

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

ODUTAYO, OLUWATOFUNMI ESTHER

(16PCP01327)

DECEMBER, 2021

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

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.

ACKNOWLEDGEMENTS

First and above all, I say an uncountable thank you to the Almighty God, the incomparable one, my maker, all in all, the giver of all things, my help in ages past, and hope for years to come. With Him, this work was possible. Be thou glorified forever. I gratefully acknowledge 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.

My sincere appreciation goes to my supervisor and co-supervisor, in the persons of Prof. Israel S. Afolabi and Prof. Olubanke O. Ogunlana respectively, words indeed are not enough to express my gratitude. I appreciate your parental concerns, time investment, guidance, push to work, constructive criticisms, the wealth of knowledge, and experience shared with me. You have been more than supervisors to me. God bless you more than you can ask or imagine. I sincerely appreciate my lecturers; Prof. Abiodun H. Adebayo, Prof. Shalom N. Chinedu, Prof. Emeka E. J. Iweala, Prof. Emmanuel N. Maduagwu, Dr. Solomon O. Rotimi and others. I know many of the things I know today because you taught me. Thank you very much for sharing your wealth of knowledge with me.

My benchwork would not have been a success without the contributions of Mr. Emmanuel A. Omonigbehin, Miss Bose E. Adegboye. Mr. Joseph Ige, Mr. Alaba O. Adeyemi, Mr. Shade J. Olorunsola, Mrs. Omowumi R. Afolabi, Mr. Taiwo, Mr. Olawale S. Ezekiel, Mr. Oluwatosin P. David, Mr. Daniel Okere, Mrs. Juliet C. Nwabueni, and all technical staff who supported me in their different capacities during the period of this research. I am deeply grateful. God bless you all abundantly. I appreciate my colleagues in the persons of Mr. Isaacson B. Adelani, Mr. Ayodeji O. Lemo, and Mr. George Ametefe, thank you for being like brothers to me. God bless you richly.

To my darling husband and daughter, Dr. Adedamola A. Odotayo and Jesuferanmi Odotayo respectively, my heart blesses the Lord for giving you both to me. The sacrifices you have made in the course of this work are memorable ones and will always linger in my heart. You will always need more rooms to contain God's blessings. Thank you very much for being there for me. My heartfelt gratitude goes to my wonderful parents; Major and Mrs. Oluseyi A. Obaseki. I thank God for choosing you to play the parental role in my life from cradle to date. Your prayers, financial support, encouragement, care, and many more have been God-driven. I definitely cannot pay you back, and it is obvious that God is your rewarder. He will bless you immeasurably here and in eternity. Thank you for playing your God-given roles in my life.

I appreciate my precious siblings Barr. Oluwadara O. Ajidasile and Mr. Oluwatosin S. Obaseki, thank you for that family spirit we have maintained to date, your show of love to me in the course of this work is unquantifiable. God bless you immeasurably. I must not fail to acknowledge my amiable parents in law; Dr. and Dr. (Mrs.) Adetokunbo Odotayo. The joy of being your daughter in law is a blessing from God to me. Thank you for your prayers, support, care, and always seeking after the welfare of this work. Your joy will know no bounds. Thank you a million times.

My sincere appreciation goes to my brothers and sisters in law; Engr. and Mrs. Ademayowa Odutayo; Mr. and Mrs. Abayomi Fasanya. Thank you for your prayers, encouragement, and support in the course of this work. You are indeed God's gifts to me. God bless you bountifully. To my dear friend turned sister, Dr. Adetutu Bello, the assistance you rendered me while I put this thesis together is a memorable one. You will never lack anything good. Thank you very much. I appreciate my brethren in the household of faith; from the Pastorate to the members of my local church; the Dream Centre of the Life Oasis International Church, Ota branch. My being part of this family in the course of this work was a sharpening one. Thank you very much.

This acknowledgement will not be complete without me appreciating my father and mother in the Lord; Rev. and Rev. (Mrs) Olusola A. Areogun. I am indeed a partaker of the anointing you carry. Thank you for giving to the Lord. This list will be endless if I keep mentioning names; I say a big thank you to everyone who contributed in different capacities to the success of this work. Once again, thank you very much.

TABLE OF CONTENTS

CONTENT	PAGE
COVER PAGE	
TITLE PAGE	i
ACCEPTANCE	ii
DECLARATION	iii
CERTIFICATION	iv
DEDICATION	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	ix
LIST OF FIGURES	xv
LIST OF TABLES	xvii
LIST OF PLATES	xviii
LIST OF APPENDICES	xix
LIST OF ABBREVIATIONS	xxi
ABSTRACT	xxii
CHAPTER ONE	23
INTRODUCTION	23
1.1 Background to the study	23
1.2 Statement of the research problem	27
1.3 Research questions	28
1.4 Aim and specific objectives	28
1.4.1 Aim	28
1.4.2 Specific objectives	28
1.5 Justification for the study	29
1.6 Scope of the study	29

CHAPTER TWO	30
LITERATURE REVIEW	31
2.1 Under-utilised seeds	31
2.2 Under-utilised seeds and food security	31
2.3 Antinutrient properties of under-utilised seeds	32
2.3.1 Tannins	34
2.3.2 Phytates	35
2.3.3 Oxalates	35
2.3.4 Alkaloids	36
2.4 Approaches to reducing antinutrients in foods	37
2.4.1 Soaking	37
2.4.2 Sprouting	38
2.4.3 Heating	38
2.4.4 Gamma radiation	39
2.4.5 Genomic technology	39
2.4.6 Fermentation	39
2.5 History of fermentation	40
2.5.1 Process of fermentation	42
2.5.2 Types of fermentation	43
2.5.2.1 Alcohol/Ethanol Fermentation	43
2.5.2.2 Lactic acid fermentation	44
2.5.2.3 Acetic acid Fermentation	45
2.5.2.4 Butyric acid Fermentation	45
2.5.2.5 Uses of fermentation	46
2.6 Fermentation as a biotechnological tool	47
2.7 Bioactive compounds as substrates for microbial metabolism in food fermentation	48
2.8 Digestive enzymes in fermentation	50
2.9 Prebiotics	54
2.10 Probiotics	55
2.11 Conventional and unconventional sources of probiotics	58
2.12 <i>Terminalia catappa</i>	63

2.13 <i>Chrysophyllum albidium</i>	68
2.14 Gaps identified in literature	75
CHAPTER THREE	76
MATERIALS AND METHODS	76
3.1 Materials	76
3.1.1 Equipment	76
3.1.2 Chemicals	76
3.2 Methods	77
3.2.1 Collection of seeds	77
3.2.2 Identification of seeds	77
3.2.3 Processing of seeds	77
3.2.4 Preparation of unfermented seeds extracts	80
3.2.5 Preparation of fermented seeds extracts	80
3.2.6 Phytochemical analysis	82
3.2.6.1 <i>Qualitative analysis</i>	82
<i>i Test for tannins</i>	82
<i>ii Test for flavonoids</i>	82
<i>iii Test for alkaloids</i>	82
<i>iv Test for anthocyanins and betacyanins</i>	83
<i>v Test for quinones</i>	83
<i>vi Test for glycosides</i>	83
<i>vii Test for cardiac glycosides</i>	83
<i>viii Test for terpenoids</i>	83
<i>ix Test for triterpenoids</i>	83
<i>x Test for phenols</i>	84
<i>xi Test for coumarins</i>	84
<i>xii Test for steroids</i>	84
3.2.6.2 Quantitative analysis	84
<i>i Determination of total phenolic content</i>	84
<i>ii Determination of total flavonoid content</i>	85

<i>iii Estimation of total alkaloid content</i>	86
<i>iv Total tannin content determination</i>	86
<i>v Oxalate content determination</i>	87
<i>vi Phytate determination</i>	87
3.2.7 Assessment of in vitro antioxidant activity	88
3.2.7.1 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging activity	88
3.2.7.2 Ferric reducing antioxidant power (FRAP)	89
3.2.7.3 Total antioxidant capacity (TAC)	90
3.2.8 Procedure for GCMS analysis	90
3.2.9 Determination of activities of digestive enzymes from the unfermented and fermented seeds	91
3.2.10 pH and titratable acidity	92
3.2.11 Quantification of protein in the seeds samples	93
3.2.12 Determination of alpha-amylase activity	94
3.2.13 Determination of protease activity	95
3.2.14 Determination of lipase activity	96
3.2.15 Isolation of Lactic acid bacteria from the fermented seeds	97
3.2.16 Bacterial cultures and maintenance	97
3.2.17 Safety evaluation of bacteria Isolates	98
3.2.18 Preliminary characterisation of isolates	98
<i>i Gram staining</i>	98
<i>ii Catalase test</i>	99
3.2.19 Biochemical characterisation of the isolated organisms	99
3.2.20 Acid tolerance assay	100
3.2.21 Bile tolerance assay	100
3.2.22 Cellular hydrophobicity assay	101
3.2.23 Cellular auto-aggregation assay	101
3.2.24 Bacteriocin production test	102
3.2.25 Molecular characterisation of isolates	103
3.2.26 Sequencing of the 16sRNA amplicons	105
3.2.27 Computational analysis of the genomic sequences	105
3.3 Methods of statistical analysis	105

CHAPTER FOUR	107
RESULTS	107
4.1 Qualitative phytochemical screening	107
4.2 Quantitative phytochemical analysis	107
4.3 Antioxidant analysis	107
4.3.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity	107
4.3.2 Ferric reducing assay property (Frap) and total antioxidant capacity (TAC)	112
4.4 GC/MS analysis of extracts	112
4.5 pH and titratable acidity	121
4.6 Activities of alpha-amylase, protease, and lipase extracted from the fermented and unfermented seeds	121
4.7 Isolated organisms	126
4.8 Biochemical characterisation of isolates	126
4.9 Identification of isolates	126
4.10 Acid tolerance of LAB isolates	126
4.11 Bile tolerance of LAB isolates	137
4.12 Bacteriocin activity by isolates	137
4.13 Cellular Hydrophobicity	141
4.14 Cellular auto-aggregation	141
4.15 Molecular characterisation	141
CHAPTER FIVE	147
DISCUSSION	147
CHAPTER SIX	173
CONCLUSION AND RECOMMENDATIONS	173
6.1 Summary	173
6.2 Conclusion	174
6.3 Contributions to knowledge	174
6.4 Recommendations	175

REFERENCES
APPENDICES

176
196

LIST OF FIGURES

Figures	Title of figures	Page
2.1	Generalised scheme showing fermentation of glucose to lactic acid	44
2.2	Uses of fermentation	46
2.3	Probiotic sources and selection criteria	62
3.1	Flow chart illustrating the preparation of flour, unfermented and fermented extracts from <i>T. catappa</i> nuts and <i>C. albidum</i> kernels.	81
4.1	The DPPH scavenging activities of the extracts from the <i>T. catappa</i> and <i>C. albidum</i> seeds	111
4.2a-f	Peculiar compounds from the unfermented <i>C. albidum</i> seeds	115
4.3a-g	Peculiar compounds from the fermented <i>C. albidum</i> seeds	116
4.3h	2, 5-methano-2H-furo [3, 2-b] pyran, hexahydro (a new isomer of di-hetero tricyclodecane) identified for the first time in the fermented <i>C. albidum</i> seeds	117
4.4a-f	Peculiar compounds from the unfermented <i>T. catappa</i> seeds	119
4.5a-e	Peculiar compounds from the fermented <i>T. catappa</i> seeds	120
4.6a	Activities of α -amylase extracted from the unfermented and fermented seeds of <i>T. catappa</i> and <i>C. albidum</i>	123
4.6b	Activities of protease extracted from the unfermented and fermented seeds of <i>T. catappa</i> and <i>C. albidum</i>	124
4.6c	Activities of lipase extracted from the unfermented and fermented seeds of <i>T. catappa</i> and <i>C. albidum</i>	125
4.7	Phylogenetic tree based on 16S rRNA gene sequences of the isolates	134
4.8a-d	Acid tolerance of Probt A1-B4b at pH 3.5, pH 5.5, pH 6.2 (control)	

	and pH 7.5	135
4.8e:	Acid tolerance of Probt A1-B4b at pH 9.5	136
4.9a-d	Bile tolerance of Probt A1-B4b in 0% (control), 0.1%, 0.3% and 0.5% bile	138
4.9e-f	Bile tolerance of Probt A1-B4b in 0.7 % and 1% bile	139
4.10	The bacteriocin production by Probt B1a-B4b	140
4.11	The percentage of cellular hydrophobicity of Probt A1-B4b	142
4.12	The percentage cellular auto-aggregation of Probt A1-B4b	143
4.13a	The characterisation of the LAB isolates with 16sRNA	144
4.13b	The detection of bile salt hydrolase (<i>bsh</i>) in the LAB isolates	145
4.13c	The detection of fibronectin binding protein (<i>fbp</i>) in the LAB isolates	146
5.1	The proposed biochemical mechanisms during the fermentation of seed of <i>C. albidum</i>	169
5.2	The proposed biochemical mechanisms during the fermentation of seed of <i>T. catappa</i>	170
5.3	A newly proposed fermentation pathway for the seeds of <i>C. albidum</i>	171
5.4	A newly proposed fermentation pathways for the seeds of <i>T. catappa</i>	172

LIST OF TABLES

Tables	Title of tables	Page
3.1	List of primers used in this study	104
4.1	Qualitative phytochemical screening of extracts from the seeds of <i>C. albidum</i> and <i>T. catappa</i>	109
4.2	Quantitative phytochemical analysis of extracts of the <i>C. albidum</i> and <i>T. catappa</i> seeds	110
4.3	Ferric reducing assay property (FRAP) and total antioxidant capacity (TAC) of extracts from the <i>C.albidum</i> and <i>T.catappa</i> seeds	113
4.4	Summary of the classes of metabolised compounds during the fermentation of the <i>C. albidum</i>	114
4.5	Summary of the classes of metabolised compounds during the fermentation of <i>T. catappa</i> seeds	118
4.6	pH and titratable acidity of the unfermented and fermented <i>C. albidum</i> and <i>T. catappa</i> seeds	122
4.7	Preliminary characterisation of isolates	128
4.8	The sugar metabolism pattern of the isolated probiotics (Probt A1-B4b)	131
4.9	The identified names of the isolated probiotics	133

LIST OF PLATES

Plates	Title of plates	Page
2.1	<i>T. catappa</i> fruits	64
2.2	<i>T. catappa</i> nuts	65
2.3	<i>C. albidium</i> fruits	70
2.4	<i>C. albidium</i> seeds	71
3.1	Ground <i>T. catappa</i> seed	78
3.2	Ground <i>C. albidum</i> seed	79
4.1a-f	The microscopic view of the isolated probiotics	129
4.1g-j	The microscopic view of the isolated probiotics contd	130

LIST OF APPENDICES

Appendices	Title of Appendices	Page
Appendix 1	CHREC ethical permit certificate	196
Appendix 2	Bioactives detected from the GC/MS analysis of the unfermented extract of the <i>C. albidum</i>	197
Appendix 3	The GC/MS chromatogram for the unfermented extract of the <i>C. albidum</i> seeds	202
Appendix 4	Bioactives present in the GC/MS analysis of the extract of fermented <i>C. albidum</i>	203
Appendix 5	The GC/MS chromatogram for the fermented extract of the <i>C. albidum</i> seeds	207
Appendix 6	Bioactives detected from the GC/MS analysis of the unfermented extract of the <i>T. catappa</i> seeds	208
Appendix 7	The GC/MS chromatogram for the unfermented extract of the <i>T.cattapa</i> seeds	213
Appendix 8	Bioactives detected from the GC/MS analysis of the extract of the fermented <i>T. catappa</i> seeds	214
Appendix 9	The GC/MS chromatogram for the unfermented extract of the <i>T.cattapa</i> seeds	219
Appendix 10	Preparation of media for acid tolerance	220
Appendix 11	Preparation of media for bile tolerance	220
Appendix 12	Preparation of sterile catalase from cow liver	220

Appendix 13	Preparation of cow bile extract powder from cow gall bladder	221
Appendix 14	Table for gallic acid standard curve (n = 3)	221
Appendix 15	Table for rutin standard curve (n = 3)	222
Appendix 16	Table for ascorbic acid standard curve (n = 3)	222
Appendix 17	Standard curve table for Bovine Serum Albumin (BSA), n = 3	223
Appendix 18	Table for maltose standard curve (n = 3)	223
Appendix 19	Table for tryptophan standard curve (n = 3)	224
Appendix 20a	Statistical analysis of the cellular hydrophobicity assay	225
Appendix 20b	Test details of the cellular hydrophobicity assay	226
Appendix 21a	Statistical analysis of the cellular auto aggregation assay	228
Appendix 21b	Test details of the cellular autoaggregation assay	229
Appendix 22a	Statistical analysis of the acid tolerance assay at pH 3.5	231
Appendix 22b	Test details of the acid tolerance assay at pH 3.5	232
Appendix 23a	Statistical analysis of the bile tolerance assay at 1 % cow bile powder extract	234
Appendix 23b	Test details of the acid tolerance assay at 1 % cow bile powder extract	235

LIST OF ABBREVIATIONS

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