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

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OCTOBER, 2021

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES 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, 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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(Secretary, School of Postgraduate Studies**)** Signature and Date

Professor Akan B. Williams ……...

(Dean, School of Postgraduate Studies) Signature and Date

DECLARATION

I, AMETEFE, GEORGE DZORGBENYA (16PCP01325), declare that I carried out this research under the supervision of Prof. Shalom N. Chinedu and Prof. Emeka E. J. Iweala of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria. I attest that this thesis has not been presented either wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in this thesis have been duly acknowledged.

AMETEFE, GEORGE DZORGBENYA ……

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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(External Examiner) Signature and Date

Prof. Akan B. Williams ……
(Dean, School of Postgraduate Studies) Signature and` Date

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.

ACKNOWLEDGEMENTS

I owe God thanks for His unfailing presence in my life right from the beginning of this programme to this moment, and I know He would unfailingly guide me aright; to Him be all the glory. I also owe a great deal of gratitude to the Chancellor of Covenant University, Dr. David O. Oyedepo, for following God's leading which birthed this great institution of learning. To the Vice-Chancellor of Covenant University, Prof. Abiodun H. Adebayo, the Registrar, Dr. Oluwasegun P. Omidiora, the Dean School of Postgraduate Studies, Prof. Akan B. Williams, the Dean College of Science and Technology, Prof. Temidayo V. Omotosho, and the entire management staff of Covenant University; thank you for the exemplary leadership that has facilitated the actualisation of my academic dream. May His grace and favor continually be with you all.

I would not have reached this stage without the support, contributions, and guidance of my highly esteemed supervisors, Prof. Shalom N. Chinedu and Prof. Emeka E. J. Iweala. I am grateful for your investment of time, despite other pressing issues demanding your attention, and many other valuable inputs to the success of this work. I have benefitted from your wealth of experience through constructive criticisms, drive, patience, and I very much look forward to collaborating with you as my life mentors going forward. God richly bless you and your families in Jesus' Name, Amen.

At this juncture, I acknowledge the academic prowess of the Head of Department Prof. Israel S. Afolabi and the immediate past Head of Department, Prof. Olubanke O. Ogunlana. I appreciate you for your guidance thus far. I also appreciate the valuable contributions of the faculty. Their constructive criticisms right from the proposal stage added to the success of the work and my development in this great institution. I am indeed privileged to be under your watchful eyes. Thank you all.

My bench work would not have been a success without the open arms of the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos State, Nigeria. I very much thank the past Director-General of FIIRO, Prof. (Mrs.) Gloria Elemo, as she approved the acceptance letter to undertake this study in the Enzymology Division of the Department of Biotechnology. I also thank the Head of the Department of Biotechnology, Dr. Lawal Adekunle, for the concern shown through visitations to enquire about the progress of the work in the laboratory and his inputs thus far. I am

very grateful to the Head of the Enzymology division, Dr. Frank A. Orji, for his involvement from the beginning to the end of the bench-work in FIIRO. He guided me on the appropriate techniques to use in micro-organisms' isolation and subculture, confirmation and identification of the isolated fungi, and his assistance in connecting me to relevant personnel and locations, which further fast-tracked the success of the project. I very much owe thanks to Miss Fashola Folake, and the entire staff of the Enzyme Division of the Biotechnology department in FIIRO for their cooperation in the course of my stay. I also thank the National Youth Service Corp members that undertook their national service within the period of my stay in the division and the numerous interns for their enthusiasm, as their questions and availability to help aided this work in no small measure. I also thank the Department of Biosciences, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, for identifying the microbes isolated using molecular techniques. God bless you all.

I appreciate the management and staff of the Covenant University library for their support in aiding my programme through the creation of a conducive atmosphere for learning. It is also appropriate that I thank the various authors cited for their significant contributions in enlightening me on areas investigated, thereby directing me to untapped areas beginning from the proposal stage to the final stage of the thesis.

To my colleagues in the department, and other colleagues in sister departments in this great institution, Mrs. Harriet Ugboko, Mrs. Joan Iriabor, both in the Department of Biological Sciences, and Mr. Emmamuel Ozordi, Department of Accounting, I must say that your presence has impacted my life for good, as we shared both pleasant and challenging times. The push we gave each other has made us more robust from the challenges we have faced. I pray for the same togetherness even after graduation from this prestigious institution.

This would not have been possible without the support of my parents Dr. and Dr. (Mrs.) Gilbert T. D. Ametefe. They financed my studies to this point in my educational development without assistance from any funding agency. Their genuine love and encouragement are exemplary, even when I felt like keeping the programme on hold due to the financial pressure; yet, they demanded that I continue. Our faith has indeed birthed my successful graduation from this prestigious institution. I also appreciate my siblings Jerry, Faith, Divine, and Marvelous for their

understanding and encouragement along the way despite the odds. Sadly, my sister, Happy, is not present to celebrate with me as I lost her to death. I believe you are in heaven.

Above all, I owe God thanks for everything that I am. He indeed has been with me all the way. To Him be all the honor, praise, and adoration for evermore, Amen!

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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_2HPO_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_2SO_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	*Thermus aquaticus*
TE	Tris-ethylenediaminetetraacetic
UV-Vis spectrophotometer	Ultraviolent 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 ^{o}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 ^{o}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.