BIOTRANSFORMATION DURING FERMENTATION OF UNDER-UTILISED SEEDS FROM *Chrysophyllum albidum* LINN, and *Terminalia catappa* LINN

ODUTAYO, OLUWATOFUNMI ESTHER

(16PCP01327)

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BY

ODUTAYO, OLUWATOFUNMI ESTHER

(16PCP01327)

B.Tech Biochemistry, Federal University of Technology, Akure

M.Sc Biochemistry, Babcock University, Ilishan-Remo

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT 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, OGUN STATE, NIGERIA

DECEMBER, 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 in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota.

Mr. John A. Phillip …….

(Secretary, School of Postgraduate Studies) Signature and Date

Prof. Akan B. Williams …….

(Dean, School of Postgraduate Studies) Signature and Date

DECLARATION

I, **ODUTAYO, OLUWATOFUNMI ESTHER (16PCP01327)** declare that this research was carried out by me under the supervision of Prof. Israel S. Afolabi and Prof. Olubanke O. Ogunlana of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

ODUTAYO, OLUWATOFUNMI ESTHER ……

Signature and Date

CERTIFICATION

We certify that this thesis titled "**BIOTRANSFORMATION DURING FERMENTATION OF UNDER-UTILISED SEEDS FROM *Chrysophyllum albidum* LINN, and *Terminalia catappa* LINN**" is an original research work carried out by **ODUTAYO, OLUWATOFUNMI ESTHER (16PCP01327)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Israel S. Afolabi and Prof. Olubanke O. Ogunlana. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Biochemistry.

Prof. Israel S. Afolabi ……

(Supervisor) Signature and Date

Prof. Olubanke O. Ogunlana ……

(Co-Supervisor) Signature and Date

Prof. Israel S. Afolabi ……

(Head of Department) Signature and Date

Prof. Adenike T. Oladiji ……

External Examiner Signature and Date

Prof. Akan B. Williams ….……..

(Dean, School of Postgraduate Studies) Signature and Date

DEDICATION

This work is dedicated to God, the giver of wisdom, knowledge, and understanding, my help in ages past, and hope for years to come.

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LIST OF ABBREVIATIONS

BSH-	Bile salt hydrolase
DPPH-	2,2-diphenyl-1-picrylhydrazyl
FBP-	Fibronectin binding protein
FCA-	Fermented *Chrysophyllum albidum*
FRAP-	Ferric reducing assay property
FTC-	Fermented *Terminalia catappa*
GC/MS-	Gas Chromatography/Mass Spectrometry
GIT-	Gastro-intestinal tract
LAB-	Lactic acid bacteria
MUB-	Mucin binding protein
TAC-	Total antioxidant capacity
UFCA-	Unfermented *Chrysophyllum albidum*
UFTC-	Unfermented *Terminalia catappa*
FAO/WHO-	Food and Agricultural Organisation/World Health Organisation

ABSTRACT

The majority of plant seeds are under-utilised because of their excessive antinutrient levels. As the quest for food security increases, approaches to reduce these antinutrients with food processing methods is now of importance. This study aims to investigate the biochemical changes in the natural fermentation of the *C. albidum* and *T. catappa* seeds, and characterise probiotic lactic acid bacteria associated with the fermentation process. The phytochemical and antioxidant assessments of extracts from the unfermented and fermented seeds were carried out and followed by the use of Gas Chromatography/Mass Spectrometry (GC/MS) for identification of phytochemical compounds. Their digestive enzymes (α-amylase, protease, and lipase) activities were also determined spectrophotometrically. Strains of lactic acid bacteria (LAB) were isolated from both fermented seeds and assessed for probiotic characteristics using biochemical and molecular methods. Significant ($p<0.05$) reductions in the levels of oxalate, phytate, tannin, and alkaloid in both seeds were observed after fermentation. The GC/MS analysis revealed a decrease from sixty-two compounds in the unfermented *C. albidum* seeds to thirty-nine in the fermented *C. albidum* seeds. There was also an increment from fifty-two compounds, in the unfermented *T. cattapa* seeds to fifty-three in the fermented *T. catappa* seeds. A significant decrease ($p<0.05$) was observed in the 2,2-diphenyl-1-picrylhydrazyl scavenging abilities of the fermented extracts from both seeds. *C. albidum* seeds had a significant ($P<0.05$) increase in only ferric reducing assay property (FRAP), but *T. catappa* seeds had significant reductions ($P<0.05$) in total antioxidant capacity (TAC) and FRAP after fermentation. There was a significant reduction ($P<0.05$) in the α-amylase activity in fermented *C. albidum* seeds, while fermentation significantly ($P<0.05$) increased the α-amylase activity in the fermented *T. catappa* seeds, and the activities of lipase in both fermented seeds. The protease activity was significantly increased in the fermented *C. albidum* seeds, while no significant difference was observed in the protease activities of the unfermented and fermented seeds of *T. catappa*. Three potential probiotic LAB strains isolated from the fermented *C. albidum* seeds, and seven from the fermented *T. catappa* seeds were identified using API 50 CHL and 16S rRNA sequencing. All strains were non haemolytic, which indicated their safety. Seven isolates grew in the acidic environment (pH 3.5) during the 48 hr incubation time, and all the ten strains grew in 1 % bile. All isolates from the fermented *T. catappa* seeds showed bactericidal activities against some selected pathogens, while all the strains showed good auto-aggregation properties. Fibronectin binding protein was detected in three of the isolates, while mucin binding protein was not detected in any, and bile salt hydrolase was detected in all the strains. A new isomer of di-hetero tricyclodecane, namely 2,5-methano-2H-furo[3,2-b]pyran,hexahydro- was identified in the fermented *C. albidum* seeds. The study established the prebiotic potentials of some phytochemicals present in the unfermented *C. albidum* and *T. catappa* seeds and thus reavealed that the isolated probiotics were unique to each seed, indicating that the growth of beneficial bacteria during the fermentation was partly dependent on their phytochemical components.

Keywords: Under-utilised seeds, *Chrysophyllum albidum*, *Terminalia catappa*, Fermentation, Probiotics