identified using molecular techniques. They included two moulds (*Aspergillus niger* and *Penicillium sp.*) and three yeasts (*Pichia kudriavzevii* strain F2-T429-5, *Pichia kudriavzevii* strain CY902, and *Saccharomyces cerevisiae*). The five fungal isolates were screened for pectinase production using six agricultural wastes as substrates and three extraction solvents (distilled water, citrate buffer and 0.1 M NaCl). Pectinase production and optimisation studies were carried out using three fungi (*Aspergillus niger*, *Penicillium sp.*, and *Pichia kudriavzevii* strain F2-T429-5) which showed larger zones of pectin hydrolysis relative to the other fungi; three agricultural wastes with high pectinase activity (*Citrus sinensis* 'orange' peel, *Triticum aestivum* 'wheat' bran, and the miracle berry '*Thaumatococcus danielli*' fruit wastes), and best extraction solvent (0.1 M NaCl). Box-Behnken design, a response surface method (RSM), was used to predict the optimal conditions for enzyme production. The parameters investigated included fermentation duration, pH, temperature, particle size, the volume of inoculum, and agitation of the fermentation substrate (during enzyme extraction). The highest pectinase activity was achieved at approximately six days of solid-state fermentation using *Aspergillus niger* and orange peel. The optimal production conditions were: pH 4.0, temperature 21 °C, particle size 0.06 inches, inoculum volume 1.0 ml, and agitation duration 11.4 min. The best pectinase activity at the optimal conditions was 5.02 U/ml. The goodness of fit from the agreement of the predicted and adjusted R^2 values in the analyses of variance (ANOVA) indicated the suitability of the quadratic models used for this study. The pectinase properties included: optimal pH 4.0, and temperature stability 20 °C. The pectinase was best activated by Na^+ at 10 mM; beyond this level, there was reduction in the enzyme activity. The kinetic studies showed a V_{max} of 4.40 U/ml and K_m of 0.36 mg/ml. The optimised pectic enzyme preferentially ($p < 0.05$) clarified orange juices compared to pineapple juices and the control (without the enzyme). There is a good prospect of producing pectinase from microbes isolated from local agricultural wastes. The pectinases can be used in the clarification of fruit juices.

Keywords: Agricultural wastes, Box-Behnken design, Clarification, Fungal isolates, Optimisation, Pectinase production.