PRODUCTION OF SINGLE CELL PROTEIN FROM PINEAPPLE AND

WATERMELON WASTE USING Saccharomyces cerevisiae

SALAMI, ABIMBOLA OLUWADARASIMI (19PCQ02044)

OCTOBER, 2021

PRODUCTION OF SINGLE CELL PROTEIN FROM PINEAPPLE AND

WATERMELON WASTE USING Saccharomyces cerevisiae

BY

SALAMI, ABIMBOLA OLUWADARASIMI (19PCQ02044)

B.Sc., Microbiology, Landmark University, Omu Aran

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN MICROBIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY

OCTOBER, 2021

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfillment of the requirements for the award of the degree of Master of Science in Microbiology in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria.

Mr. John A. Philip	
(Secretary, School of Postgraduate Studies)	Signature and Date
Prof. Akan B. Williams	
(Dean, School of Postgraduate Studies)	Signature and Date

DECLARATION

I, SALAMI, ABIMBOLA OLUWADARASIMI (19PCQ02044) declare that this research was carried out by me under the supervision of Prof. Obinna C. Nwinyi of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the dissertation 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 dissertation are duly acknowledged.

SALAMI, ABIMBOLA OLUWADARASIMI

Signature and Date

CERTIFICATION

We certify that this dissertation titled "PRODUCTION OF SINGLE CELL PROTEIN FROM PINEAPPLE AND WATERMELON WASTE USING Saccharomyces cerevisiae" is an original research work carried out by SALAMI, ABIMBOLA OLUWADARASIMI (19PCQ02044) in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Obinna C. Nwinyi. We have examined and found this work acceptable as part of the requirements for the award of Master of Science in Microbiology.

Prof. Obinna C. Nwinyi	
(Supervisor)	Signature and Date
Prof. Solomon U. Oranusi	
(Head of Department)	Signature and Date
Prof. Oluwatoyin R. Afolabi	
(External Examiner)	Signature and Date
Prof. Akan B. Williams	
(Dean, School of Postgraduate Studies)	Signature and Date

DEDICATION

I dedicate this research to God Almighty, my Creator, and strong pillar, source of inspiration, strength, wisdom, knowledge, and understanding.

ACKNOWLEDGEMENTS

I want to acknowledge God Almighty for the grace and strength to complete this program. My appreciation also goes to the Chancellor, Bishop David Oyedepo, the present Vice-Chancellor, Prof. Abiodun H. Adebayo, and the entire management and staff of Covenant University for providing me the facilities for the successful completion of my program.

To the current Dean of the School of Postgraduate Studies, Prof. Akan Williams, and the Postgraduate School staff, I appreciate you for the diverse training and programs organized towards capacity building for a successful postgraduate research. I sincerely appreciate the Head of Department Biological Sciences, Prof. Solomon U. Oranusi, for his support and guidance.

My special appreciation goes to my supervisor, Prof. Obinna C. Nwinyi, whose advice, insightful criticisms, patience, and encouragement aided this project's success in numerous ways. I am sincerely grateful to Dr Oniha, Mr Adeyemi, Mrs Adekeye, Mr Taiwo, Miss Kuye, Mr Mike of the Department of Biological Sciences for their immeasurable assistance during this research work.

I sincerely appreciate my parents, Mr and Mrs. Salami, who laid the foundation for my education and gave it all it takes to ensure I always get the best. To my siblings, Ayobami and Ibukunoluwa, thank you for your unquantifiable love and support.

A big thank you to my loving friends, Bolaji, Ayomide, Busola and Ife for their numerous support, encouragement and advice throughout this phase. Finally, I am grateful to my colleagues, Bolu, Dozie, Dominion and Nifemi who have encouraged me throughout the process.

TABLE OF CONTENTS

CON	NTENT	P	age
COVER PAGE			
TIT	LE PAGE	ii	i
ACC	CEPTANCE	ii	ii
DEC	CLARATION	iv	V
CER	RTIFICATION	V	,
DED	DICATION	V	i
ACK	KNOWLEDGEMENTS	V	ii
TAB	BLE OF CONTENTS	V	iii
LIST	Γ OF FIGURES	X	iv
LIST	Γ OF TABLES	X	V
LIST	LIST OF PLATES		vi
APPENDIX		vii	
LIST	Γ OF ABBREVIATIONS	X	viii
ABSTRACT		ix	
CHA	APTER ONE: INTRODUCTION	1	
1.1.	Background to the Study	1	
1.2.	Statement of the Problem	3	
1.3.	Research Questions	3	
1.4.	Aim and Objectives	3	
1.5.	Justification of the Study	4	
1.6	Scope of the study	4	
CHA	CHAPTER TWO: LITERATURE REVIEW		
2.1	Food insecurity	5	

2.2	Agricultural wastes	8
2.2.1	Watermelon and Pineapple wastes	9
2.2.2	Biochemical composition of watermelon and pineapple wastes	10
2.2.3	Applications of pineapple and watermelon wastes	10
2.2.3.1	Animal feed	10
2.2.3.2	Phenolic compounds	11
2.2.3.3	Dietary fiber and functional polysaccharides	11
2.2.3.4	Vinegar production	12
2.2.3.5	Single-cell protein production	12
2.2.3.6	Minerals	12
2.3	Impacts of Agricultural wastes on the environment	14
2.3.1	Air pollution	14
2.3.2	Water pollution	14
2.4	Bioremediation of agricultural wastes	15
2.5	Concept of Single-cell protein (SCP) or Microbial protein	17
2.5.1	Nutritional Benefits of SCP	17
2.5.2	Substrates used for the production of Single-Cell Protein	18
2.5.2.1	Lignocellulose wastes	18
2.5.2.2	Liquid wastes	18
2.5.2.3	Urban bio-wastes	18
2.5.3	Microorganisms used as Single-Cell Protein	19
2.5.3.1	Microalgae	19
2.5.3.2	Yeast and other fungi	19
2.5.3.3	Bacteria	20
2.5.4	Production of Single-Cell Protein	20

2.5.4.1 S	Submerged fermentation	21
2.5.4.2 S	Solid state fermentation	22
2.5.4.3 S	Substrates	22
2.5.4.3.1	Pretreatment and hydrolysis of substrates	22
2.5.4.4 N	Media Preparation	22
2.5.4.4.1	Media Preparation Using Fruit and Vegetable Waste	22
2.5.4.4.2	Media Preparation Using Lignocellulose Wastes	23
2.5.4.4.3	Media Preparation Using Liquid Wastes	23
2.5.4.5	Enrichment of the Media/ Nutritional parameters	24
2.5.4.5.1	Supplementation with Molasses	24
2.5.4.6	Sterilization of Growth Media	25
2.5.4.7	Inoculum Isolation and Growing	25
2.5.4.8	Inoculation and Incubation	25
2.5.4.9	Harvesting and Protein Content Determination	26
2.6 Sac Production	ccharomyces cerevisiae as starter in Single-Cell Protein	26
2.6.1 Gr	owth of Saccharomyces cerevisiae	27
2.6.2 Sou	arces of Saccharomyces cerevisiae	27
CHAPTEI	R THREE: MATERIALS AND METHODS	29
3.1 List of	f culture media, equipment and reagents	29
3.1.1 Equ	nipment	29
3.1.2 Cor	nsumables	29
3.1.3 Med	lia, Buffers and Reagents	29
3.2 Meth	ods	30
3.2.1 San	nple collection and preparation	30

3.2.2	Proximate analysis of the sample	31
3.2.2.1	Determination of Carbohydrate Content	31
3.2.2.2	Determination of Protein Content	31
3.2.2.3	Determination of Moisture Content	32
3.2.2.4	Determination of Crude Fibre	32
3.2.2.5	Determination of Ash Content	32
3.2.2.6	Determination of Crude Fat	33
3.3 Is	solation and characterization of yeast	33
3.3.1	Preparation of Potato Dextrose Medium	33
3.3.2	Inoculation of Samples on Medium	33
3.4 M	Iorphological Characterization	34
3.4.1	Macroscopic Characterization	34
3.4.2	Gram's Staining	34
3.4.3	Lactophenol Cotton Blue Staining	34
3.5 B	iochemical Characterization	34
3.5.1	Sugar Fermentation Test	34
3.6 M	Iolecular characterization of yeast isolate	35
3.6.1	Genomic DNA Isolation	35
3.6.2	Quantification of DNA	35
3.6.3 Isolate	PCR Amplification of the ITS 1 and ITS 4 Genes of the Yeast	36
3.6.4	Agarose Gel Electrophoresis	36
3.6.5	Purification of PCR amplicons for sequencing	37
3.6.6	Sequence Analysis	37
3.7 Pr	eparation of fruit hydrolysates	37

3.8 Fermentation and harvesting of single-cell protein	39
3.8.1 Mass production and harvesting of yeast cells	39
3.8.2 Fermentation	39
3.8.3 Growth profile of organism	40
3.8.4 Harvesting of yeast biomass	40
3.9 Protein content determination of biomass produced	41
CHAPTER FOUR: RESULTS	42
4.1 Proximate analysis of fruit peels	42
4.2 Isolation and characterization of yeast isolate	44
4.2.1 Morphological characterization of isolates	44
4.2.2 Biochemical characterization	44
4.2.3 Molecular characterization	45
4.3 Growth profile of the organism during fermentation	48
4.4 Cell dry weight of the biomass obtained	54
4.5 Protein content of biomass produced	56
CHAPTER FIVE: DISCUSSION	58
CHAPTER SIX: CONCLUSION AND RECOMMENDATION	63
6.1 Summary	63
6.2 Conclusion	63
6.3 Contributions to Knowledge	63
6.4 Recommendations	64
REFERENCES	65
APPENDIX	

LIST OF FIGURES

Figure	Title of Figures	Page
2.1	Prevalence of severe food insecurity in Nigeria from 2014-2020	6
2.2	Percentage of under-fives in Nigeria who are underweight, stunted or wasting	7
2.3	The percentage of food loss from Post-harvest to distribution in 2016	8
2.4	Worldwide production of pineapple, melon, pumpkin and watermelon from 2008	9
	to 2017	
2.5	General steps during industrial production of single cell proteins.	21
4.1	Proximate compositions of Pineapple (Ananas comosus) peels	43
4.2	Proximate compositions of Watermelon (Citrullus lanatus) peels	43
4.3	An unrooted Phylogenetic tree of Saccharomyces species with accession number	47
	OK172582.	
4.4	Optical densities of yeast against time measured at 600nm during fermentation	49
	utilizing different media from Pineapple peel substrate	
4.5	Optical densities of yeast against time measured at 600nm during fermentation	49
	utilizing different media from Watermelon peel substrate	
4.6	Comparison of optical densities on day 1 of yeast grown in different fermentation	51
	media from Pineapple and Watermelon peels.	
4.7	Comparison of optical densities on day 3 of yeast grown in different fermentation	51
	media from Pineapple and Watermelon peels.	
4.8	Comparison of optical densities on day 5 of yeast grown in different fermentation	52
	media from Pineapple and Watermelon peels.	
4.9	Comparison of optical densities on day 7 of yeast grown in different fermentation	52
	media from Pineapple and Watermelon peels.	
4.10	Cell Dry Weight of the yeast biomass produced from different media of Pineapple	55
	and Watermelon peels.	
4.11	Protein content of the biomass produced from different media of Pineapple and	57
	Watermelon peels.	

LIST OF TABLES

Table	Title of Tables	Page
2.1	Pineapple waste utilization	13
2.2	An overview of some different value added products produced from food and fruit wastes	16
3.1	Composition of the medium used in the study	40
4.1	Microscopic characteristics of the yeast isolate	44
4.2	Biochemical characteristics of the yeast isolate	44
4.3	Similarity of the yeast isolate from this study to isolates in the NCBI Genbank ITS database based on BLASTn analysis	46
4.4	The fermentation medium employed in the study	48

LIST OF PLATES

Plates	Title of Plate	Page
Plate 3.1	The fruit powders (Pineapple and Watermelon)	30
Plate 3.2	The fruit hydrolysates after filtration and after dilution to $10\%\;v/v$	38
Plate 4.1	Agarose gel electrophoresis for the confirmation of Amplicons of ITS genes	45
Plate 4.2	Day 3 of fermentation	53

APPENDICES

Appendice	Title of Appendice	Page
A	Morphological and Biochemical characteristics of the yeast isolate	83
В	Fermentation media used in the study	84
C	Day 1 and Day 3 of fermentation using Pineapple and Watermelon	85
	peel media	
D	PCR reaction mixture and PCR amplification conditions	86
E	Composition of Biuret Reagent	86
F	Mean Proximate compositions of Pineapple and Watermelon peels	87

LIST OF ABBREVIATIONS

BLAST Basic Local Algorithm Search Tool

BSA Bovine Serum Albumin

CDW Cell Dry Weight

FHM Fruit Hydrolysate Media

GM Glucose Supplemented Media

GNF Glucose-Nitrogen Supplemented Media

ITS Internal Transcribed Spacer

MF Molasses Supplemented Fruit media

NCBI National Center for Biotechnology Information

OD Optical Density

PCR Polymerase Chain Reaction

PDA Potato Dextrose Agar

rDNA ribosomal Deoxyribonucleic acid

SCP Single-cell Protein

SF Supplemented Fruit Media

ABSTRACT

The fast growing population of the world has led to an increased demand for protein rich food. The conversion of fruit wastes into single-cell protein is an alternative solution to the worldwide protein deficiency and environmental pollution concerns. In this study, watermelon and pineapple fruit peels were used to produce single cell protein by submerged fermentation. The organism used for the single cell production was isolated from Palm wine. The organism was characterized via standard microbiological methods - morphological, biochemical and molecular characterization. For the molecular characterization the internal transcribed spaces ITS 1 and ITS4 genes were targeted and sequenced using Sanger Sequencing method. In addition, the isolate evolutionary relatedness was performed on the basis of rDNA comparison of the obtained data sequence with known sequences in the GenBank. Five different hydrolysate media were compounded and used to determine the growth profile of the isolate and single cell protein production. The formulates from the fruits include: FHM, GH, GHM, GNF and MF. The rDNA sequence comparison identified the organism as Saccharomyces cerevisiae with accession number OK172582 with 99.62 % homology to the type strain of Saccharomyces cerevisiae strain YBA 08. The pineapple and watermelon fruit hydrolysate media supplemented with molasses recorded high concentrations of total protein (0.53-0.66 mg/mL) respectively on the 7th day of fermentation, as compared to that of the fruit hydrolysate media without supplementation which produced 0.01-0.06 mg/mL of protein respectively. Similarly, fruit hydrolysate media supplemented with glucose and nitrogen recorded a high content of protein (0.24-0.52mg/mL) while fruit hydrolysate media supplemented with glucose produced (0.07-0.20mg/mL) of protein and the supplemented fruit hydrolysate media produced (0.12-0.17mg/mL) protein. It was observed that supplementation with molasses as a carbon source greatly increased SCP production in both fruit combinations. This suggest that microbial growth on fruit peels can be best improved by addition of molasses as a nutrient supplement rather than just the fruit peels. The findings in this study indicated that pineapple and watermelon peels could serve as a good substrate for SCP production.

Keywords: Fermentation, Pineapple, Saccharomyces cerevisiae, Single Cell Protein, Watermelon.