QUALITY ASSESSMENT AND SAFETY EVALUATION OF PROVITAMIN A CASSAVA (*Manihot esculenta*) STARCH HYDROLYSATE AS PROBIOTIC CARRIER FOR ENCAPSULATED AND FREE *Lactobacillus rhamnosus GG*

BY

MODUPEOLA ADELAJU OGUNTOYE

Matric Number: 134971

B.Sc. Food Science and Technology (FUTA); M.Sc. Food Technology (Ibadan)

A THESIS IN THE DEPARTMENT OF FOOD TECHNOLOGY

SUBMITTED TO THE FACULTY OF TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

OF THE

UNIVERSITY OF IBADAN, NIGERIA

JANUARY, 2021

ABSTRACT

Incorporation of probiotics such as *Lactobacillus rhamnosus GG (LGG)* into non-dairy food matrices (fruits and vegetables) has recently been explored but with negative impact on the viability of organisms due to low pH. Provitamin A cassava starch hydrolysate could be a better vehicle in enhancing the viability of *LGG,* owing to its high glucose content which improves probiotic viability when exposed to low pH. However, there is paucity of information on incorporation of *LGG* into provitamin A cassava hydrolysates as non-dairy probiotic carrier. This study was designed to evaluate the quality and safety attributes of provitamin A cassava starch hydrolysate as carrier for *LGG*.

Starches from three provitamin A cassava varieties (IITA-TMS-I011368, IITA-TMS-I070593 and IITA-TMS-I011371) were extracted and hydrolysed using α-amylase and glucoamylase. The hydrolysates were inoculated with free and alginate-encapsulated *LGG,* and analysed after fermentation (48 h) and during storage (4^{o}C, 90 days). Chemical [residual hydrogen cyanide (HCN) and β-carotene retention], antioxidant [2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity] and microbiological [coliforms, *Salmonella* spp*., Shigella* spp*.* and survival/viability of *LGG* in Simulated Gastric Juice (SGJ), Simulated Intestinal Juice (SIJ) and hydrolysates] properties were determined. Hydrolysate with the most significant properties was selected and administered to adult Wistar rats (120-150 g). Acute toxicity (n=65 females; 24 h) and sub-acute toxicity (n=40 males; 30 days) studies of hydrolysate were conducted. Signs of acute toxicity were monitored, while blood and organ samples (kidney and liver) were collected on Day 31 for sub-acute toxicity and compared with the control. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Residual HCN decreased significantly from 25.06-38.70 to 1.00-1.80 mg/kg in the hydrolysates, making it safe for consumption. β-carotene retention and DPPH assay ranged from 47.37 to 94.44% and from 83.30 to 86.97%,indicating strong antioxidant activity. No coliforms*, Salmonella* and *Shigella* were observed for 30 days of storage. Encapsulated *LGG* had better percentage survival in SGJ (77.36%) and SIJ (31.21%) than free *LGG* at 6.53% and 1.22%, respectively. Viable counts of *LGG* in hydrolysates before and after fermentation were 7.32-7.48 and 9.12-9.23 Log CFU/mL (free), and 7.51-7.73 and 9.28-9.32 Log CFU/mL (encapsulated), respectively.

Viability of free (91.45%) and encapsulated *LGG* (94.01%) was maintained after 30 days, but reduced significantly to 35.77 and 57.78%, respectively by the end of 90 days of storage. No sign of toxicity was observed. The IITA-TMS-I011368 hydrolysate groups with free *LGG*, encapsulated *LGG* and control had blood urea nitrogen of 1.15, 2.09 and 1.28 g/dL, and albumin:globulin ratios were 1.43, 1.90 and 1.55 respectively. Aspartate aminotransferase, Alanine transaminase and Alkaline phosphatase in IITA-TMS-I011368 hydrolysate groups with highest dose of *LGG* were 65.00, 54.80 and 39.60 IU/L (free), 72.40, 62.20 and 52.80 IU/L (encapsulated), and 74.40, 62.60 and 49.20 IU/L (control) respectively, indicating no kidney and liver damage.

A high quality, safe and microbiologically stable probiotic carrier with properties that supported the growth of *Lactobacillus rhamnosus GG* was developed from hydrolysed provitamin A cassava. Encapsulated *Lactobacillus rhamnosus GG* maintained significant viability in fermentation and storage.

Keywords: Non-dairy probiotic carrier, Provitamin A cassava hydrolysates, Alginate encapsulation, *Lactobacillus rhamnosus GG*.

Word count: 493

DEDICATION

To the Almighty God, the Beginning and the Ending. It was His grace and strength that saw me through this work. To Him be all the glory.

And to my husband, Victor Oluwagbeminiyi Oguntoye, and children- Feyisayo, Fikayomi and Fayokunmininu, thank you for your support, understanding and numerous sacrifices.

ACKNOWLEDGEMENTS

I am very thankful to Professor A.A. Olapade, the Head of Department of Food Technology for his support. I would like to express my sincere thanks and appreciation to my Supervisor, Dr. Olufunke O. Ezekiel for her excellent supervision, encouragement, and understanding and for having confidence in me. Your advice on both my research and life generally have been priceless. I am immensely grateful.

I am thankful to the immediate past Head of Department of Food Technology, Professor K.O. Falade, for his assistance and understanding during the course of my Teaching and Research Assistantship.

My appreciation goes to the Post Graduate College, University of Ibadan for granting me the award of Teaching and Research assistantship during the course of this research. Those stipends went a long way.

I am thankful to all the academic staff of the department - Prof. O.C. Aworh, Prof. G.O. Adegoke, Prof. Rahman Akinoso, Dr. Phyllis Uzo-Peters, Dr. G.L. Arueya, Dr. Folasade O. Adeboyejo and Dr. A.O. Oladunjoye.

My gratitude also goes to the laboratory and administrative staff of the Department of Food Technology, University of Ibadan for their useful contributions and assistance at one time or the other during the course of my research.

I am deeply grateful to Prof. V.O. Adetunji of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for her magnanimity in providing the lyophilised *Lactobacillus rhamnosus GG* that was used for this research.

Words would not convey my deep appreciation to Dr. Olayinka Oridupa of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan, who held nothing back in providing assistance and guidance with the toxicological aspects of this work.

Many thanks to Dr. Naomi Fega, Dr. Oyagbemi, Dr. Jeremiah Afolabi and everyone in the Cardio-Renal Central Laboratory of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan, for all the assistance and careful way you put me through while working on the Toxicological aspect of the work.

My appreciation goes to Mr Niyi Alao of Microbiology Department, University of Ibadan and Mr Ale Oluwatosin of the Pharmaceutical Chemistry Department, University of Ibadan for their support and assistance at the time of carrying out the bench work.

Many thanks to the following staff of Multidisciplinary Central Research Laboratory (MCRL) of the University of Ibadan assisted at the time of carrying out bench work: Mrs Korede, who assisted with the work relating to HPLC; Mr Abiola, who assisted with the use of light microscope on morphological studies of the Microsphere.

I am grateful to Mr Peter Illuebbey and the entire Cassava Breeding Unit if the International Institute of Tropical Agriculture (IITA), Ibadan for providing the Provitamin A cassava varieties used in this research.

My wonderful friend, Dr. Folasade O. Adeboyejo, who has been such a great friend to have. Thank you always for your sincere love, encouragement and motivation.

My parents, Barrister and Mrs Adeboye Olatunji, who were always enthusiastic and keen to know my progress, thank you. Thank you for laying the foundation of my educational career, for your love and for always encouraging me.

I am also grateful to my second Mum, Mrs Ayodele Oguntoye, who showed me an exceptional love and support, especially with the care of the children during the course of my study.

My thanks go to my siblings, Engr & Mrs Adeboye Olatunji, Barr. & Mrs Adebayo Olatunji and Oluwatoyin Adejare. Thank you for your love and support all these years.

I sincerely appreciate and thank everyone who has contributed in various ways in the course of this research, all of whom I cannot mention here for space constraints.

CERTIFICATION

I certify that this research was carried out by Mrs Modupeola Adelaju, Oguntoye in the Department of Food Technology, University of Ibadan.

Supervisor

Olufunke O. Ezekiel Ph. D

Senior Lecturer, Department of Food Technology,

University of Ibadan.

TABLE OF CONTENTS

TITLE PAGE		i
ABSTRACT		ii
DEDICATION		iv
ACKNOWLEDGEMENTS		v
CERTIFICATION		vii
TABLE OF CONTENTS		viii
LIST OF TABLES		xvii
LIST OF FIGURES		xix
TABLE OF CONTENTS		
CHAPTER ONE		1
INTRODUCTION		1
1.1	Background of the study	1
1.2	Problem statement and Justification of the Research	4
1.2.1	Problem statement	4
1.2.2	Justification of the Research	4
1.3	Objectives of the research	6
CHAPTER TWO		7
LITERATURE REVIEW		7
2.1	Cassava	7
2.1.1	Utilization of cassava	7
2.1.2	Antinutrient factors in cassava	10
2.1.3	Cyanide detoxification in cassava	10
2.2	Biofortification	11
2.2.1	Provitamin A carotenoids	11
2.2.2	Beta-carotene	13
2.2.2.1	Advantages of Beta carotene	13
2.2.2.2	Chemical properties of beta carotene	13
2.2.2.3	Bioavailability of β-carotene	14

2.2.2.4 Factors affecting the stability of β-carotene 15
2.3 Provitamin A cassava 15
2.3.1 Consumers' acceptance of biofortified cassava 16
2.4 Starch hydrolysis 16
2.4.1 Amylolytic enzymes 19
2.5 Fermentation 20
2.5.1 Lactic Acid Fermentation 21
2.5.2 Production of beverages by lactic fermentation 21
2.5.3 Factors affecting production of lactic acid by lactic acid bacteria during fermentation 24
2.5.3.1 Fermentation process conditions 24
2.5.3.2 Carbon source 24
2.5.3.3 Nutrient sources 24

2.6 General overview of lactic acid bacteria 25
2.6.1 Lactic acid bacteria (LAB) as starter culture 25
2.7 Probiotics: consumption of live lactic acid bacteria 26
2.7.1 Health benefits of probiotics 28
2.7.2 Characterisation of an organism as a probiotic 27
2.7.3 Gut microflora 29
2.8 *Lactobacillus rhamnosus GG (LGG)* 30
2.8.1 Taxonomy of *Lactobacillus rhamnosus GG* 30
2.8.2 Characteristics of *Lactobacillus rhamnosus GG* 30
2.8.3 Clinical importance of *Lactobacillus rhamnosus GG* 30
2.9 Immobilisation of probiotics 32
2.9.1 Alginate-encapsulation of probiotics 36
2.9.2 Effect of encapsulation on fermentation 36
2.10 Recent trends of probiotic applications in beverages 37
2.10.1 Beverages used as probiotic carriers and their limitations 37
2.10.2 Use of encapsulating agents in non-dairy probiotic beverages 38
2.11 Phytochemicals 38

2.11.1 Classes of phytochemicals 38
2.11.1.1 Alkaloids 39
2.11.1.2 Carotenoids 39
2.11.1.3 Phenolics 39
2.11.1.4 Saponins 40
2.12 Colour intensity in beverages 40
2.13 Toxicological studies 42
2.13.1 Food toxicology 42
2.13.2 Animal toxicity tests 43
2.13.3 Oxidative stress 44
2.13.3.1 Reactive oxygen species (ROS) 45
2.12.3.2 Oxidative stress markers 45
2.12.4 Antioxidant defense systems 45
2.13.4.1 Enzymatic and Non-enzymatic Antioxidants 45
2.14 Rheological properties 46
2.14.1 Kinematic Viscosity 47
2.15 Glycemic Index 47
CHAPTER THREE 50
MATERIALS AND METHODS 50
3.1 Source of raw materials 50
3.2 Preparation of Provitamin A cassava hydrolysate from cassava roots 50
3.2.1 Starch Extraction 50
3.2.2 Hydrolysis of Provitamin A cassava starch 52
3.3 Culturing and harvesting of *L. rhamnosus GG* before inoculation 52
3.3.1 Inoculation of the provitamin A cassava hydrolysate with starter culture *L. rhamnosus GG.* 52
3.4 Microencapsulation of probiotics 54
3.4.1 Microencapsulation of *L. rhamnosus GG* in alginate beads by emulsion technique 54
3.4.2 Microencapsulation of *L. rhamnosus GG*in alginate beads by

extrusion method 54
3.4.3 Optical examination of calcium alginate beads containing *L. rhamnosus GG* 54
3.4.4 Calcium alginate bead size determination 54
3.4.5 Release of entrapped cells 55
3.4.6 Enumeration of probiotic cells 55
3.4.7 Determination of survival rate and encapsulation efficiency 55
3.5 simulation of In-vitro gastric and intestinal conditions 55
3.5.1 Preparation of simulated gastric and intestinal juices 55
3.5.2 Sequential Incubation of Free and Microencapsulated *Lactobacillus rhamnosus GG* in Simulated Gastric and Intestinal Juices. 56
3.6 Chemical Analysis 56
3.6.1 Determination of pH 56
3.6.2 Determination of % total titratable acidity (TTA) 57
3.6.3 Organic acids quantification 57
3.6.4 Estimation of Total Sugars by Anthrone Method 57
3.6.5 Determination of Reducing Sugar (Dinitrosalicylic Acid method) 58
3.6.6 Total soluble sugars determination 58
3.6.7 Elemental Mineral Analysis 58
3.7 Phytochemical, carotenoid and antinutrient analysis 59
3.7.1 Determination of total phenolic Content 59
3.7.2 Determination of Total Flavonoids 59
3.7.3 Antioxidant capacity [2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay] 59
3.7.4 Determination of carotenoid content 60
3.7.4.1 Extraction of carotenoids 60
3.7.4.2 β-carotene content determination 60
3.7.5 Determination of Cyanide Content 61
3.8 Colour measurement 61
3.9 Microbiological Analysis 62

3.9.1 Determination of presence of pathogenic organisms in provitamin A cassava hydrolysate samples 62
3.9.1.1 Media Preparation 62
3.9.1.2 Coliform count 62
3.9.1.3 Total fungal (yeast and mould) counts 63
3.9.1.4 Enumeration of *Salmonella* and *Shigellaspp*. 63
3.9.2 Total viable counts of *Lactobacillus rhamnosus GG* 63
3.10 Evaluation of microbiological safety of provitamin A cassava hydrolysate during storage 64
3.11 Determination of viscosity 64
3.12 Animal study 64
3.12.1 Ethical Statement 64
3.12.2 Experimental Animals 65
3.12.3 Evaluation of *In vivo* effect of administration of provitamin A cassava hydrolysate with or without *L. rhamnosus GG* on faecal microbial ecology of Wistar rats 65
3.12.3.1 Evaluation of Growth Direction Index (GDI) 65
3.12.4 Toxicological evaluation of Provitamin A cassava hydrolysate 66
3.12.4.1 Study design 66
3.12.4.2 Acute toxicity study (LD_{50}) 66
3.12.4.3 Sub-Chronic toxicity study 67
3.12.4.3.1 Physical observation and mortality 67
3.12.4.3.2 Body weight, feed and water consumption 67
3.12.5 Sample collection 67
3.12.5.1 Relative Organ Weight 68
3.12.5.2 Preparation of tissues homogenates for lipid peroxidation assay 68
3.12.6 Haematological and biochemical analysis 68
3.12.6.1 Determination of haematological parameters 68
3.12.6.2 Determination of serum biochemical parameters 69
3.12.6.2.1 Assessment of lipid profile 69
3.12.6.2.2 Estimation of serum creatinine and blood urea nitrogen 69

3.12.6.2.2.1 Estimation of serum creatinine 69
3.12.6.2.2.2 Estimation of blood urea nitrogen 69
3.12.6.2.2.3 Assessment of liver function parameters 70
3.12.7 Assessment of markers of oxidative stress on the kidney, heart and liver tissues 70
3.12.7.1 Determination of total protein content in serum and tissue samples 70
3.12.7.2 Estimation of Nitric Oxide (NO) in tissue samples 71
3.12.7.3 Hydrogen Peroxide Generation 71
3.12.7.4 Estimation of superoxide dismutase (SOD) activity 71
3.12.7.5 Determination of reduced glutathione (GSH) level 72
3.12.7.6 Determinaton of Glutathione-S-Transferase (GST) activity in tissues of rats 72
3.12.7.7 Glutathione Peroxidase (GPX) Determination 72
3.12.7.8 Assessment of Lipid Peroxidation 73
3.13 Determination of glycemic index (GI) using Wistar rats 73
3.13.1 Blood collection and determination of blood glucose level 74
3.13.2 Calculation of glycemic index 74
3.14 Studies involving Human subjects 74
3.14.1 Ethical statement 74
3.14.2 Determination of Glycemic Index using human subjects 75
3.15 Sensory analysis 75
3.15.1 Development of descriptors for product attributes 75
3.15.2 Evaluation of sensory attributes of provitamin A cassava hydrolysate with and without *L. rhamnosus GG* 76
3.16 Statistical Analysis 76
CHAPTER FOUR 78
RESULTS AND DISCUSSION 78
4.1 Encapsulation of *L. rhamnosus GG*. 78
4.1.1 Properties of calcium-alginate beads 81
4.1.2 Effect of microspheres stored at 4^{o}C on the viability of *L. rhamnosus GG* 83

4.2 Effect of microencapsulation on the survival of probiotic cells in simulated gastric and intestinal conditions 83
4.3 Changes in chemical attributes of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* during fermentation 88

4.3.1 Changes in pH of probiotic provitamin A cassava hydrolysate 88
4.3.2 Changes in the rate of lactic acid production during fermentation 91
4.3.3 Effect of fermentation on the total sugar, reducing sugar total soluble sugars (brix) content of provitamin A cassava hydrolysate 94
4.3.4 Dextrose equivalents of provitamin A cassava hydrolysates 96
4.4 Kinematic viscosity (mm^2/s) of provitamin A cassava hydrolysates 96
4.5 Mineral analysis 99
4.6 Phytochemical and anti-nutrients of provitamin A cassava hydrolysates 102
4.6.1 Total flavonoid content, total phenolic content and total antioxidant capacity as measured by DPPH free radical scavenging activity 102
4.6.2 Effect of fermentation on beta carotene content 106
4.6.3 Residual Hydrogen cyanide content of provitamin A cassava hydrolysates 108
4.7 Hunter L, a, b colour attributes of provitamin A cassava hydrolysates 110
4.8 Microbial analysis 115
4.8.1 Total viable count of *L. rhamnosus GG* in Provitamin A cassava hydrolysates during fermentation 115
4.8.2 Microbiological safety of provitamin A cassava hydrolysate after fermentation 116
4.9 Storage studies 116
4.9.1 Effect of Probiotic on the pH of provitamin A cassava hydrolysates during storage at 4^oC 116
4.9.2 Changes in the organic acid concentration of the provitamin A cassava hydrolysates during storage at 4^oC 119
4.9.3 Changes in the Hunter L, a, b (ΔL, Δa and Δb) colour attributes of probiotic provitamin A cassava hydrolysate during storage 122
4.9.4 Viability of free and encapsulated *L. rhamnosus GG* in

provitamin A cassava hydrolysate during storage at 4°C 127
4.9.5 Microbiological safety of provitamin A cassava hydrolysate during storage 132

4.10 Animal study 133
4.11 Effect of administration of provitamin A cassava hydrolysate on faecal microbial ecology and growth direction index of Wistar rats 134
4.12 Toxicological evaluation of probiotic provitamin A cassava hydrolysate 139
4.12.1 Acute toxicity of probiotic provitamin A cassava hydrolysate 139
4.12.2 Sub-chronic toxicity of provitamin A cassava hydrolysate 141
4.12.2.1 Effect of provitamin A cassava hydrolysate on the weight gain of Wistar Rats 141
4.12.2.2 Effect of provitamin A cassava hydrolysate with or without *L. rhamnosus GG* on the haematological parameters of Wistar rats 143
4.12.2.3 Effect of Provitamin A cassava hydrolysate on serum biochemical indices of Wistar rats 148
4.12.2.4 Relative Organ weights 154
4.12.2.5 Assessment of Markers of Oxidative Stress in Wistar rats administered with probiotic provitamin A cassava hydrolysate 156
4.13 Glycemic index 163
4.13.1 Estimation of glycemic index of provitamin A cassava hydrolysate containing free and encapsulated *Lactobacillus rhamnosus GG* in rats 163
4.14 Descriptive sensory analysis 169
CHAPTER FIVE 173
CONCLUSIONS, RECOMMENDATIONS AND CONTRIBUTIONS TO KNOWLEDGE 173
5.1 Conclusions 173
5.2 Recommendations 175
5.3 Contributions to knowledge 175
REFERENCES 177
APPENDICES 216

LIST OF TABLES

Table 2.1:	Global production of cassava	8
Table 2.2:	Summary of various effects of probiotics on human health	33
Table 3.1:	Product attributes, definitions and corresponding reference standards used for sensory evaluation	77
Table 4.1:	Properties of *L. rhamnosus GG* microspheres	79
Table 4.2:	The effect of fermentation on the total sugar, reducing sugar, total soluble sugars (^{o}Brix) content of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG*	98
Table 4.3:	Mineral elements composition of provitamin A cassava hydrolysates (mg/kg)	100
Table 4.4:	Phytochemical, antioxidant capacity and carotenoid content of provitamin A cassava hydrolysates with and without *L. rhamnosus GG*	103
Table 4.5:	Residual hydrogen cyanide content (mg/100g fresh root weight) of provitamin A cassava hydrolysates with and without *L. rhamnosus GG*	111
Table 4.6:	Hunter L, a, b colour attributes of provitamin A cassava Hydrolysate with and without *L. rhamnosus GG*	112
Table 4.7:	Pearson correlation between beta carotene and colour attributes of provitamin A cassava hydrolysates	114
Table 4.8:	Changes in Organic acid content of provitamin A cassava hydrolysate Containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C	120
Table 4.9:	Changes in the Hunter L, a, b colour attributes of TMS-I011368 hydrolysates containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C	123
Table 4.10:	Changes in the Hunter L, a, b colour attributes of TMS-I070593 hydrolysates containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C	124

Table 4.11: Changes in the Hunter L, a, b colour attributes of TMS-I011371 hydrolysates containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C 125

Table 4.12: Acute Toxicity of provitamin A cassava hydrolysate with or without *L.rhamnosus GG* in Wistar Rats: Toxicity and Mortality 140

Table 4.13: Effect of provitamin A cassava hydrolysate with or without *L. rhamnosus GG*on percentage (%) body weight gain of rats 142

Table 4.14: Effect of provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on the haematological parameters of Wistar rats 144

Table 4.15: Effect of provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on the differential white blood cell parameters of Wistar rats 145

Table 4.16: Effect of Provitamin A cassava hydrolysate with or without *L. rhamnosus GG*on Plasma protein 149

Table 4.17: Effect of provitamin A cassava hydrolysate with or without *L. rhamnosus GG*on liver enzymes of Wistar rats 150

Table 4.18: Effect of Provitamin A cassava hydrolysates with or without *L. rhamnosus GG*on blood lipid profile of Wistar rats 151

Table 4.19: Effect of Provitamin A cassava hydrolysates with or without *L. rhamnosus GG*on Relative Organ Weights of Wistar rats 155

Table 4.20: Effect of Provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on kidney oxidative stress profile of Wistar rats 157

Table 4.21: Effect of Provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on Heart oxidative stress profile of Wistar rats 158

Table 4.22: Effect of Provitamin A cassava hydrolysates with or without *L. rhamnosusGG*on Liver oxidative stress profile of Wistar rats 159

Table 4.23: Blood glucose indices of normoglycemic Wistar rats administered TMS-I011368 hydrolysate with or without *L. rhamnosus GG* 164

Table 4.24: Blood glucose indices of normoglycemic humans administered TMS-I011368 hydrolysate without or with *L. rhamnosus GG* 165

Table 4.25: Sensory evaluation and descriptive analysis of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* 170

LIST OF FIGURES

Figure 2.1:	Structures of major carotenoids	12
Figure 2.2:	Optical isomers of Lactic acid	23
Figure 2.3:	The encapsulation process of probiotics by extrusion technique	34
Figure 2.4:	The encapsulation process of probiotics by emulsion technique	35
Figure 2.5:	Classification of dietary phytochemicals	41
Figure 2.6:	Ostwald viscometer	48
Figure 3.1:	Provitamin A cassava starch production	51
Figure 3.2:	Provitamin A cassava hydrolysate production	53
Figure 4.1:	Microspheres of *L. rhamnosus GG* produced by extrusion and emulsion methods	80
Figure 4.2:	External structure of Ca-alginate encapsulated *L. rhamnosus GG* produced by extrusion and emulsion methods as viewed under the Light microscope	81
Figure 4.3:	Stability of *L. rhamnosus GG* microspheres stored at $4^{o}C$ for 7 days	84
Figure 4.4:	Rate of survival of *L. rhamnosus GG* cells during sequential incubation in simulated gastric and intestinal juice	85
Figure 4.5:	Rate of cell release of encapsulated *L. rhamnosus GG* cells in simulated Gastro-intestinal conditions	86
Figure 4.6:	Changes in pH of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* during fermentation	89
Figure 4.7:	Changes in lactic acid content of provitamin A cassava hydrolysates containing free and encapsulated *L. rhamnosus GG*during fermentation	93
Figure 4.8:	Dextrose Equivalent of Provitamin A cassava hydrolysates containing free and encapsulated *L. rhamnosus GG*	97
Figure 4.9:	Kinematic viscosity (mm2/s) of provitamin A cassava hydrolysates containing free and encapsulated *L. rhamnosus GG*	98
Figure 4.10:	Regression analysis showing the relationship between total	

phenolic, total flavonoid content and free radical scavenging activity of provitamin A cassava hydrolysate 104

Figure 4.11: Rate of Beta carotene retention in the provitamin A cassava hydrolysates containing free and encapsulated *L. rhamnosus GG*with respect to fresh cassava roots 107

Figure 4.12: Percentage (%) reduction in cyanide contents during processing of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* in comparison with fresh roots 109

Figure 4.13: Total viable counts of encapsulated and free *L. rhamnosus GG* in provitamin A cassava hydrolysates during fermentation 117

Figure 4.14: Changes in pH of provitamin A hydrolysates containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C 118

Figure 4.15: Effect of packaging on the viability of free and encapsulated *L. rhamnosus GG* in TMS-I070593 hydrolysate during storage at 4^{o}C 128

Figure 4.16: Effect of packaging on the viability of free and encapsulated *L. rhamnosus GG* in TMS-I011371 hydrolysate during storage at 4^{o}C 129

Figure 4.17: Effect of packaging on the viability of free and encapsulated *L. rhamnosus GG* in TMS-I011368 hydrolysate during storage at 4^{o}C 130

Figure 4.18: Evaluation of presence of spoilage and pathogenic organisms in provitamin A cassava hydrolysate during storage at 4^{o}C for 60 days 133

Figure 4.19: Microbial population of faecal microflora of Wistar rats administered TMS-I011368 hydrolysate with encapsulated or free *L. rhamnosus GG*for 30 days 135

Figure 4.20: Growth direction index (GDI) of faecal microflora of Wistar rats administered TMS-I011368 hydrolysate with encapsulated or free *L. rhamnosus GG* for 30 days 137

Figure 4.22: Blood glucose response curves of TMS-I011368 hydrolysates with or without *L. rhamnosus GG* in normoglycemic rats 166
Figure 4.23: Blood glucose response curve of TMS-I011368 hydrolysate with or without *L. rhamnosus GG* in normoglycemic human subjects 167

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Probiotic food and beverages are products with the inclusion of live bacteria, which are considered to be beneficial to the consumers, if ingested in sufficient amounts. These category of food products are increasing in popularity as a result of increase in demands due to increased awareness of health benefits that can be derived from consuming such products. *Lactobacillus* or *Bifidobacterium*are examples of lactic acid bacteria utilised as probiotics. Products of dairy origin, such as yogurts, ice cream, cheese, etc. have been the conventional matrices for the incorporation of probiotics. However, the major limitation of probiotic beverages of dairy origin is the unsuitability for consumers who are lactose intolerant, hypercholesterolemia or vegetarian in nature (Bayless *et al.*, 2017). As a result of this, creating new or innovative products of non-diary origin as alternative carriers for probiotics is an evolving line of research in the food industry, as it will satisfy the growing demands of consumers who desire non-dairy probiotic foods.

In recent times, food matrices of non-dairy origin have been explored as alternative food carriers for probiotics. The nature of food carrier is essential to the viability of probiotic organism. There are existing research in which vegetables and fruits, such as carrots, cabbage, water-melon, Tomato, beet root, apple, orange, pineapple, etc. (MaiaCosta *et al.*, 2013; Sivudu *et al.*, 2014; Tamminen *et al.*, 2013; Yoon *et al.*, 2006) have been established as excellent probiotic carriers. Development of these functional probiotic foods or beverages often involves lactic acid fermentation.

Fermentation can be explained as an advantageous biochemical process whereby alteration or modification of the chemical composition of primary food products occur, by the metabolism of microorganisms and enzymes (Karovicova and Kohajdova, 2003). Lactic acid fermentation is a process through which the foods are pre-digested

by the useful enzymes, fungi and beneficial bacteria which metabolise sugars and primarily

forms lactic acid as the metabolite (Fugelsang and Edwards, 2007). Beverages produced by lactic acid fermentation therefore, are non-alcoholic as they contain mainly organic acids, especially lactic acid. Non-alcoholic beverages play a dominantpart in the dietary patterns of many Nigerians as refreshing drinks, especially during the dry season. Beverages can be served for pleasure after eating or taken as functional foods for health purposes. Yoghurt and other lactic acid fermented products of dairy origin were the most commonly consumed functional food matrices with probiotics in time past (Vella *et al.*, 2013).

Exploration of food matrices of plant origin for development of functional probiotic beverages through lactic fermentation has been extended to roots and tubers crops such as orange fleshed sweet potatoes (Panda and Ray, 2007).

Roots and tuber crops such as cassava and sweet potatoes are highly perishable. Therefore, a major pathway to ensure nutrition and food security in Sub-Saharan Africa is to reduce postharvest losses (PHL) (Affognon *et al.*, 2014). In a bid to reduce post-harvest losses, there is a need to increase utilisation of root/tuber crops such as cassava by the means of product diversification.

Cassava (*Manihot esculenta crantz*) is a staple root crop in India, Asia, Africa and South. It is important as an energy-giving food for over five hundred million people (Njoku and Obi, 2010). Traditionally, cassava roots can be cooked in many ways and it can be formed into products such as tapioca, starch, *attieke, lafun* flour or *gari*. Other products from cassava include dried cassava chips, etc. Cassava leaves can be used to make soup or as feed for livestock. Industrially, the production of bioethanol from cassava has also been achieved (Yuwa-Amornpitak, 2010). Asides biofuel, the starch can be used as raw material in the paper and drug making industries. Cassava can also be used to produce syrup concentrates for soft drinks production. The flour can be used in the confectioneries industry to make cookies, biscuits, bread, etc.

Cassava roots generally serve as a staple food that has high carbohydrates and dietary fibre but low vitamin A contents. The deficiencies of micronutrients such as vitamin A, iodine and iron are the most predominant in the world (Montagnac *et al.*, 2009) but the micronutrient deficiency of highest importance in Nigeria and Africa is vitamin A which is a fat-soluble vitamin essential in clear vision, reproduction, bone growth, and for maintaining healthy hair, skin, and mucous membranes (Uchendu, 2013). Uchendu,

(2013) stated that most cases of childhood blindness are caused by vitamin A deficiency.

In recent years, International Institute of Tropical Agriculture (IITA), Ibadan, with National Root Crops Research Institute (NRCRI), Umudikeand in support of the effort of Nigeria government in alleviating micronutrient deficiency of vitamin A, developedbio-fortified root/tuber crops e.g. cassava(Omodamiro and Aniedu, 2012).

Bio-fortification refers to improving the quality of nutrients of food crops using conventional plant breeding methods either solely or in combination with biotechnology(Uchendu, 2013). Bio-fortification of staple food crops has a major impact in reducing micronutrient deficiency in developing nations. It is a new approach which is employed in public health to alleviate deficiencies of micronutrients in poor countries (Penelope *et al.*, 2014). Examples of crops that have been bio-fortified with β carotene which is a precursor of vitamin A include provitamin A cassava and Orange-fleshed sweet potatoes. The orange-fleshed sweet potato has a deep orange coloration while the provitamin A cassava has a yellow flesh colouration. The variation from the usual white flesh colour of root crops is because of the heavy presence of carotenoids in bio-fortified crops.

There is an ongoing concern in value addition of bio-fortified cassava varieties and derivative products (Ayetigbo *et al.*, 2018). Provitamin A cassava hydrolysate is obtained from the hydrolysis of provitamin A cassava starch. It is rich in beta-carotene, and other phytochemicals compounds which are bioactive compounds (Orrù *et al.*, 2018). It is further enriched with probiotic *L. rhamnosus GG.* Thus it can be regarded as a functional food. European Commission describes functional foods as a food product that offers, asides the basic nutrients, additional beneficial effects on the human physiological functions, resulting in decreasing the risk of diseases or improvement of general physical wellbeing of an individual (Orrù *et al.*, 2018). The intrinsic human-health benefits of probiotics are well known.

Therefore, supplementation of provitamin A cassava hydrolysate with probiotic bacteria represents a new option for non-dairy probiotic beverages and it will serve as a good alternative for people that are lactose intolerant. The development of a lactic acid fermented beverage such as provitamin A cassava hydrolysate from provitamin A cassava would provide more value addition for cassava, as it is different from the traditional uses of these crops. Provitamin A cassava hydrolysate is an innovative

product which can also be considered a functional food due to the bioactive components such as carotenoids. These are antioxidants which have physiological characteristicssuch as of preventing oxidation, cancer, night blindness, ageing and other degenerative disorders like injury to the liver (Panda *et al.*, 2007, Martarelli *et al.*, 2011). Lactic acid fermentation enhances digestibility and nutritive content of hydrolysate produced from cassava(Montagnac *et al.*, 2009) thus imparting the product with attributes of robust stability and safety (Rattanachaikunsopon and Phumkhachorn, 2010, Wang *et al.*, 2015). Provitamin A hydrolysate with the added advantage of probiotics thus provides the consumer with a beverage that is rich in bioactive compounds.

1.2 Problem statement and Justification of the Research

1.2.1 Problem statement

Most non-dairy probiotic beverages currently reported in literatures are from soy, cereals, fruits and vegetables sources. These are good probiotic carriers, however, their major limitation is the negative impact of these carriers on the viability of probiotic organisms during processing and storage as well as through the harsh gastrointestinal conditions (Ranadheera *et al.*, 2017). It is thus important to improve value addition and expand utilisation of non-dairy sources that enhance the viability of probiotic organisms as probiotic carriers.

Value addition of cassava products is necessary in order to reduce postharvest losses. Asogwa *et al* (2013) stated that the major challenge in the cassava sector is finding new uses for cassava. Cassava hydrolysates have been utilised industrially as sweeteners, and in the production of bioethanol (Ajibola *et al.*, 2012; Wangpor *et al.*, 2017).However, there is sparse information on the use of provitamin A cassava starch hydrolysate as ready-to-drink lactic acid fermented beverages.

There is a paucity ofinformation in the literature which reported study regarding the use of provitamin A cassava hydrolysate as carrier for *Lactobacillus rhamnosus GG* or other probiotic strain.

1.2.2 Justification of the Research

In recent years, exploration of non-dairy food matrices as probiotic carriers has increased due to increased demands of consumers for non-dairy probiotic products as a result of increased health awareness or vegetarianism(Rivera-Espinoza and Gallardo-

Navarro, 2010). Improved interest in non-dairy probiotic beverage products development exists. Functional non-dairy beverages with probioticsare believed to be healthy and refreshing (Okereke *et al.*, 2016).

Incorporation of probiotics such as *Lactobacillus rhamnosus GG (LGG)* into non-dairy food matrices (fruits and vegetables) has recently been explored but with negative impact on the viability of the organisms due to low pH. Provitamin A cassava starch hydrolysate could be a better vehicle in enhancing the viability of *LGG,* owing to its high glucose content which improves probiotic viability during low pH exposure (Corcoran *et al.*, 2005). However, there is paucity of information on incorporation of *LGG* into provitamin A cassava hydrolysates as non-dairy probiotic carrier.

In a bid to expand the list of food matrices that are suitable as probiotic carriers, it is important to explore root and tuber sources such as provitamin A cassava, as regards their ability to support the viability of probiotics through these conditions. Provitamin A cassava roots are low-cyanide varieties (sweet varieties), rich in bioactive compounds such as beta carotene which can be absorbed by humans from dietary sources and converted to retinol (Talsma *et al.*, 2016). These varieties have been established as vehicles to mitigate micronutrient deficiency of vitamin A. Provitamin A cassava varieties can be boiled and eaten and have been used for several food products(Oboh and Elusiyan, 2007). Based on this established knowledge as an added advantage, provitamin A hydrolysate was selected as vehicle for probiotics.

Probiotics have the tendency to lose viability during gastrointestinal transit and storage. Therefore, a major consideration in producing functional foods containing probiotic cells is the maintenance of functionality and viability of the probiotics in the gastrointestinal transit until they get to the colon (Heidebach, 2009). Hence, the need for microencapsulation.

Probiotic microencapsulation have mostly been applied to milk and its derivative products such as cheese, frozen desserts and yogurt until recently, when the matrix is expanding to vegetable or fruitjuices (Rokka and Rantamäki, 2010). Product innovation/development from new sources is therefore very imperative. The innovation must be a food matrix which in combination with encapsulation, supports viability of organisms in the gastrointestinal tract (Ranadheera *et al.*, 2017). From the foregoing, provitamin A cassava hydrolysates is being explored to serve as a suitable carrier for probiotic *L. rhamnosus GG*. This would in turn be of use as a healthy non-

dairy beverage for a wide population of consumers and it would be a healthy alternative for consumers who may not be able to consume probiotic dairy products.

As a ready-to-drink beverage, it is important to evaluate the safety attributes in line with product innovation. Hence, this research covers the evaluation of physical, chemical, microbial and toxicological properties as it affects the health of the consumers.

1.3 Objectives of the Research

The general objective of this research was to develop a probiotic beverage from provitamin A cassava by starch hydrolysis, modify the hydrolysate by lactic acid fermentation using *Lactobacillus rhamnosus GG* (as a probiotic starter culture) and evaluate its physicochemical, phytochemical, microbial, toxicological and sensory properties.

Specifically, this study intended to:

- hydrolyze provitamin A cassava starch using α-amylases and gluco-amylase and apply the hydrolysate as carrier for probiotic *Lactobacillus rhamnosus GG.*
- evaluate the physicochemical, microbial, and phytochemical properties of the hydrolysate without and with *L. rhamnosus GG*.
- determine the effect of encapsulation on *L. rhamnosusGG (LGG)* before and during storage at 4^{o}C.
- determine the effect of encapsulation on the viability of *L. rhamnosus GG* in simulated gastric and intestinal conditions.
- evaluate in-vivo toxicity of the provitamin A cassava hydrolysate using changes in the haematology, serum biochemistry and markers of oxidative stress.
- determine the glycemic index and sensory properties of provitamin A cassava hydrolysate.

CHAPTER TWO
LITERATURE REVIEW

2.1 Cassava

Cassava (*Manihot esculenta crantz*), of thefamily *Euphorbiaceae*is a starchy and shrubby perennial food crop grown in numerous countries. It serves as an essential food whichgives energy toover 800 million people globally (Ladeira *et al.*, 2013), especially in the tropical countries (Echebiri and Edaba, 2008).It can survive under harsh growing conditions. Cassava is a crop that is rich mainly in carbohydrate but low in protein and fat. Cassava ranks as the 5th notable crop in the world, has a global production of 278 million tons in 2016 and 2017 (FAO, 2017), with Nigeria ranking as the largest cassava producing nation in Africa (Table 1.1).

Cassava roots are easily decomposable due to high moisture contents (62%) of fresh roots(Ofor, 2011) which predisposes the roots to high microbiological activities and physiological changes that promotes unfavourable biochemical changes and microbial deterioration. This is because roots continue to respire and metabolise after harvesting since they are living organs. This also leads to high post-harvest losses especially during storage. Cassava has a shelf life ranging from 24-48 hours after harvest. Therefore, all the plans relating to processing must be adequately prepared for, even before the tubers are harvested, so as to prevent deterioration (Asonye, 2001). Deterioration in cassava could be in the form of internal discolouration or microbial spoilage (Westby, 2002). Internal discolouration is a primary physiological deterioration which could be caused by tissue damage in the process of harvesting. Usually after harvesting, fresh cassava roots are immediately eaten on the farm - either cooked or roasted.

2.1.1 Utilisation of cassava

Food constitutes the major end use of cassava. Although non-food markets for cassava products (such as animal feed and energy for industrial use) exist at both local and regional levels in sub-Saharan Africa, they are of minimal significance (FAO, 2017). Therefore, cassava production effectively translate into levels of food uses for humans.

Table 2.1: Global production of cassava

	2014 (000 tonnes)	**2015**	**2016**	**2017**
World	**276766**	**276995**	**278754**	**277957**
Africa	**154900**	**152833**	**155398**	**155962**
Nigeria	56328	57643	57855	55000
DRC (Congo)	16817	15300	15200	14550
Ghana	16524	17213	17798	19139
Angola	7639	7727	7788	7140
Mozambique	12700	8103	9100	10920
Tanzania	4993	5886	6000	5500
Uganda	2812	2898	2400	2450
Malawi	5013	4997	5000	5050
Benin	4067	3421	4096	4150
Cameroon	4836	5000	5170	5345
Rwanda	3117	3000	3060	3200
Madagascar	2930	2677	2629	2700
Côte d'Ivoire	4239	5087	4548	5367
Other Africa	12885	13881	14753	15451
Latin America	**32334**	**32299**	**32908**	**29407**
Brazil	23254	23060	23710	20110
Paraguay	3000	3000	3167	3168
Colombia	2186	2092	2117	2125
Other Latin America	3894	4147	3914	4004
Asia	**89365**	**91689**	**90274**	**92418**
Thailand	30022	32358	31161	30936
Indonesia	23436	21801	20745	20330
Viet Nam	10210	10740	10201	10650
India	8139	4373	4421	4645
China, Mainland	4593	4500	4548	5000
Cambodia	7933	11944	13298	14820
Philippines	2540	2711	2755	2825
Other Asia	2490	3261	3145	3212
Oceania	**249**	**252**	**252**	**247**

Source: (FAO, 2017)

Both cassava roots and leaves can be utilised as food. The leaves are utilised for human consumption as a protein supplement in form of vegetables or as a condiment in sauces. Due to its high protein value, the leaves are also used in animal feed formulations or as fresh forages (El-Sharkawy, 2003). Westby (2002) stated that cassava roots are more important than the leaves. The majority of cassava roots produced in Africa is utilised as human food. The percentage of cassava production for human consumption is 65% while 25% used as industrial raw materials- mostly animal feed (19%) and starch (6%) and waste is 10% (Ajala *et al.*, 2012). Asogwa*et al*. (2013) stated that the major challenge in the cassava sector has shifted from supply to demand and thus improving value addition of cassava therefore remains a greater challenge. Various food products can be obtained from cassava either as fermented foods or unfermented food products. Traditionally, *Fufu*, *Gari*,*Lafun*, *Chickwanghe*, *Attieke*, *Agbelima*,*Kivunde*, *Tape,* 'coated peanut', cheese bread, etc. are the fermented foods obtained from cassava(Ray and Sivakumar, 2009). Unfermented food products from cassava include chips, tapioca, pellets, starch and flour (Falade and Akingbala, 2011). Cassava flour is useful in the confectionery industry for making biscuits, bread,confectionery, and pasta. Cassava starch is being utilised industrially to produce a vast array of products which include production of dextrins and sweeteners such as glucose, dextrose, fructose, and maltose syrups for soft drinks production using cassava starch as the raw material, production of starch-based biodegradable plastics and polymers, production of ethanol from cassava. Cassava starch has characteristics such as high purity and solubility. It is easily gelatinised, having a high viscosity and a low retrogradation tendency compared to rice, potato or corn starches (Zamora *et al.*, 2010). These characteristics favour the use of cassava starch industrially as raw material in ethanol production.

Various enzyme treatments and pretreatments of cassava starch have been studied only with the aim of significantly improving the ethanol yield from cassava starch. Cassava starch residues have been treated with several enzyme preparations including α-amylase, amyloglucosidase (Ajibola *et al.*, 2012), cellulase, β-D-glucosidase, D-xylanase, and pectinase. All these enzymes have been used to produce cassava starch hydrolysate with increased ethanol production. The non-food uses of cassava include the use of cassava starch in textile and paper industries, and in production of adhesives and other cassava-starch based gums (Premkumar *et al.*, 2014).

2.1.2 Antinutrient factors in cassava

Utilisation of cassava roots have three major limitations which include: high moisture content which leads to high rates of postharvest deterioration, low content and quality of its protein and their naturally occurring cyanogenic glycosides, linamarin and lotaustralin. These cyanogenic glycosides are the major antinutritional factors in cassava. They break down when cellular structures are disrupted resulting from mechanical damage such as bruising of cassava tissues due to poor handling (Falade and Akingbala, 2011). Under these conditions, these cyanogenic glycosides come in contact with the hydrolytic enzyme linamarase, are hydrolyzed to produce acetonecyanohydrin which decomposes spontaneously at high temperature (> 72^{o}C and > pH 4.0) to produce hydrocyanic acid (HCN) or prussic acid. Linamarin could be ingested undecomposed but once it comes in contact with a β-glucosidase, it decomposes to produce HCN. However, in the absence of β-glucosidase, it can be excreted intact in the urine. Cassava varieties have been categorised with two descriptors into "sweet" and "bitter" on the basisof their hydrogen cyanidecontent. Sweet cassava varieties are the varieties that are low in cyanogens (< 50-100 mg HCN/kg). The highest quantity of these cyanogens exist in the peels. Cassava varieties that are designated 'bitter' are those varieties that are high in cyanogens (>100mg HCN/kg) where the cyanogens are apportioned evenly throughout the roots (Egwim *et al.*, 2013). The symptoms of cyanide toxicity in humans are: vomiting, abdominal pain, etc. A lot of diseases such as dwarfism, goitre, and dysfunction of sensory nerves (ataxia) have been linked to prolonged cyanide exposure through consumption of cyanide that has not been properly detoxified in cassava or cassava products (Kobawila *et al.*, 2005).

2.1.3 Cyanide detoxification in cassava

Zidenga *et al.* (2017) explained the detoxification pathways of cyanide as follows. Cyanide is detoxified through condensation with cysteinein plants. The condensation reaction is catalyzed by β-Cyanoalanine Synthase (CAS) to form β-cyanoalanine and hydrogen sulfide. Nitrilase is then involved in the conversion of cyanoalanine into aspartate, asparagine, and ammonia. This pathway leads to the take up of cyanide into the amino acid biosynthesis pathway (Zidenga *et al.*, 2017).

Detoxification of cyanide can also occur through condensation with thiosulfate, which is a derivative of sulfur metabolism. This is catalyzed by the rhodanese. In mammals,

detoxification of cyanide by rhodanese frequently occurs where the excretion of thiocyanate takes place through the urine (Zidenga *et al.*, 2017). Residual cyanide can be detoxified in cassava by adequate processing thereby converting cassava into products that are safe for human consumption. The various processing methods of cassava include: peeling, grating, fermentation, sun-drying, heat processes such as roasting, etc. Levels of cyanide reduction or retention depends on type and processing duration of products (Montagnac e*t al.,* 2009).

2.2 Biofortification

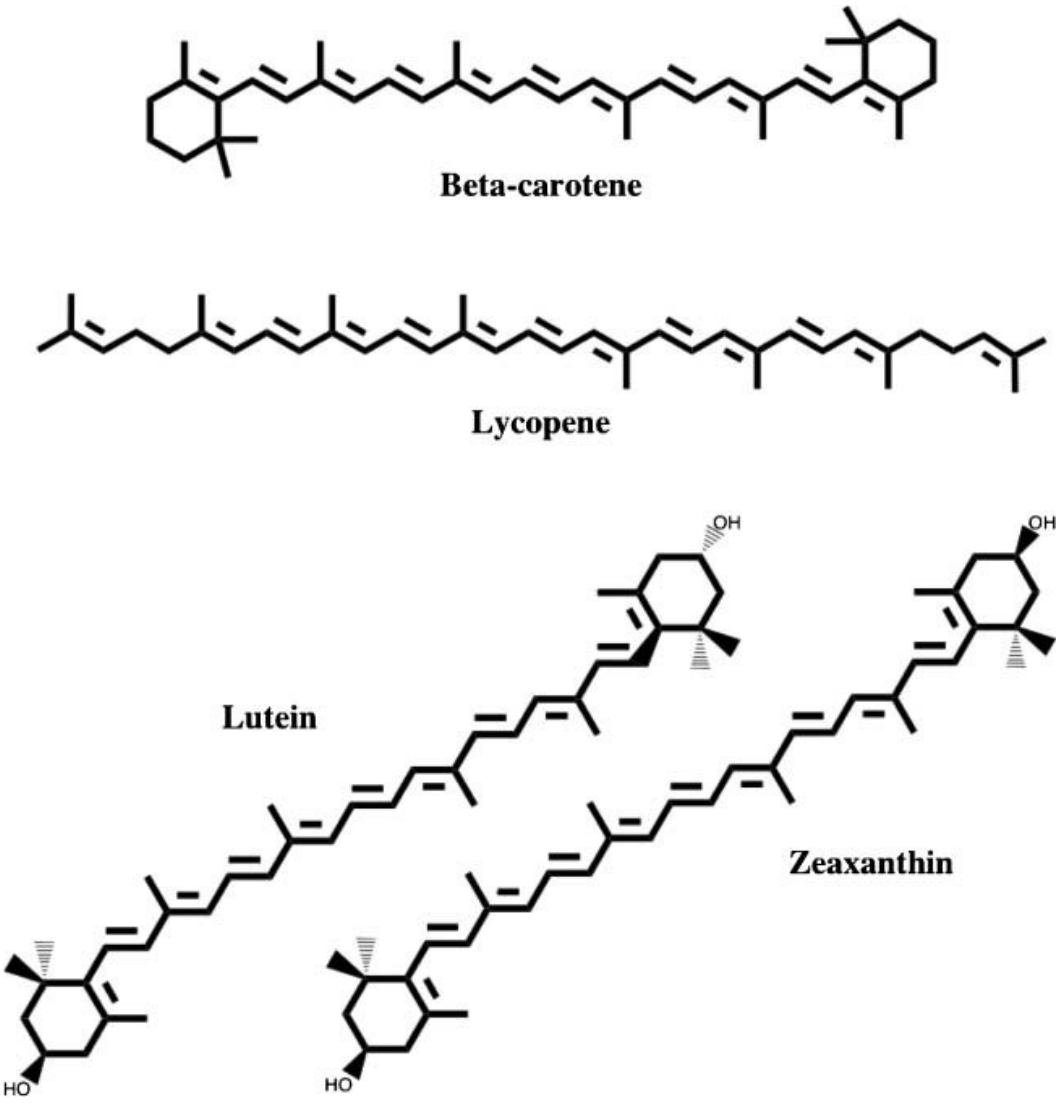
Biofortification involves improving the quality of micronutrients of food crops using conventional plant breeding methods either solely or with biotechnology (Tumuhimbise *et al.*, 2013; Uchendu, 2013, Penelope *et al.*, 2014). It is a process that focuses on increasing the levels of nutrient in crops as plant grows instead of nutrient supplementation in processing (WHO, 2014) thus having better prospects than supplementation by reaching the malnourished population more feasibly than conventional fortified or supplemented foods.

Biofortification that targets staple food crops is a new approach in public health to mitigate the deficiencies ofiron, vitamin A, and zinc in developing countries (Penelope *et al.*, 2014) through the development such as iron-biofortification of legumes, beans, rice, sweet potato and cassava; zinc-biofortification of rice, maize, wheat, beans and sweet potato; amino acid and protein-biofortification of sorghum and cassava, and provitamin A carotenoid-biofortification of maize, sweet potato, and cassava (Shahriari *et al.*, 2013, WHO, 2014).

2.2.1 Provitamin A carotenoids

Carotenoids are lipid-soluble pigments which may be red, yellow, or orange colours found in plants. They could be produced by photosynthetic bacteria (such as algae) through biosynthesis and also by yeasts and some non-photosynthetic bacteria (Carvalho *et al.*, 2012). Carotenoids contribute to the very bright colours of flowers, vegetables (e.g. carrots, watermelons, etc), and fruits. Examples include α-carotene,β-carotene, β-cryptoxanthin,zeaxanthin and lutein. Carotenoids serves to protect plants against potentially harmful photo-oxidative processes (Namitha and Negi, 2010). Due to their provitamin A activity, carotenoids have high antioxidant properties, which serve to prevent the onset of certain cancers as well as other degenerative diseases (Gul

et al., 2015). Animals rely on dietary sources of carotenoids because of their inability to



synthesise these carotenoids (Namitha and Negi, 2010). Provitamin A carotenoids initiate the synthesis of retinol (vitamin A) from diets. They are absorbed through the diet and metabolised into vitamin A. Vitamin A that has been pre-formed can only be found in foods derived from animals while dietary carotenoids are located in foods from plant sources such as oils, fruits and vegetables, and now by biofortification, in roots and tuber crops.

Carotenoids are commercially useful as colourants in dietary supplements; in agriculture, pharmaceutical, and cosmetic industry (Namitha and Negi, 2010).

2.2.2 Beta-carotene

Beta carotene is a very important carotenoid, and it is the most widely studied among the carotenoids (Liang *et al.*, 2013). It is abundant in fruits and other plants with a deep red or orange-colored pigment. β-carotene is the main source through which humans derive vitamin A because it is the predominant carotenoid found in human diet (Grune *et al.*, 2010). It is a precursor of vitamin A, capable of yielding two molecules of retinol when oxygen is present(Gul *et al.*, 2015). The main sources of β-carotene are fruits and vegetables (such as carrots, apricots, grapefruits, bell pepper or sweet pepper, fluted pumpkin, broccoli etc).

2.2.2.1 Advantages of β-carotene

β-carotene is a phytochemical, thus it exerts several benefits such as antioxidant properties with the potential of preventing the body against cellular damage, degenerative diseases like cancers and cardiovascular diseases (Kim *et al.*, 2014, Gul *et al.*, 2015). It is a potent antioxidant scavenging lipid peroxides and it is quenches singlet oxygen due to its unique structure (Kim *et al.*, 2014). β-carotene is capable of quenching a thousand free radicals per molecule (Kim *et al.*, 2014). Furthermore, β-carotene has the most efficient vitamin A conversion hence the best vitamin A activity compared to other carotenenoids (cryptoxanthins and α-carotenes) (Grune *et al.*, 2010). It is also an attractive natural colorant and has been regarded as safe because high amounts of β-carotene intakes from diets can be well tolerated by humans without possible harm. Long-term exposure to β-carotene is thus beneficial while deficiency of β-carotene can result in blindness,xerophthalmia, and premature death(Kim *et al.*, 2014).

2.2.2.2 Chemical properties of beta carotene

β-carotene exists as red, violet, or brownish-red crystals or in powdery form. It is heat-labile thus readily degraded by heat, oxygen or ultraviolet light. β-carotene consists of all trans (Z) isomer which is the natural form, with changing amounts of the cis-isomer based ondifferences in formulations. The relative abundance of the β-carotene isomers occur with all-trans being the highest, followed by 9-cis isomer, then 13-cis and 15-cis isomers respectively (Namitha and Negi, 2010). All trans-β-carotene easily isomerises into the cis-isomers due to the highly instable nature of the former. Immediately trans-β-carotene is exposed tolight, oxygen, or high temperature, it undergoes isomerisation, photosensitisation, thermal and chemical oxidation (Liang *et al.*, 2013).

2.2.2.3 Bioavailability of β-carotene

Bioavailability means the proportion of a consumed nutrient whichisaccessible by the body either for storage or to perform physiological functions (Donhowe *et al.*, 2014).β-carotene is metabolised to retinol that is the required form for normal cell division and turnover in adult stem cells(Grune *et al.*, 2010). One of the important factors that influences β-carotenes bioavailability is the type of food matrix in which they are localised since they could either exist as crystals or may be localised in complexes of fat or protein which have incompletedigestion in the GI tract (Sy *et al.*, 2013, Donhowe *et al.*, 2014). Other factors determining the assimilation of β-carotene in the humanintestine include the physicochemical properties of the β-carotene (i.e. trans or cis form), amount consumed in the meal, fat content in the meal, transport, host-related factors such as genetic factors, along with the nutrient condition of the host (Stinco *et al.*, 2012, Sy *et al.*, 2013).

Nestel *et al.* (2006) gave the bioconversion ratio of β-carotene to retinol as 12:1 (in mixed foods) or 6:1 (in oils), and the estimated average requirements (EARs) of retinol activity equivalents (RAE)/d of vitamin A are 210 µg RAE/day for children 1-3 years, up to 275 µg RAE/day for 4-8 years, and an average of 500 µg RAE/day for older ages. However, a re-evaluation of bioconversion efficiency of pro-vitamin A to vitamin A established recently that 21 mg of β-carotene correspond to the vitamin A activity of 1 mg of retinol instead of 12 mg (Namitha and Negi, 2010). This shows that the intake of vitamin A is lower than the required amount to prevent vitamin A deficiency. Therefore, to mitigate the deficiency of vitamin A, its supplementation

alone is insufficient, thus necessitating approaches that are based on food fortification, and the production of varieties of crops with improved activity of provitamin A.

2.2.2.4 Factors affecting the stability of β-carotene

Carotenoid such as β-carotene are highly unsaturated and labile compounds (Liang *et al.*, 2013), thus unstable (Boon *et al.*, 2010). Loss of β-carotene could occur as a result of oxidation or isomerisation on exposure to light, oxygen, and enzymes (Liang *et al.*, 2013; Sy *et al.*, 2013). This makes the retention of β-carotene difficult during food processing and storage since destruction that occurs mostly during food processing and storage could be due to enzymatic and non-enzymatic oxidation (Chatterjee and Janarthan, 2012). Factors determining β-carotene stability include processing time and temperature, oxygen content and water activity. When oxygen is present, it could interact with metals (Sy *et al.*, 2013), enzymes, and unsaturated lipids producing free radicals leading to oxidation (Boon *et al.*, 2010). The bioavailability and biological activity of β-carotene is severely affected during thermal processing due to the trans-cis isomerisation of β-carotene which occurs as a result of heat, light, and acid (Chatterjee and Janarthan, 2012, Liang *et al.*, 2013). Other factors that could hasten the loss of β-carotene include unfavorable relative humidity and temperature during storage. Controlling factors such as exposure to light, processing time and temperature, severity of processing, packaging material, and storage conditions could enhance the retention of carotenoids in general (Chatterjee and Janarthan, 2012).

2.3 Provitamin A cassava

Provitamin A cassava were biofortified with total carotenoid content ranging from 100 to 10,000 μg per 100 g fresh cassava, as a sustainable means to alleviate vitamin A deficiency in Nigeria through the joint effort of IITA and NRCRI, Nigeria(Talsma *et al.*, 2013). Biofortified cassava are yellow-fleshed cassava rootsdifferent from the conventional cassava which is white-fleshed or cream root colour. This change in colour is due to the presence of provitamin A carotenoids, especially beta carotene (Talsma *et al.*, 2013, Talsma *et al.*, 2016). Since cassava is an essential food in Africa, yellow cassava varieties have been reported to have great potential in alleviation of Vitamin A deficiency (VAD). This deficiency is a public health problem, causing preventable blindness during childhood and contributes mostly to cases of illnesses from infections that could result in death, especially in pregnant women and children.

It affects those in low and middle income countries due to poor economic situation(WHO, 2009). VADresults from low vitamin A intake from diets, mal-absorption or high vitamin A excretion due to common illnesses.

Vitamin A is a fat-soluble vitamin that exists in many different forms such as retinol, retinal, retinoic acid or retinyl ester. It is an important micronutrient which helps in the maintenance of clear vision, immune system, and proper growth and development.

2.3.1 Consumers' acceptance of biofortified cassava

The colour changes brought about by the process of biofortification of cassava roots does not influence the appearance of the products only, but as well as the taste (Adenle *et al.*, 2012). These changes could also influence the acceptance of biofortified crop by both consumers and farmers (Saltzman *et al.*, 2013, Ayetigbo *et al.*, 2018). There is, however, sparseinformation about the consumer acceptance of biofortified cassava varieties (Talsma *et al.*, 2013) and there are diverse opinions as regards the little information that is available. In 2011, Inter Press Service (IPS) news agency wrote under the column of Busari Bafana that the consumer acceptance of pro-vitamin A cassava in Nigeria was low (Bafana, 2011). Adenle *et al.* (2012) explained that media reports on genetically modified cassava can be negative, unsubstantiated and lacking balanced views. This is because the various information they disseminate comes from sources that may not have been properly investigated. Consumer acceptance depends on the amount of information available to the public about the product, the nutritional and sensory characteristics of the crop as well as the beliefs and cultural practices in the community (Nestel *et al.*, 2006).

2.4 Starch hydrolysis

Starch is the most abundant form in which carbohydrates (polysaccharides) are stored in higher plants (Uthumporna *et al.*, 2010, Xu *et al.*, 2013). It is mainly found in the seed, root, tubers, and fruits of plants (Xu *et al.*, 2013).

Starch is primarily composed of a mixture of two polysaccharides- amylose and amylopectin. Amylose is linear (15 -20% of starch) while amylopectin is highly branched (75- 85% of starch) (Aderibigbe and Adejumo, 2015).

Starches are categorised into three based on the rate and extent of susceptibility to hydrolytic enzymes, namely:

- Rapidly digestible starch (RDS)
- Slowly digestible (SDS), and
- Resistant starch

Rapidly digestible starch is the fraction of starch that is digested and absorbed rapidly in the small intestine, thus elevating the blood glucose rapidly with a subsequent lowering of blood sugar below the baseline (hypoglycemia). It is the starch that is rapidly converted to glucose molecules within 20 min of enzyme digestion. Starchy foods cooked using moist heat usually contains large amounts of rapidly digestible starch. Rapidly digestible starch has been shown to have a positive correlation to glycemic index (Zhu *et al.*, 2011).

Slowly digestible is the fraction of ingested starch that is slowly but completely digested in the small intestine with a low initial rise in blood sugar level, resulting in a sustained and prolonged glucose release (Simsek and El, 2015). This makes energy available for a prolonged time. It can be measured as the starch that is broken down to glucose after 100 min of enzyme digestion. SDS is beneficial in providing stable glucose metabolism that is important in diabetes management.

RS is the starch fraction that is resistant to enzyme hydrolysis. It is not digested in the small intestine but enters the large intestine along with dietary fiber and fermented by the gut microflora to yield short chain fatty acids that provide more energy for the body. This category of starches resist digestion by digestive enzymes either because such starches are physically inaccessible in the native form, (e.g. whole grains) or by retrogradation or modification of the starch. These could be further categorised into four types (Deepa *et al.*, 2010).

Hydrolysis is the first stage of the metabolism of starch. It is basically the breaking down of starch to glucose.Enzymatic and acid hydrolysis are two methods mostly used for converting starch to glucose (Ayoola *et al.*, 2013).

Conventionally, starch is hydrolyzed by acid catalysis. Acid hydrolysis is generally done by 6N hydrochloric acid. Acid hydrolysis is disadvantageous because it requires the use of materials that are corrosion-resistant, increases ash content and colour (after neutralisation), requires more heat energy and it is relatively more difficult to control (Betiku *et al.*, 2013). However, the method has now largely been replaced by enzymatic processes (van der Maarel *et al.*, 2002).

In enzymatic reaction, the diffusion of the enzyme to solid surface is essential. The enzyme needs to pass across the boundary between the solid and aqueous phases before attaching to the starch granule. This diffusion takes place before the adsorption of the enzyme. The adsorption step is also essential before the subsequent catalytic step, which is the final event (Uthumporna *et al.*, 2010).

Hydrolysis occurs with each attacked layer of starch granule being completely hydrolysed before the next layer. This happens in a layer by layer pattern (Uthumporna *et al.*, 2010).

Amylose and amylopectin have two different types of linkages within the structures as follows: α-1, 4 and α-1, 6 linkages. Amylose is asingle and unbranched polymer, which contains up to 2000 glucose units that are linked with only α-1,4 glycosidic bonds(Kanlaya and Jirasak, 2004). Amylopectin is a highly branched polymer having α-1, 4 short chains with α-1, 6 glycosidic linkages.

In enzymatic hydrolysis, amylases attacksα (1→4) and α (1→6) linkages of amylose and amylopectin in gelatinised starch releasing maltodextrin after saccharification (Mazumder and Bera, 2013). Amylases possess active centers with sub-sites, which can bind glucan chains through several units of glucose in the substrates to their sub-sites (Dhital *et al.*, 2015).

Enzymatic hydrolysis involves the following three steps: gelatinisation, liquefaction and saccharification. The gelatinisation of the starch, is the first step in which high temperatures (90-110°C) is used to initiate the α-amylase for the breakdown of starch granules (Marwati *et al.*, 2018). During this step, the structure of starch granules swell and undergo disruption with subsequent melting of their crystalline structure (Li *et al.*, 2016) leading to an increase in viscosity.

Liquefaction step allows the conversion of starch into soluble, short-chain dextrins, then high glucose syrup. During this step, the α-amylase enzyme cleaves the α-1,4 glycosidic bonds to yield shorter chains of soluble dextrins, thus increasing the rate of starch hydrolysis (Marwati *et al.*, 2018). The interaction of starch granules with the enzyme leads to a decrease in viscosity which creates a better condition for the saccharification process (Li *et al.*, 2016). Saccharification is the third step during which an exo-acting gluco-amylase hydrolyses α-1,4 glycosidic bonds from the non-reducing ends of starch and α-1,6-glycosidic linkages consecutively, yielding glucose as the end-product (van der Maarel *et al.*, 2002). For saccharification process to be

efficient, pH of the liquefiedstarch is adjusted to 4.5, because pH and temperature conditions for the stability of gluco-amylase enzyme is 4.2 and 60 °C respectively. The liquefiedsyrup is converted to a high glucose syrup, having above 95% glucose, which could serve as a feedstock during biological processes of fermentation (Pereira and Gibson, 2002, Betiku *et al.*, 2013).

Only dextrose or glucose formation is the result of a complete hydrolysis process. The sweetness of a hydrolysate syrup is dependent upon the degree of hydrolysis, and it is measured as dextrose equivalent (DE) (Li *et al.*, 2016). The dextrose equivalent (DE) refers to the content of reducing sugar (dextrose or glucose) in a syrup and it is expressed in percent glucose on a dry basis (Soto *et al.*, 2012). Maltodextrins are defined by food and drug administration (FDA) as a mixture of non-sweet nutritive carbohydrate having varying degree of polymerisation, consisting of d-glucose units joined by glycosidic bonds- α-(1,4) and α-(1,6), which together have a dextrose equivalent, (DE) < 20, and are presented as white powders or concentrated solutions and they are classified as ingredients generally recognised as safe (GRAS) (De Keersmaecker *et al.*, 2006)..

2.4.1 Amylolytic enzymes

Enzymes which are capable of hydrolysing α-glycosidic linkages of starch are referred to as amylolytic enzymes. These enzymes represent a group of starch hydrolases which act on the α-glycosidic bonds in starch and similar polysaccharides and oligosaccharides (Falade and Akingbala, 2010). Amylolytic enzymes, α-amylase, β-amylase and glucoamylase, are very important in biotechnology (Khosrokhavar and Mortazavian, 2010). Enzymes used industrially for starch hydrolysis have been broadly categorised into α-1,4 and α- 1,6 amylases. Further classifications give rise to two groups under each broad category. Endo- α-1, 4 amylases (e.g. α-amylases) and exo- α -1, 4 amylases (e.g. glucoamylase, isopullulanase, β-amylase, maltohydrolase) are enzymes that cleave the α-1, 4-glycosidic bonds of polysaccharides. They cannot cleave the α-1, 6 linkages. Endo- α 1, 6-amylases (e.g isoamylase and pullulanase) and exo- α 1, 6-amylases (e.g exopullulanase) are enzymes that act on the α-1, 6 glycosidic bonds and are referred to as debranching enzymes.

The endo-amylases (e.g. α-amylases) act on the internal linkages of their substrates. There are two types of α-amylases: thermo-stable and thermo-labile α-amylases. The thermo-stable α-amylases is obtained from *Bacillus licheniformis.* They can act on

starch solutions and they are stable at temperatures 90^{o}C and above. This makes it possible to liquefy starch solutions at temperatures above 100^{o}C. Their stability is not really dependent on calcium ions while the thermo-labile α-amylases is from fungal sources. It is not as stable as the thermo-stable α-amylase, it requires calcium ions for stabilisation and it is active only in the temperature range of 65-70^{o}C. It is more difficult for amylases to act on raw starch granules than on gelatinised starch (Nigam and Singh, 1995, Guzmán-Maldonadoa and Paredes-Lópezb, 1995)

The exoamylases (e.g. glucoamylase) act on the external linkages of their substrates. They can act on α 1, 4- and also α 1, 6-glycosidic linkages, although at a slower rate than α-1, 4 linkages. Glucoamylase is obtained from fungal sources- *Aspergillus niger and Rhizopusspp*. It is used on liquefied starch to produce high-glucose and high fructose syrups. Glucoamylase acts in synergy with α-amylase during starch hydrolysis. Enzymatic starch hydrolysis yields low degree of conversion to fermentable or simpler sugars e.g. Glucose, fructose, or maltose (Chen *et al.*, 2011).

2.5 Fermentation

In fermentation, primary food components such as simple sugars and other carbohydrates are biochemically modified into secondary compounds such as alcohols and lactic acids, through the action of microorganisms and their enzymes (Karovicova and Kohajdova, 2005; Kohajdová *et al.*, 2006). It is a desirable process utilised by mosthumans as a wayof improving the organoleptic attributes and nutritional value of food as well as preservation (Colehour *et al.*, 2014).

The modification of sugars and other polysaccharides to end products that are useful is the most important benefit of fermentation (Egwim *et al.*, 2013). The fermentation process also produces foods that are more easily digested (by pre-digesting the food), with dense composition of bioavailable nutrients, reduced toxic or anti-nutritional components, generally enhancing the nutritional quality, textural and organoleptic characteristics. There is also an added advantage of preservation and shelf-life extension of foods by secondary metabolites e.g. lactic acid, alcohol and acetic acid.

Fermentation brings about chemical changes in food composition. There may be a decomposition of certain food components, while other components may undergo a significant increase and there may also be a re-combination of some to form new bio-

nutrients (Manea *et al.*, 2009). Fermented foods are considered very safe. The safety is based on the principle that during fermentation, desirable, edible microorganisms overgrow on food substrates making it become resistant to invasion by pathogenic or spoilage microorganisms, which are toxic. This makes it difficult for disease producing organisms to compete or grow (Steinkraus, 2002).

2.5.1 Lactic Acid Fermentation

Lactic acid (2-hydroxypropanoic acid) is a natural organic acid with the formular, CH_3-CH(OH)-COOH. It has been used industrially for food and non-food uses such as cosmetics, pharmaceutical, and agro-allied industries (Sobrun *et al.*, 2012). It is a three-carbon carboxylic acid having carboxyl group, methyl or hydrocarbon group and an alcohol group. It can be obtained from chemical synthesis or the process of carbohydrate fermentation (Narayanan *et al.*, 2004). D (-) and L (+) lactic acid are the two isomeric forms in which lactic acid occurs. L (+) lactic acid is the only isomer that the human body can metabolise.

Lactic acid fermentationinvolves the conversion of fermentable sugars to lactic acid by lactic acid organisms e.g.*Lactobacillus spp., Streptococcus spp., Leuconostoc mesenteroides, Bifidobacterium* and so on (Steinkraus, 2002).

The two major types of lactic acid fermentation pathways are homofermentative and heterofermentative pathways (Lahtinen *et al.*, 2011). The homofermentative pathway involves those bacteria that primarily ferment glucose to pyruvate, eventually yielding lactic acid while heterofermentative organisms yield lactic acid asides other metabolites such as ethanol and CO_2 (Lahtinen *et al.*, 2011). Homolactic or homofermentative pathway is dependent on glycolysis (Embden–Meyerhof–Parnas pathway) while heterolactic or heterofermentative fermentation is also known as hexose monophosphate shunt,pentose phosphoketolase pathway, or 6-phosphogluconate pathway (Lahtinen *et al.*, 2011; Wang *et al.*, 2014). Fermentation of other hexoses apart from glucose (e.g. galactose, mannose and fructose) could enter the homo- or heterofermentative pathways, while pentoses can only be fermented through the heterofermentative pathway, but without the production of CO_2.Homofermentation yields 2 moles of ATP for every mole of glucose consumed while heterofermentation yields only 1 mole of ATP (Lahtinen *et al.*, 2011). The requirement for this pathway are limited oxygen and excess sugar (Martinez *et al.*, 2013). Homofermentative

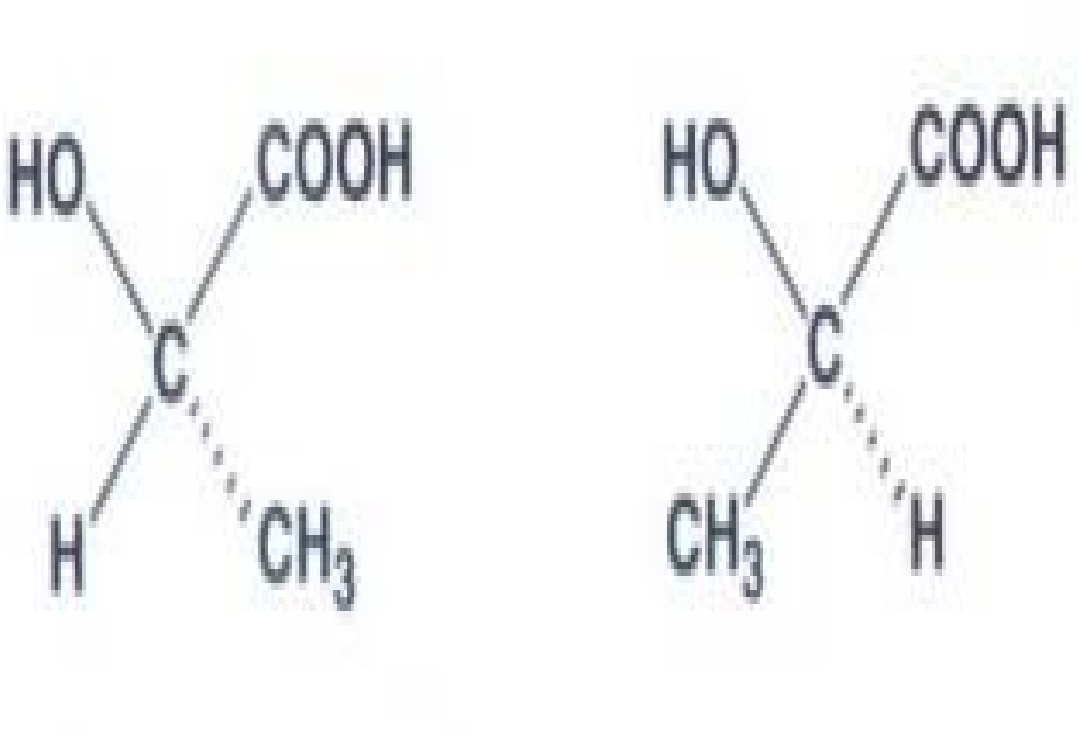
metabolism of carbohydrates in food yields lactate as sole or major product of metabolism (Ganzle, 2015).

2.5.2 Production of beverages by lactic fermentation

Vegetables such as tomato, celery, cucumbers, carrots, radishes and tropical fruits such as apples, berries, papaya, lemon and oranges, etc have been used across the world for the production of juices, either consumed fresh or lactic fermented as a means of preservation.Fermentation can be achieved either by the addition of starter cultures in a controlled fermentation process where selected strains of lactic acid bacteria are applied as specific starter cultures to an already heat-preserved (pasteurised) substrate; or by spontaneous fermentation by natural microflora; or a combination of both (Yoon *et al.*, 2004, Yoon *et al.*, 2006). An example of acid fermentation of vegetables is sauerkraut produced from cabbage. The process in sauerkraut fermentation involves salt addition which provide a conducive environment for lactic acid bacteria to grow, giving the vegetable an acid flavour. Thus, influencing the final product quality.

Cereals and roots and tubers have also been used in the development of alcoholic and non-alcoholic beverages (Marshall and Mejia, 2012). Maize, millet, sorghum, guinea corn, etc. can be used to produce gruels such as ogi (pap), which is non-alcoholic while sorghum based fermented foods includes *pito,burukutu, bogobe, injera, kisra,* etc which are mostly alcoholic(Mbajiuka *et al.*, 2010).Panda and Ray (2007) fermented β-carotene fortified sweet potatoes for 48 hours and produced a non-alcoholic beverage which was referred to as lacto juice. Non boiled and fully boiled sweet potatoes were grated into juice. Pectinase enzyme was employed to facilitate juice extraction and the resultant juice was fermented with *Lactobacillus plantarum*. Carrot and red beet were processed by lactic acid fermentation into a probiotic juice using *Lactobacillus acidophilus* and *Bifidobacterium* sp. (Buruleanu *et al.*, 2009).Development of non-alcoholic beverage from sweet potatoes was also carried out by Wireko-Manu *et al.* (2010) by hydrolyzing sweet potatoes with the use of α- and β- amylases.Non-alcoholic beverage from sweet potatoes was equally produced by Sohail *et al.* (2013) using a method very similar to the one used by Wireko-manu *et al*. (2010). Both methods carried out starch hydrolysis using α-amylase enzyme.Jeng *et al.* (2012) produced both alcoholic and non-alcoholic beverages using freeze dried sweet potatoes powder which was reconstituted to 25% solids.

The mixture was then hydrolysed and filtered without being fermented to obtain the non-alcoholic beverage. The alcoholic beverage was obtained by fermenting the filtrate with yeasts for 48hours. It was centrifuged and the supernatant was distilled. In the case of cassava, the most common beverage developed from cassava is alcoholicbeverage. Simultaneous saccharification and fermentation procedure was employed in the producing ethanol from cassava after starch hydrolysis (Yuwa-Amornpitak, 2010).



L (+) lactic acid D (-) lactic acid.

Figure 2.2: optical isomers of lactic acid

Beer production from cassava has also been achieved (Rajagopal, 2006). However, there is limited information as to the previous researches conducted on the possibility of production of non-alcoholic beverage from cassava.

2.5.3 Factors affecting production of lactic acid during fermentation

2.5.3.1 Fermentation process conditions

In order to achieve optimum lactic acid production, bacteria cell growth, and desired lactic acid yield, it is imperative to ensure the process conditions such as temperature, pH and inoculum size are within the optimum range. Temperature influences the microbial activity and rate of substrate conversion (Kim *et al.*, 2012). LAB are mesophilic bacteria, which can grow and produce lactic acid at an optimum temperature of approximately 20-45^{o}C. Temperature can have significant influence on biomolecules, by favoring a higher yield of one metabolite or the other (Souza *et al.*, 2017). pH 5-7 is the ideal initial pH for the growth of LAB, as the final pH requirement for LAB is pH < 5 (Lahtinen *et al.*, 2011). Uncontrolled initial pH reduction may induce early substrate utilisation. As fermentation progresses, the concentration of lactic acid gradually increases with decreasing substrates concentration, causing acidification of fermentation broth. This slows down the process of fermentation,leading to the prevention of cell growth and lactic acid production (Martinez *et al.*, 2013).

2.5.3.2 Carbon source

The presence of sugars as a source of carbon is essential during lactic acid fermentation. Although, glucose is the most preferred source of carbon for most strains of LAB, it is not the sole carbon source (Wang *et al.*, 2015). Carbon sources are essential to generate energy necessary for proliferation. Mixed sugars such as hexoses

and pentoses can be used as carbon source in lactic acid fermentation although in the presence of glucose, the utilisation of other mixed sugars are repressed. This means that in co-fermentation with other sugars, most lactic acid bacteria preferentially utilise glucose as a source of carbon to produce lactic acid. This phenomenon is referred to as carbon catabolite repression (Abdel-Rahman *et al.*, 2015).

2.5.3.3 Nutrient sources

LAB have limited ability to synthesise the nutritional requirements such asminerals, vitamins and amino acidsfor cell growth, maintenance and lactic acid production; and thus depend on nutrient sources present in the growth medium (Lahtinen *et al.*, 2011). Therefore, an improved lactic acid production can be achieved by the addition of sources of nitrogen such as peptone, yeast extract, and meat extract. Minerals and vitamins may selectively enhance the metabolism of LABin lactic acid fermentation (Ganzle, 2015).

2.6 General overview of lactic acid bacteria (LAB)

LAB are non-spore forming, gram-positive, cocci or rods, which yield lactic acid as the primary end product during carbohydrate fermentation (Rattanachaikunsopon and Phumkhachorn, 2010, Souza *et al.*, 2017). They comprisethe genera *Lactobacillus, Leuconostoc, Streptococcus, Pediococcus,* and *Lactococcus.*Lactic acid bacteria are found on mucosal surfaces of humans, animals and plants, in association with nutrient rich environments especially carbohydrate-rich environments. (Colehour *et al.*, 2014). LAB varies widely in ecology but have a major similarity in their production of lactic acid as the major metabolite when they metabolise sugar (Fugelsang and Edwards, 2007). The natural habitat of LAB are widespread, including plants and gastrointestinal microflora.

LAB have significant importance in the food industry for their ability to utilise fermentable sugars for normal metabolism, yielding lactic acid, ethanol and other metabolites depending on the fermentation pathway.

Generally, it can be said that different LAB can ferment different carbohydrate substrates depending on the type of sugar. Sucrose can be fermented by *Lactobacillus delbreuckii* subspecies *delbreuckii.* Lactose can be fermented by *Lactobacillus delbreuckii* subspecies *bulgaricus. Lactobacillus helveticus* can utilise lactose and galactose while *Lactobacillus lactis* can utilise glucose, galactose and sucrose.

Lactobacillus rhamnosus GG can metabolise glucose and fructose but not lactose or sucrose (Zhu *et al.*, 2013).

2.6.1 Lactic acid bacteria (LAB) as starter culture

Starter culture refers to preparation comprising of a large population of one organism singly or in combination with other organisms, inoculated into a raw material to accelerate and control its process of fermentation (Florou-Paneri *et al.*, 2013). Lactic acid bacteria have been used as starter cultures in foods and beverages fermentation for age-long, owing to their safety and ability to enhance the organoleptic, nutritional, technological and shelf-life characteristicsof foods (Florou-Paneri *et al.*, 2013). They are considered to be harmless or even to improve human and animal health (probiotics) and this has earned them the generally recognised as safe (GRAS status) (Tanasupawat, 2009).

Lactobacillus spp. is the main genus of LAB that is used as starter culture. They are facultative anaerobic, gram-positive, catalase-negative, may be long, short, plump or slender non-spore-forming rods (Goldstein *et al.*, 2015). They are important in balancing microflora in the gut ecosystem of human and animals (Brashears *et al.*, 2005). Organisms that must be used as starters in lactic acid fermentation must be able to yield lactic acid in a medium and their consumption even at a very high dose must be safe regardless of the immunity status of the consumers (Molin, 2010). They should be able to contribute significantly to the improvement of processing conditions of pH and processing times (Ali and Mustafa, 2009).

2.7 Probiotics: consumption of live lactic acid bacteria

Probiotics are live microorganisms that induce health benefits when ingested in adequate quantities(Guzmán-Maldonadoa and Paredes-Lópezb, 1995). They are consumed either in fermented foods or as food supplement. They exert favourable effects on human health by colonising the gastro-intestinal (GI) tract (Willowski *et al.*, 2001). Over time, a regular part of human food intake includes the consumption of live cells of LABespecially in fermented foods(Molin, 2014). The ingestion of probiotics can positively influence the balance of microflora in the gastrointestinal tract.A lot of the probiotics used in recent times are members of the *Bifidobacterium and Lactobacillus* genera. Most of the species in these genera are regarded as safe and such

species include *Lactobacillus rhamnosus* (Dokic *et al.*, 2004). These organisms have longstanding use owing to their safety (Nigam and Singh, 1995). It is required that the viability of the probiotic bacteria should be maintained at a sufficient level throughout storage of probiotic product so as to be able to obtain a desirable therapeutic effect in the products(Cruz *et al.*, 2012).

The most known probiotic carriers are food matrix of dairy origin e.g. yogurts and fermented milks. However, recent advances in research have featured non-dairy food matrix that may be supplemented with probiotic microorganisms include foods e.g. soy, meat, cheeses, fruits, cereal and fruit juices, chocolate and various juices e.g. tomato, cabbage, grape, orange, carrot, water melon, beet juice, etc(Rivera-Espinoza and Gallardo-Navarro, 2010,Nahaisi et al., 2014).Incorporation of probiotic strains into food products with significant acceptability and retention of their viability and functionality have been successfully carried out(Nahaisi *et al.*, 2014). Examples of probiotics include strains of *Lactobacilli, Lactococcus and Bifidobacterium*. Minimum recommended amounts at which probiotics should be present in food products is 10^6 CFU/g, representing a daily dose of 10^8 CFU/g, to offset any possible reduction in cell number during passage through the gastrointestinal tract (Cruz *et al.*, 2012). Research have shown that probiotics can significantly prevent and treat gastrointestinal disease (Ritchie and Romanuk, 2012).

2.7.1 Health benefits of probiotics

Some health benefits derived from the ingestion of probiotic organisms include: prevention of disease, reduction or lowering the levels of blood cholesterol, alleviation of symptoms associated with lactose intolerance, alleviation of diarrhea associated with antibiotics, inhibition of the proliferation of pathogens and improvement in nutrient. Probiotics exert several health benefits on their host and they achieve this by various means which includes their ability to lower the pH of the intestine, their ability to dominate the gut microflora, thereby preventing pathogens from invading, and eventually modifying the host immune response (Williams, 2010).

2.7.2 Characterisation of an organism as a probiotic

Probiotics are initially screened to satisfy the suitable genotyping and phenotyping identification. They are then tested to satisy the other important attributes that further characterises them as probiotics. These attributes include their tolerance to high acid and bile salt concentrations, adhesion to the epithelial cells of the intestine, their

patterns of sugar utilisation, ability to produce substances with antimicrobial activity, ability to prevent pathogens in the gut, antibiotic resistance patterns, etc (Mishra and Sharma, 2014, Goldstein *et al.*, 2015, Reid, 2016). The ability of a probiotic organism to resist bile acids is the property which guarantees that an organism survives through the intestinal tract alive (Anandharaj *et al.*, 2018). The ability of a probiotic microorganism to adhere to epithelial cells by protein surface layers gives the organism its ability to colonise the human gastrointestinal tract, and exhibit a competitive advantage over pathogenic organisms (Rivera-Espinoza and Gallardo-Navarro, 2010). The functionality of probiotics can only exert maximum beneficial therapeutic effects if the probiotics are viable and maintained at a dose of at least 10^8–10^9cfu per ml or per gram of product, be viable through the human gastrointestinal transit and reach the colon (Anandharaj *et al.*, 2018).Probiotics can modulate the immune system by modifying the microflora of the intestine (Klemashevich *et al.*, 2014). This is achieved by their ability to colonise the intestine,provide antimutagens and antioxidants, and displace enteric pathogens (Sanders, 2011). Many species of *Lactobacillus*produce bacteriocins or other anti-bacterial compounds which hinder the growth of other organismscomprising pathogens and spoilage organisms in the intestine(Soetan *et al.*, 2010).

Possession of these antimicrobial properties by LAB is attributable to production of compounds such as hydrogen peroxide, organic acids and bacteriocins which are inhibitory(De Keersmaecker *et al.*, 2006). Production and accumulation of lactic acid by LAB in a medium lowers the pH and exerts a bacteriocidal or bacteriostatic effect (Makras *et al.*, 2006, Sarika *et al.*, 2010). The human stomach is characterised by a very low pH due to the acidity, which may further enhance the antimicrobial activity of LAB (Soleimani *et al.*, 2010).

The health benefits expressed by probiotics are strain-specific i.e. not all probiotic organisms can impart the desirable properties. Hence, continuous screening is carried out (Reid, 2016). Factors that could influence the survival of probiotics through the gastrointestinal tract include the physiological state of the probiotic organism, the concentration of the probiotic organism in the food product during storage, the storage conditions of pH and temperature in the carrier product, and the concentration of the probiotic organism at consumption time(Min *et al.*, 2018).The mechanisms of action of probiotics are fully dependent on the viability. However, recent advances in probiotics

research show that there are emerging new terms such as post-biotics and para-biotics (Klemashevich *et al.*, 2014, Aguilar-Toalá *et al.*, 2018). These new terms suggest that the functionality of probiotic strains in attaining health-promoting effects is not limited to bacterial viability.

Parabiotics, refer to non-viable probiotics such as inactivated probiotic cells that confer benefits to consumers when taken in sufficient quantities(Taverniti and Guglielmetti, 2011). Post-biotics refer to the metabolites or cell-free supernatants secreted by either live or lysed bacteria cells, which provides additional bioactivity to consumers thereby imparting physiological benefits (Aguilar-Toalá *et al.*, 2018). Post-biotics have been reported to be naturally abundant in some fermented foods like *kefir*, yogurt, *kombucha*, pickled vegetables, etc (Chaluvadi *et al.*, 2016). Li *et al.*(2009) stated that inactivated *L. rhamnosus GG* and their metabolic products were capable ofexerting similar advantageous effects as the live *L. rhamnosus GG* in vivo.

2.7.3 Gut microflora

The indigenous microflora characterising the gastrointestinal tract of mammals, especially humans is referred to as gut microflora (Maity *et al.*, 2012). It is composed of a variety of microorganisms with complex diversity. The human and mouse microbiome is dominated by *Bacteroidetes* and *Firmicutes*. *Firmicutes* are related to energy harvest and storage, whereas*Bacteroidetes* have the capacity for energy consumption. Variation in the *Firmicutes/Bacteroidetes* ratio could therefore affect the energy balance of the host (Chen *et al.*, 2019). The microbial activity of gut microflora is beneficial to the host in the stimulation of intestinal maturation, breaking down of undigested food, synthesis of vitamins (especially vitamin K and biotin), drug metabolism, improved immunity of the host, and resistance of colonisation by pathogens (Maity *et al.*, 2012). The growth of gut microbes can be differentially promoted by the availability of different carbohydrate types and protein, which provides fermentative substrates (Roberfroid *et al.*, 2010). Therefore, diet composition could alter the gut microflora (Karl *et al.*, 2013). Other factors such as disease conditions, stress, drugs or antibiotics, diet or environmental contaminant or host induced interactions- could also be responsible for alterations in gut microflora. Alterations or disruption in gut microflora- a condition referred to as dysbiosis- could negatively affect the health of the host by producing symptoms such as bloating,

flatulence, abdominal pain, food intolerance, incontinence, loss of appetite, cramps, blood in stools, etc (Adak *et al.*, 2013).

Probiotics such as *Bifidobacterium*and *Lactobacillus* can restore altered gut microflora to a healthy gut microbiota, thereby exerting a protective effect on the gut, owing to the ability of *Lactobacillus* to initiate the expression of anti-inflammatory genes, which improves gut motility and overall function, and modulating the response of the immune system(Tintore *et al.*, 2017). A healthy gut microbiota refers to a microbial community in which healthful microorganisms predominate, contrary to dysbiosis which is described by a predominance of harmful microorganisms(Roberfroid *et al.*, 2010; Karl *et al.*, 2013).

2.8 *Lactobacillus rhamnosus GG (LGG)*

2.8.1 Taxonomy of *Lactobacillus rhamnosus GG*

Lactobacillus rhamnosus GG is a probiotic strain belonging to the kingdom *Bacteria,* subkingdom *Posibacteria,* phylum *Firmicutes,* class *Bacilli*, order *Lactobacillales,* family *Lactobacillaceae,* Genus *Lactobacillus* and Species *Lactobacillus rhamnosus,* strain *GG*. The 'GG' is derived from the names of Sherwood Gorbach and Barry Goldinwho originally isolated *Lactobacillus rhamnosus* strain *GG (L. rhamnosus GG* or*LGG)* from faecal samples of a healthy human adult in 1985 (Gorbach *et al.*, 2017).

It is most commonly found in the human faeces and vagina flora (Makras *et al.*, 2006). They also belong to the normal mucosal flora of the mouth (Zhang *et al.*, 2011). One of the most studied probiotic strains is *Lactobacillus rhamnosus GG,* originally isolated from human intestinal flora (Valik *et al.*, 2008).

2.8.2 Characteristics of *Lactobacillus rhamnosus GG*

L. rhamnosus GG is acid and bile stable, and it attaches to the human intestine while temporarily and effectively colonising it (Goldhaber, 2003).

2.8.3 Clinical importance of *Lactobacillus rhamnosus GG*

In humans, *LGG* has been shown to modulate immune responses (Ross *et al.*, 2011). It is also effective in the treatment of several forms of diarrhea including rotaviral and

acute non-rotaviral diarrhea in children, 'traveler's diarrhea' in children (Nguyen *et al.*, 2007), *Clostridium difficile* and diarrhea caused by the use of antibiotics. Diarrhea is often a primary symptom of gastrointestinal tract disorder which may cause serious problems ranging from mild to severe in children and adults (Helland *et al.*, 2004). According to the concise oxford dictionary, diarrhoea is defined as a condition in which faeces are discharged from the bowels frequently and in a liquid form. It can be a very debilitating disease and it has been reported as a major cause of over 5 million deaths per year (Helland *et al.*, 2004). *LGG* may be beneficial in the treatment of ulcerative colitis, pouchitis, andinflammatory bowel disease in humans. Nguyen *et al.* (2007)conducted a study by isolating *Lactobacillus rhamnosus GG* from the faeces of healthy individuals and administering a daily dose of 2 x 10^7CFU/mL of the *Lactobacillus GG*either in capsules containing the powder or as a placebo with similar capsules containing ethyl cellulose powder. The study reported that the probiotic *Lactobacillus rhamnosus* GG decreased the incidence of traveler's diarrhea. These results suggest that probiotics, particularly *LGG*, might be useful in treating gastrointestinal disease.*LGG* might be also be useful in the prevention of atopic sensitisationand other allergic diseases among children. The possibility of *Lactobacillus rhamnosus* HN001 to impart protection against eczema between ages 0-2 years was investigated (Shetty and Jespersen, 2006). Supplementation of *Lactobacillus rhamnosus HN001* at a dose of 6 x 10^9 CFU/day to a pregnant woman from gestation age of 35 weeks until 6 months of breastfeeding,with further supplementation to the infant until age 2, significantly reduced the cumulative occurrence of eczema and rhino-conjunctivitis in the children by age 4. It was therefore concluded that *Lactobacillus rhamnosus* exhibited a protective effect against rhino-conjunctivitis and eczema, up till 2 years after birth. *L. rhamnosus GG* might be useful in the resistance of antibiotics-associated gastro-intestinal imbalance (Agamennone *et al.*, 2018). There has been great concerns in the use of antibiotics as inappropriate use of antibiotics may cause an imbalance in the gastrointestinal flora and prolong the duration of the diarrhea with other associated problems such as antibiotics resistance (Oboh and Elusiyan, 2007). *L. rhamnosus GG* produces a broad inhibitory spectrum bacteriocin that is clinically useful in replacing antibiotics(Sarika *et al.*, 2010).

Although *L. rhamnosus GG* is regarded as non-pathogenic, various cases of associated morbidity have been reported in adult and children who use probiotics, and have pre-existing underlying diseases (Robin *et al.*, 2010). *L. rhamnosus* has been the most frequently identified strain in blood cultures (Ambesh *et al.*, 2017). This is a condition referred to as *Lactobacillus* bacteremia. Cases of *Lactobacillus* bacteremia have been reported in individuals with pre-existing diabetes mellitus, structural heart disease, leukemia and other types of cancer, chronic kidney disease,HIV infection, inflammatory bowel disease, pancreatitis, individuals undergoing chemotherapy, liver transplantation, broad spectrum antibiotic use, etc (Gouriet *et al.*, 2012, Sherid *et al.*, 2016).

Lactobacillus bacteremiais rarely associated with pathology in individuals that are immuno-competent, thus immunosuppression has been reported as a major risk factor. Meini *et al.* (2015) suggested that the use probiotics is safe in patients that are healthy but caution is necessary with the administration in patients that have pre-existing risk factors. Franko *et al.* (2017) advised that *Lactobacillus* bacteremiashould not be regarded as a contaminant, it should however guide doctorsin diagnosing pre-existing diseases and infections.

2.9 Immobilisation of probiotics

The term immobilisation refers to a physical entrapment of microbial cells within a polymer matrix. It is used interchangeably with encapsulation and it means to enclose a material in a matrix as if in a capsule. It therefore refers to a process in which probiotic living cells are protected against severe conditions of the environment such as temperature,pH, organic solvents, poison and even water molecules (Borgogna *et al.*, 2010). When cells are immobilised in a matrix, a micro-environment is created which protects the cells and keep them viable during processing and storage up to the appropriate time of release in the intestine (Fijałkowski *et al.*, 2016).

Immobilisation of cells offer several advantages which includes decreased susceptibility of the microorganism to contamination, improved fermentation rate, improved microorganism handling characteristics and reusability of the cells (Zhao *et al.*, 2016).There are different methods of achieving encapsulation/immobilisation such as methods that apply the emulsion techniques, extrusion and spray drying methods

(Burgain *et al.*, 2011).The encapsulation material is often called the carrier, coating membrane, shell, wall material, matrix or external phase while the encapsulated substance could be referred to as active agent, core material, filler agent or internal phase (Heidebach *et al.*, 2012).

Table 2.2: Summary of various effects of probiotics on human health

Improved immunity
Reduction of symptoms of food allergy in infants
Alleviation of inflammatory bowel diseases
Lowering of serum cholesterol
Improvement of symptoms of lactose tolerance
Reduction in the risk factors for colon cancer
Inhibition of endogenous pathogens
Repression of exogenous pathogens, such astraveler's diarrhoea

Source: (Rokka and Rantamäki, 2010)

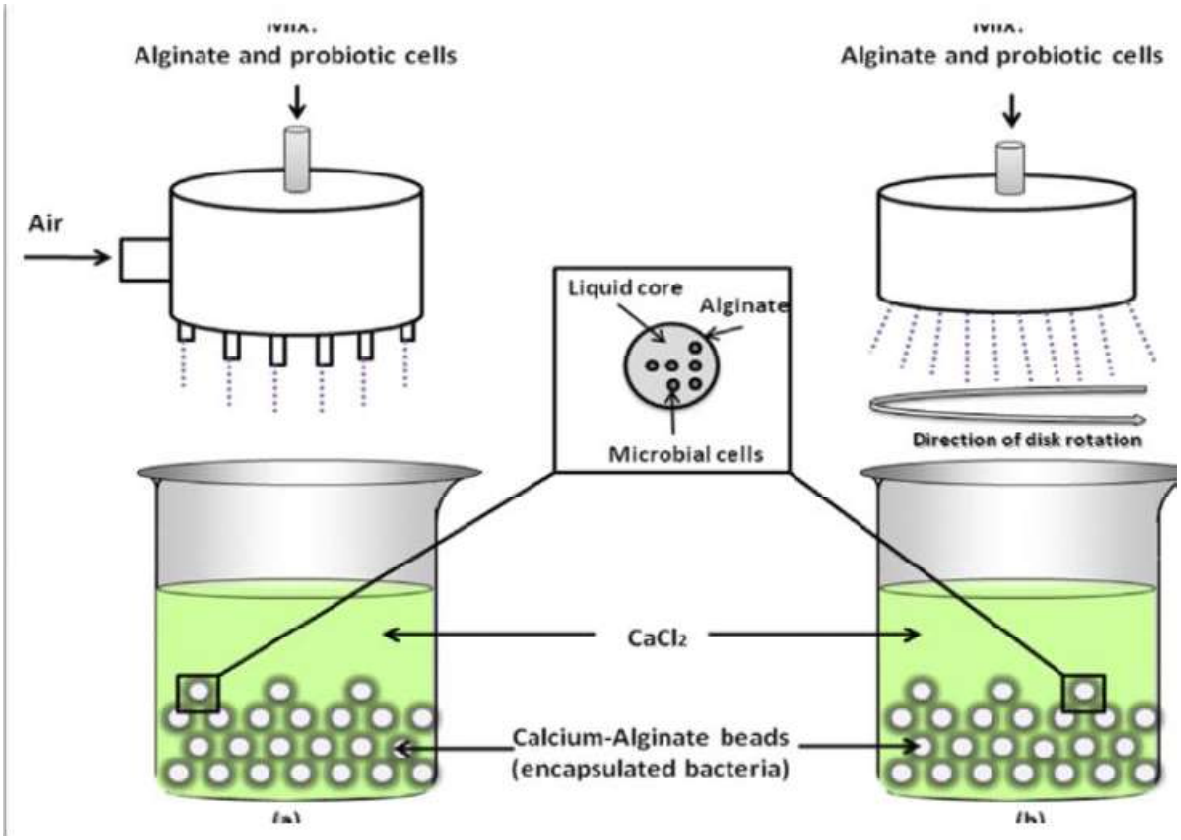


Figure 2.3: The process of probiotic encapsulation by extrusion technique (Burgain *et al.*, 2011)

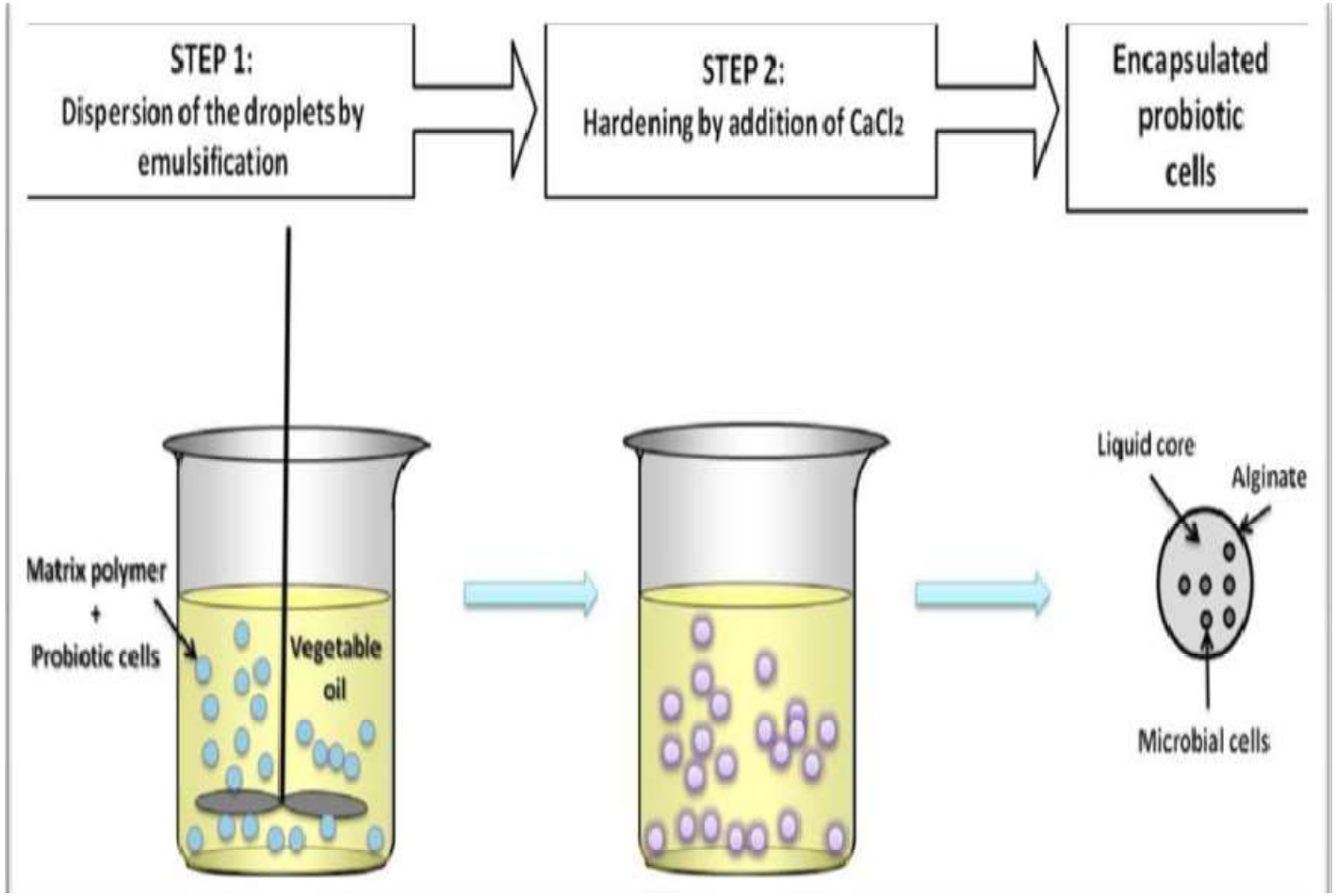


Figure 2.4: The process of probiotic encapsulation by emulsion technique (Burgain *et al.*, 2011)

2.9.1 Alginate-encapsulation of probiotics

Alginate refers to alginic acid, its salts and its derivatives. It is an anionic, water soluble polysaccharide which can be obtained from natural sources such as brown seaweed, or synthesised by some bacteria (Nestel *et al.*, 2006, Lee and Mooney, 2012). Alginate has a linear copolymer structure which is made up of two different blocks at alternating positions: mannuronic acid (M block) and guluronic acid (G block)units (Nestel *et al.*, 2006) in various proportions(Anal and Singh, 2007)depending on the natural source of the alginate. Interaction with divalent cations like calcium instantly causes an interfacial polymerisation with alginate resulting in the formation of calcium alginate precipitates, with a subsequent but gradual gelation of the interior (Rokka and Rantamäki, 2010).

Several literatures have reported alginate as the most commonly used biopolymer for microencapsulation (Krasaekoopt *et al.*, 2003, Mokarram *et al.*, 2009, Li*et al.*, 2009,Chávarri *et al.*, 2010) owing to its numerous advantages.

Advantages of sodium alginate over the other encapsulating agents include: its ability to react with calcium chloride to form a matrix which traps sensitive materials such as live microorganisms, its non-toxic, its simple and cheap(Soto *et al.*, 2012). The choice of alginate for food application systems is based on the fact that it is non-toxic.

The limitations of alginate gel include the fact that alginate beads are not resistant to acid and based on research, it has been established that the beads may shrink and during lactic fermentation, it reduces in its mechanical strength (Mazumder and Bera, 2013). It is susceptible to disintegration in harsh chemical environments such as in the presence of calcium ion chelating agents and excess monovalent ions (Ayoola *et al.*, 2013). However, this disadvantage could also be an advantage in that its susceptibility to disintegration offers a form of reversibility of encapsulation making it possible for alginate gel to be solubilised when entrapped cells are needed to be released in the human intestine.

2.9.2 Effect of encapsulation on fermentation

During fermentation, fermentation times and the rates of lactic acid production could be delayed in encapsulated/immobilised cells as compared to free cells. This could be due to several reasons including mass transfer resistances which could be dependent on the choice and thickness of encapsulating materials (Zhou *et al.*, 1998).

2.10 Recent trends of probiotic applications in beverages

2.10.1 Beverages used as probiotic carriers and their limitations

Dairy products such as milk and its derivative products e.g. yoghurt, fresh milk, fermented milk, cheese, and whey-based products have been conventionally used in the delivery of probiotics (Song *et al.*, 2012). This is because milk proteins create a suitable environment that provide excellent protection for probiotic strains (Ayichew *et al.*, 2017). *L. rhamnosus GG* is a probiotic strain of wide importance in the dairy industry, although several other strains have been used. However, the major limitation of dairy-based probiotic products is lactose intolerance and increasing consumers who tend towards vegetarianism. Lactose intolerance is a condition which arises as a result of lactase deficiency in a consumer (Malik and Panuganti, 2019). Lactose, present in milk is broken down by the enzyme lactase into glucose and galactose upon consumption. Failure to break down lactose into absorbable units results in the passing of undigested components directly into the colon. This leads to symptoms such as abdominal bloating, diarrhea, flatulence, abdominal pain, etc. The statistics of global prevalence of lactose intolerance is 65% (Bayless *et al.*, 2017). This has led to recent interest in development of non-dairy probiotic foods.

Non-dairy probiotic beverage have been developed from sources such as cereals, vegetables, fruit and soy. Fruits and vegetables are good probiotic carriers with the various nutrients that can be obtained from such sources. However, the major challenge encountered with fruits as carriers of probiotics is the low pH which creates a harsh condition of acidity that impact negatively on the viability of probiotic strains (Galgano *et al.*, 2015). Vegetables require low incubation temperatures which may not favour the viability of probiotics.

Recent trends in probiotic beverages have reported the application of several fruit and vegetables as carriers for probiotic. Examples include tomato juice and cabbage juice which were reported as carriers for *L. plantarum*, *L. delbrueckii*, and *L. casei* (Yoon *et al*., 2004, Yoon *et al*., 2006). Orange and pineapple juices have been reported to support the proliferation of probiotics such as *L. casei*, *B. lactis*, and *L. paracasei.* Cashew apple juice was used as carrier for *L. casei.* Free and microencapsulated probiotic bacteria have been reported to show significant viability in apple and orange

juices(Ding and Shah 2008) and probiotic pomegranate was also developed by Mousavi *et al.* (2010).

2.10.2 Use of encapsulating agents in non-dairy probiotic beverages

Several researches exist in literatures on the incorporation of immobilised probiotic organisms into foods, and beverages in particular. This was done to enhance the viability of probioticsin processing and during storage. Sohail *et al.* (2012)evaluated the effect of *Lactobacillus rhamnosus GG* and *Lactobacillus acidophilus NCFM* encapsulated in alginate in orange juice, and reported excellent survival of *L. rhamnosus GG. Lactobacillus acidophilus was* immobilised in calcium alginate for fermentation of banana puree (Tsen *et al.*, 2004). Nualkaekul *et al.* (2013) investigated the survival of *Bifidobacterium longum* and *Lactobacillus plantarum* encapsulated using alginate, gelatin or chitosan inoculated intocranberry and pomegranate juice, and reported that there was considerable improvement in the survival of the organisms encapsulated using alginate or chitosan. Improved viability of *Lactobacillus plantarum* encapsulated in sodium alginate inorange, grapes, and watermelon juices have been reported (Ding and Shah, 2008, Gaanappriya *et al.*, 2013).

2.11 Phytochemicals

Plant foods contain many non-nutrient plant chemicals (Liu, 2004) which are bioactive compounds produced through secondary metabolism in relatively smaller quantities apart from the conventional nutrients, such as minerals and vitamins (Rodriguez *et al.*, 2006). Examples of these compounds include: phenolics, carotenoids, alkaloids, organo-sulphur and nitrogen containing compounds, etc. They protect plants against pests and disease; and they give plants its natural flavor and colour. They can be found inplant foods such as grains,vegetables,fruits, etc. that have been linked to having antioxidant properties (Chede, 2013). Most phytochemicals possess antioxidant activity which protect the body against oxidative damage thereby reducing the risk of developing cancer and other diseases. Examples of phytochemicals that have antioxidant activity are: carotenoids, polyphenols, lycopene, flavonoids, isoflavones, etc.

2.11.1 Classes of phytochemicals

Various classes of phytochemicals exist. They can be grouped into alkaloids, flavonoids,glycosides, phenolic, saponins, terpenes,tannins, anthraquinones, steroids, essential oils (Doughari, 2012). They may operate by different mechanisms- they may modulate reactions that prevent oxidative damage, interfere with or delay some metabolic processes. Phytochemicals may either function as agents with chemo-preventive chemotherapeutic ability.

Chemoprevention is defined as the use of natural dietary compounds from common plants to prevent, reverse or block tumour multiplication at different stages of cancer formation(Ugbogu *et al.*, 2013). Phytochemicals are antioxidative which means they can also have anti-inflammatory, antimicrobial and anti-carcinogenic functions.

2.11.1.1 Alkaloids

Alkaloids refers to various group of nitrogen-containing compounds with low molecular weight, which arecommonly derived from amino acids (Aberoumand, 2012). It is recognised that alkaloids have strong antimicrobial, antibacterial and antifungal biological properties. They are comprised of secondary chemical constituents having ammonia compounds. Alkaloids are alkaline in reaction, and are used widely as pharmaceuticals, narcotics, stimulants and even poisons owing to their strongbioactive components(Doughari, 2012). Alkaloids are largely found in the seeds.

2.11.1.2 Carotenoids

Among the phytochemicals, carotenoids - which are tetraterpenoids that influence the color of many plants products, egg yolk, salmon and crustaceans such as crabs, shrimps, lobsters, etc having orange, yellow and red - have been the most studied (Rodriguez *et al.*, 2006). Carotenoids are the most essential and this is because they are converted to vitamin A (Akinwale *et al.*, 2010). Trans- β-carotene functions in the prevention of cancers and cardiovascular diseases. They decrease the risk of cataracts and muscular disorders. They also improve the immune system. They achieve all these functions due to their antioxidant properties.Provitamin A carotenoids are important components of the diets consumed by humans, and thus impart anti-cancer activity which many carotenoids have been established to possess(Bartley and Scolnik, 1995). In recent years, there has been substantial evidence that provitamin A carotenoids impart other health benefits which includes: enhancement of immunity, lowering the chances of diseases such as cardiovascular diseases (CVD), cancer, macular and

cataract degeneration (Rodriguez *et al.*, 2006) owing to their free radical scavenging ability.

2.11.1.3 Phenolics

Phenolic acids are derivatives of hydroxycinnamic acid e.g.*p*-coumaric, caffeic, and ferulic acids which are commonly found in food as simple esters withglucose or quinic acid (Doughari, 2012). Phenolic compounds consist of: simple phenols e.g. catechol, resorcinol, etc., proanthocyanidins (epicatechin), tannins, coumarins, biflavonoids (ormocarpine, etc.),phenolic acids, stilbene (resveratrol, etc.), flavonoids (quercetin, cyanidin, etc.), and anthraquinones (Bellik *et al.*, 2013).

2.11.1.4 Saponins

Saponins are glycosidic, secondary metabolites found in higher plants and some animal sources, e.g. Marine invertebrates (Podolak *et al.*, 2010). They have a range of biological properties, both beneficial and deleterious(Zohra *et al.*, 2012). Their name is derived from their behavior in aqueous solution where they can form foams that are stable and soap-like (Francis *et al.*, 2002). Saponins are used in the pharmaceutics for functions such as expectorant, lowering of cholesterol, anti-inflammatory, analgesic, vasoprotective, immunomodulatory, antispasmodic, hypoglycemic, antimicrobial, etc (Mbaebie *et al.*, 2012). Mbaebie *et al.,* (2012) explained that saponins and alkaloids have a long history of pharmacological effects for their analgesic and antispasmodic effects and thus explains why traditional healers of South Africa used *schotia latifolia jacq* for the management of chest pain and arthritis among other diseases.

2.12 Colour intensity in beverages

Food colours greatly affect the way consumers perceive food quality. Colour is a visual parameter evaluated by the consumers (Pathare *et al.*, 2013). It can determine the degree of acceptability of a food product even before it enters the mouth. A UV-VIS spectrophotometer can measure reflectance and absorbance on solid and as well asconventional liquid samples (Pedjie, 2012). Colour measurements can be made using either double monochromatic or singlemonochromatic spectrophotometer.

Colorimeters such as the Minolta Chroma meter and Hunter Lab colorimeters are some of the instruments most used in the measurement of color. The colour scale used for colour measurement is based on the CIE Lab. L* is for lightness or luminance component, while a*, which stands for quadrant red to green, and b*, which represents blue to yellow quadrant, are the chromatic components. For colour measurements, the

commissioninternationale d'eclairage (CIE) adopted the L*, a*, b*, or CIElab colour space as an international standard in 1976 (Leo´n *et al.*, 2006).

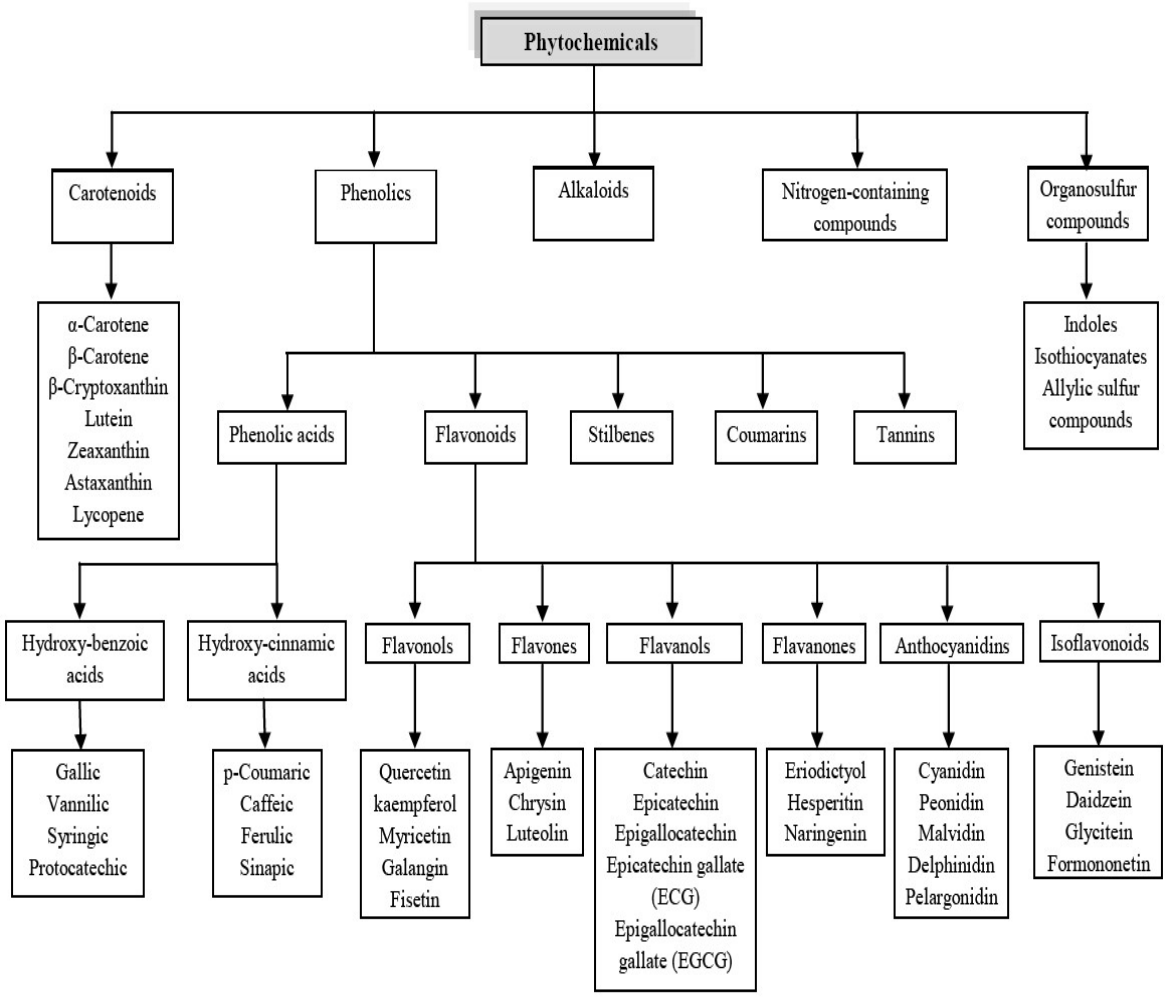


Figure 2.5: Classification of dietary phytochemicals

Source: (Bellik *et al.*, 2013)

2.13 Toxicological studies

2.13.1 Food toxicology

Toxicology refers to the study of how substances- either physical or chemical, affects living systems. The term 'toxin' refers to the toxic substances that plants animals, microorganisms or any biological systems produce. Toxicants are therefore the toxic substances that are generated by different activities carried out by humans (Eaton and Gilbert, 2008).

Food is a complex combination of nutrient (micronutrient and macronutrient) and non-nutritive substances either in the raw or processed form. Consumption of food may result in the accumulation of certain chemically toxic compounds which could exert toxic activities against the consumers (Maldonado-Pereira *et al.*, 2018). The nutritive substances present in food may be beneficial but may not necessarily be safe, considering the dose at which it is consumed. If a micronutrient (e.g. vitamin A) is consumed in amounts excess of its required recommended daily allowance (RDA) to prevent deficiencies, it may be toxic (Williams, 2012). Example of the non-nutrient substances are anti-nutrients (e.g. cyanide in cassava), hormones or any substance that the plants need for survival and growth. These anti-nutrient substances are vital to the plant but may be toxic for human consumption (Kotsonis and Burdock, 2008).

Sources of hazards found in foods may be classified into: Environmental contaminants, Microbial contaminants, natural toxic constituents in the food, and hazards resulting from food additives or novel foods ingredients (Williams, 2012). Environmental contaminant include pesticides and heavy metals. Natural toxins include anti-nutrients (such as cyanogenic glycosides), enzyme inhibitors (e.g. trypsin inhibitors and protease inhibitors), Antivitamins (such as the biotin-binding protein, avidin in raw egg white (Yuasa *et al.*, 2016)), mineral-binding (e.g. phytate, goitrogens, oxalate, etc). Microbial contaminants are the most dangerous contaminants in food (Williams, 2012) as they are characterised by infestations of pathogenic bacteria or moulds (e.g. *Clostridium botulinum, Staphylococcus aureus, Salmonella species and Clostridium perfringens*), which can produce toxins in the food. Fungi has the potential to produce a wide range of biologically active compounds that could either be advantageous as

effective antibiotics for humans, or could produce mycotoxins which constitute food hazards resulting in several diseases (Dellafiora *et al.*, 2018). Mycotoxins are highly carcinogenic and toxic compounds which are heat-stable and survive most forms of food processing (Williams, 2012). Contamination from mycotoxins occurs whenever the environmental conditions favours mould growth (Chiocchetti *et al.*, 2018). Examples of mycotoxins include *Aflatoxin* and *Fumonisins*.

Toxicity testing of a food or ingredient can reveal the potential adverse effects and the level of consumption (i.e. the dose) at which those adverse effects may occur (Williams, 2012). This informs why the toxicity evaluation of provitamin A cassava hydrolysate from cassava becomes more important, to ensure its safety as it will be consumed widely as a ready-to-drink beverage by different age-groups of humans.

The degree of toxicity of any particular toxic material on any subject can be determined by: the route of exposure and the dosage. The route is the pathway through which the toxic material enters the body e.g. by inhalation, by ingestion, through the skin which by extension, also involves intravenous routes. Intravenous routes produces the most rapid response since it goes directly into the bloodstream (Eaton and Gilbert, 2008).

The dosage refers to the duration, and frequency of exposure measured in mg/100g (for smaller animals) or mg/kg for bigger animals.

In toxicological testing procedures, the duration of exposure of experimental animals to toxic materials can be categorised into four: acute, sub-acute, sub-chronic, and chronic exposure.

Acute exposure refers to a single exposure to the chemical or toxic agent for periods less than 24 hours while repeated exposures refer to repeated administrations within a period of more than 24 hours and this covers sub-acute, sub chronic and chronic exposures.

2.13.2 Animal toxicity tests

Generally, toxicity test can be categorised into acute toxicity, sub-acute toxicity, sub-chronic or chronic exposure testing. The acute toxicity testing is determined by a single exposure (administration), usually done to determine the lethal dose (LD_{50}). LD_{50} which refers to "lethal dose" is the amount of a chemical material given all at once that is required to cause the death of half (50%) of a test group of animals (Chinedu *et al.*, 2013). The LD_{50} is used to determinethe potential of a material to

impart toxicity within a short term (Saganuwan, 2017). The lower the LD_{50}, the more toxic the test material.

Animal toxicity testing is based on the principle that the observed effects of administration of a test material on laboratory animals can be applied to humans by recalculation of body weight. However, there is a more pronounced vulnerability in humans than experimental animals.

Healthy animals not older than 8 weeks are used and the most often used animal species are rodents (mouse and rat) (Parasuraman, 2011). Animals are starved the night before dosing. A record of the number of animals that die during the observation period after a single dosage is kept. Apart from mortality and weight, any signs of toxicity, lethargy, morbidityor changes in behaviour, appetite, etc. are observed. An important tool in toxicity testing includes the evaluation of changes in the hematological profiles (such as haemoglobin concentration, erythrocyte indices and differential leukocyte count), and urinalysis. The haematological profiles include the red blood cells, packed cell volume (hematocrit), Haemoglobin,mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Assessment of the serum biochemical indices in animals is also essential in evaluating the pathological condition and functionality of the organs (Lawal *et al.*, 2016). Red blood cell (RBC) along with its membrane plays important roles in several physiological and metabolic processes (Pandey and Rizvi, 2010). RBC are the primary means of transporting oxygenfrom the lungs to the tissues of the body through the blood (Marrocco *et al.*, 2017).

Clinical biochemistry measures the levels of chemicals, enzymes, and organic waste products found in the blood (Chauhan *et al.*, 2016). Clinical biochemistry assessment includes the measurement of electrolyte balance, carbohydrate metabolism, liver and kidney functions test, etc. Aspartate Transaminase (AST), Alanine transaminoferase (ALT), Alanine phosphatase (ALP) are enzymes that are usually localised within the cells of the heart, liver and kidney, muscles and other organs, and they can be used to assess damage to the liver and heart (Ajani *et al.*, 2015).

A functional balance exists between the pro-oxidants and antioxidant system in the human body. This gives cells the ability to protect themselves from damaging effects of the oxidants. Disrupting this balance results in oxidative stress which could be triggered intra- or extra-cellular as a result of genetics, age or diet (Melekh *et al.*, 2017).

2.13.3 Oxidative stress

Oxidative stress is a condition which results from physiological shift between the levels of oxidants and antioxidants, favouring the oxidants (which are free radicals or reactive species). Unpaired electrons, atoms,ions or molecules are referred to as free radicals(Wang *et al.*, 2011).

2.13.3.1 Reactive oxygen species (ROS)

Oxygen atoms are highly prone to the formation of free radical. Oxygen radicals such as singlet oxygen, ($^1O_2^-$) andsuperoxide anion ($^{\cdot}O_2$) are generated when molecular oxygen is reduced by addition of electrons. Oxygen radicals, if surplus, are involved in several cellular processes. They are harmful to living cells and able to produce other free radicals with deleterious potentials (Ighodaro and Akinloye, 2018).

Reactive oxygen species (ROS) results in lipid peroxidation. Examples include hydrogen peroxide (H_2O_2), superoxide anions ($O^{\cdot}_2$), hydroxyl radicals ($^{\cdot}OH$), and Nitric oxide (NO_2). When there is a low antioxidant concentration, there is an increase in peroxidative processes associated with the involvement of hydroxyl radicals and super-oxides.

The mechanisms of toxicity effects of food are based on several factors which all lead to oxidative stress and chronic inflammation leading to degenerative processes such as aging, cell death, and other degenerative diseases such as Alzheimer's disease, cardiovascular diseases, etc (Gosslau, 2016). The intracellular oxidants concentration is determined by the synthesis and/or removal by the antioxidant system (Weydert and Cullen, 2010).

2.13.3.2 Oxidative stress markers

Malondialdehyde (MDA) is a usual biomarker in investigating the extent of oxidative damage on lipids as it is a major lipid peroxidation product. Lipid peroxidation is a chain reaction initiated and accelerated by reactive oxygen species. MDA can take part in several physiological reactions resulting in structural modifications, which can induce immune system dysfunction (Ighodaro and Akinloye, 2018). Such reactions include the reactions of MDA with phospholipids, free amino group of proteins, and nucleic acids. Results of such peroxidation reactions may cause cytotoxicity, carcinogenicity and certain allergies (Wang *et al.*, 2011).

2.13.4 Antioxidant defense systems

2.13.4.1 Enzymatic and Non-enzymatic Antioxidants

The body is protected against the effect of ROS by antioxidant systems, which could either be endogenous i.e. synthesised in the body or exogenous i.e. from dietary sources(Wang *et al.*, 2011). These anti-oxidant defense systems are employed in the prevention of uncontrolled ROS generation. Endogenous antioxidants include non-enzymatic components (glutathione, vitamins A, C, E, carotenoids, and polyphenols), and enzymatic antioxidants. Enzymatic antioxidants include scavengers of ROS, with superoxide dismutase (SOD),catalase (CAT),glutathione peroxidase (GPX)andglutathione s-transferase (GST) being the best-known defense systems (Marrocco *et al.*, 2017). These delay or prevent oxidation reactions either by scavenging superoxides, by activating defensive or detoxifying antioxidative mechanisms on ROS (Ebuehi and Dibie, 2015).

Glutathione (GSH) acts as a reductant. Through this mechanism, the tissue is protected from free oxygen radicals that can cause lipid peroxidation thereby protecting the biological system from oxidative damage. Glutathione (GSH) is an important antioxidant which exists in either the oxidised or the reduced form and the decrease or increase in its concentration is a good indicator of oxidative stress (Wang *et al.*, 2011).

Superoxide dismutases (SOD) destroys free radical superoxide by converting it to peroxides which can then be destroyed by GPx reactions by catalyzing its removal, converting it to non-toxic products (Mansuroˇglua *et al.*, 2015).

Glutathione peroxidases (GPx) catalyses the removal of hydrogen peroxide and converts it to non-toxic compounds (Ighodaro and Akinloye, 2018).

2.14 Rheological properties

Rheology refers to the study of flow and deformation of a body, primarily in liquid state, in response to stress. Various types and nature of the deformation is determined by the physical conditions of the material (Goodwin and Hughes, 2000). Rheological properties measure the consistency and flow behaviour of fluids. Food structures arecomplex being composed of various heterogeneous materials. In some cases, they may be made up of mixtures of liquids and solids.Determination of rheological properties of a given material is done by measuring deformation of flowas a response

to the force applied over a given time.This relates the stress applied on a body to the resultant flow and deformation(Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

Rheograms, obtained by plotting shear stress (τ) against shear rate (ɤ), are employed in describing the flow pattern or rheological properties of fluids (Guibad *et al.*, 2005). Mathematical equations or models which consist of parameters describing the flow pattern can also be used as a convenient way of describing the data obtained from rheological studies. Sometimes, more than one model may be used to describe rheological properties(Rao, 2014).

The rheometer, or viscometer, is an important tool in measuring rheological properties of a food material and it measures stress or how a material resists flow in response to a given applied force (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

2.14.1 Kinematic Viscosity

Kinematic viscosity is a measure of the flow of a fluid in resistance to gravity (Sies *et al.*, 2017). It is usually measured using a capillary viscometer e.g. Ostwald viscometer. An Ostwald viscometer is a U-tube with two reservoir bulbs separated by a capillary. The time taken for a known volume of a particular liquid to flow through the bulbs under the force of gravity is measured and multiplied by the viscometer constant. Ostwald viscometer operates on the principle of Hagen-Poiseuille law (Kennedy *et al.*, 1995) which characterises the viscosity of a Newtonian fluid (such as water) that has a laminar flow through a long cylindrical pipe that has a uniform cross-section. It is important to know the viscosity of juice in food processing operations as variations in the viscosity of juices or other liquid products could affect the energy usage during the concentration, evaporation or pumping operations (Magerramov *et al.*, 2007).

2.15 Glycemic Index

Glycemic index (GI) is the incremental area under a 2-hr blood glucose response curve of a particular test food divided by the incremental area under a 2-hr blood glucose response curve of a standard glucose (Jenkins *et al.*, 1981, Francis *et al.*, 2010). It is the measure of how a 50g carbohydratequickly raises the blood glucose of an individual compared to how quickly a 50g standard glucose raises the blood glucose level of the same person. Physical form of food, processing, preparation or cooking methods, type of sugar, nature of starch in the food, anti-nutrient and micronutrient component, etc are factors that may influence glycemic index(O'Reilly *et al.*, 2010). Other factors that could determine the glycemic index of foods include the differential

susceptibility of the starch to amylolytic enzymes (RDS, SDS or RS), the composition of the starch- amylose/amylopecting ratio (Eleazu, 2016). High level of amylose in food products has been related to low blood glucose in comparison with high amylopectin food products (Adedayo *et al.*, 2018), dietary fiber, carbohydrate, protein and fat content of food, antinutrient content of starch, processing such as milling, grinding, mashing, gelatinisation, particle size, acidity (which determines rate of stomach emptying),

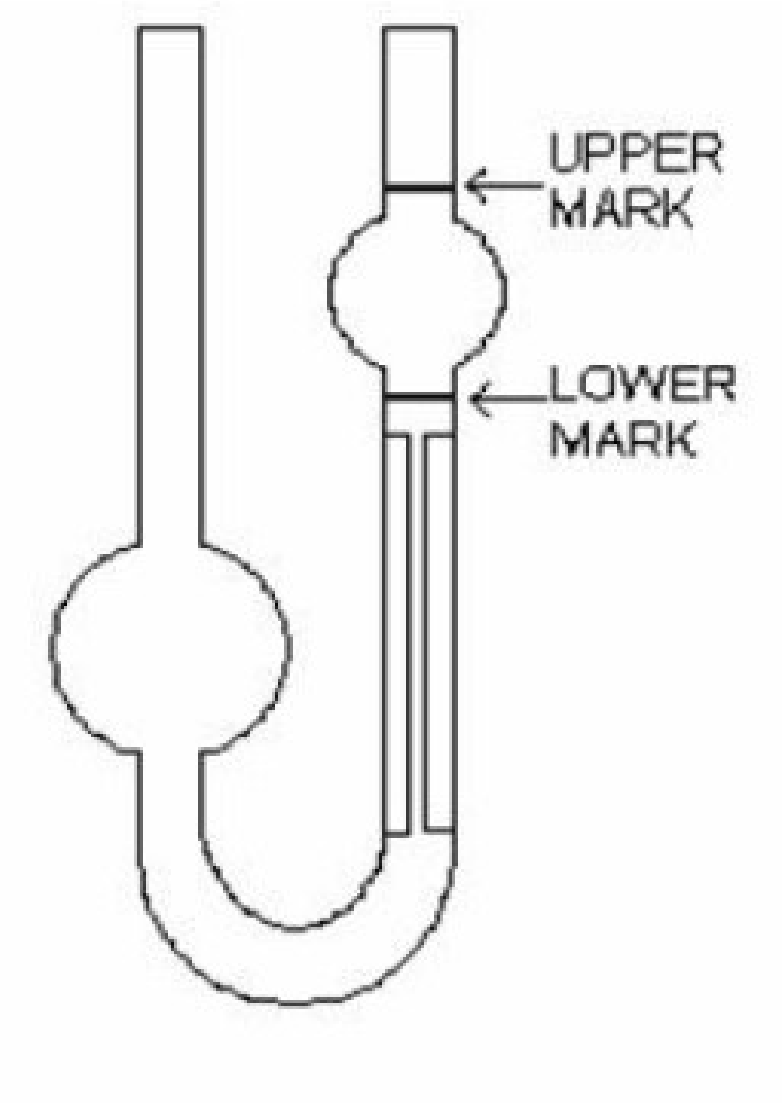


Figure 2.6: Ostwald viscometer

glycemic load, etc (Eleazu, 2016).The GI ranks foods on a scale of 0-100. The GI of glucose is 100. The higher the GI, the quicker the carbohydrate is digested and absorbed in the blood stream.The classification of foods by their glycemic index affords the opportunity to design meals that provide better postprandial glycemic control. Several factors that could influence glycemic control include the rate of gastric emptying, starch accessibility and dietary fiber (Scazzina *et al.*, 2009). Dietary advice based on GI classification is therefore of importance in the management of diabetes.

High GI diets are diets with carbohydrates that are rapidly digested, absorbed, utilised and having a GI≥70, while foods with carbohydrates that are slowly digested and absorbed, with GI ≤ 55 are referred to as low GI diets (Trinder, 1969, Bucolo and David, 1973, Francis *et al.*, 2010). High GI foods produce high glycemic rating owing to the rapid carbohydrate digestion rate in the small intestine, with high absorption of glucose to the blood stream (Kouamé *et al.*, 2015), leading to a large increase of blood glucose and insulin, while low GI foods are slowly digested, leading to a slight increase in blood glucose and insulin levels(Little *et al.*, 2009). High GI foods have been identified as risk factors in developing diabetes which is a condition with fasting blood glucose ≥200mg/dL. Although Jenkins *et al.* (1988) contested that the fasting blood glucose is more important in diabetes control than the postprandial blood glucose response since fasting blood glucose is usually not affected by the meal after, i.e. the subsequent meals. This means that if the fasting blood glucose of an individual has already been raised, it will not be ameliorated by consuming a low GI diet.

The negative effect of high GI diets is more emphasised, it is believed that high glycemic foods have some positive impacts as well. In individuals with energy-deficient situations or in clinical cases of subjects with hypoglycemia or low blood sugar, high GI foods or beverages can release a quick burst of energy into the blood

stream and offer a quick restoration of glycemia from a hypoglycemic condition to a normoglycemic condition (Eleazu, 2016). The normal glycemic range is 70-110mg/dL.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Source of raw materials

Provitamin A cassava tubers IITA-TMS-I011368, IITA-TMS-I070593, and IITA-TMS-I011371 were obtained from International Institute of Tropical Agriculture (IITA), Ibadan. Lyophilised *Lactobacillus rhamnosus GG* was purchased from Valio Ltd., Helsinki, Finland. The α-amylase enzyme with enzyme activity 36U/mg and glucoamylase enzymes with enzyme activity 62.4U/mg were both purchased from Sigma-Aldrich.

3.2 Preparation of Provitamin A cassava hydrolysate from cassava roots

3.2.1 Starch Extraction

The extraction of cassava starch was done according to the method of Adegunwa *et al*. (2010) with some modification. Fresh cassava roots (50kg) were sorted, weighed, peeled, washed, and grated in a commercial mechanical cassava grating machine. The resultant grated pulp was immediately suspended in 70 litres of water so as to facilitate sieving. The sieving was done by passing the mixture through a screen (Muslin cloth). This allowed separation of fibrous and other coarse materials from the starch. After sieving through muslin cloth into a receiving container, the starch was allowed to settle for 10-12 hours before excess water was decanted to allow for easy collection of the starch (Adegunwa *et al.*, 2010). The colour of the extracted starch that settled at the base of the container was observed to be white. However, the beta-carotene layer, which had a distinct yellow colour, was immiscible with water (being lipid soluble). It settled at the interface between the starch and the water. The beta carotene layer was extracted carefully using muslin cloth so as to prevent it from flowing off with the water that was being discarded. The beta carotene layer was then mixed back evenly into the starch and this gave a starch which is light yellow in colour. The cassava starch was collected, sealed in polythene bags and kept for further use.

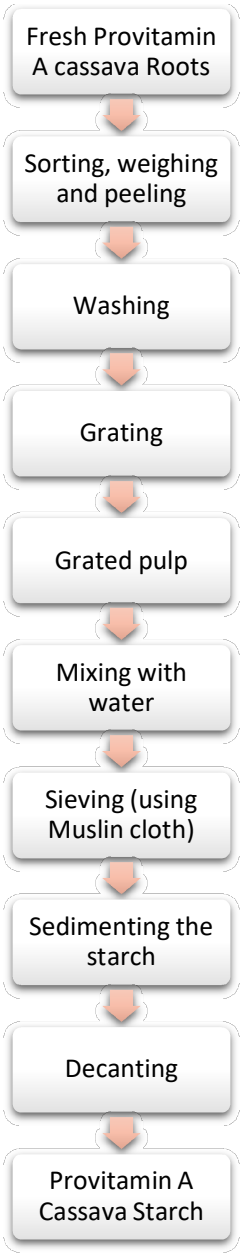


Figure 3.1 Provitamin A cassava starch production

(Source: Adapted from Adegunwa *et al.*, 2010)

3.2.2 Hydrolysis of Provitamin A cassava starch

Provitamin A cassava starch (100 g) was reconstituted with water to 15% solids. The mixture was heated to 100^{o}C, allowed to gelatinise and cooled to 90^{o}C in a shaking water bath. The pH was adjusted to 7.2 with 0.1 M NaOH, and then treated with 2700U of α-amylase corresponding to 0.1%w/w of starch. The liquefaction was done at 90^{o}Cfor 60 min after which the mixture was cooled to 60^{o}C.The pH was then adjusted to 4.5 with 0.01 M HCl and treated with 2700U of glucoamylase enzyme corresponding to 0.058% w/w of starch. The saccharification was carried out at 60^{o}Cfor 60 min. After saccharification, the starch hydrolysate was cooled and filtered for homogeneity using muslin cloth. The filtrate (hydrolysate) was then pasteurised at 72^{o}C in-bottle for 15 min before it was inoculated with the starter culture (Jeng *et al.*, 2012). The process is further explained using the flow chart presented in Figure 3.2.

3.3 Culturing and harvesting of*L. rhamnosus GG* before inoculation

A modification of the method of Ding and Shah (2008) was used. The probiotic organism, *L. rhamnosus GG* was cultured in 500 mL of Mann Rogosa Sharpe (MRS) broth for 48 hours at 37^{o}C. *L. rhamnosus GG* cells were concentrated by centrifuging at 5,000 x g for 30 min at 4^{o}C. The *L. rhamnosus GG* cell suspension was washed with 25 mL of sterile normal saline (pH 7.0) prior to either microencapsulation or addition to provitamin A cassava hydrolysate. The concentrated cells were serially diluted and enumerated by the pour plate method using MRS agar before use (Ding and Shah, 2008).

3.3.1 Inoculation of the provitamin A cassava hydrolysate with starter culture *L. rhamnosus GG.*

Lactobacillus rhamnosus GG was the probiotic culture used. The extracted provitamin A cassava hydrolysate was divided into different portions to allow the inoculation of the organisms. For the free cells, a portion of the hydrolysate was inoculated with *L. rhamnosus GG* at a ratio 1: 10 (organism: provitamin A cassava hydrolysate ratio) and a concentration of 1×10^{10}CFU mL^{-1} of *L. rhamnosus GG* culture. Another portion was inoculated with encapsulated *L. rhamnosus GG* cells at a ratio 1: 10 (organism: provitamin A cassava hydrolysate)at a concentration of 1×10^{10} CFU g^{-1}of *L. rhamnosus GG*, and incubated under microaerophilic condition at 37^{o}±2^{o}C for 48 hours.

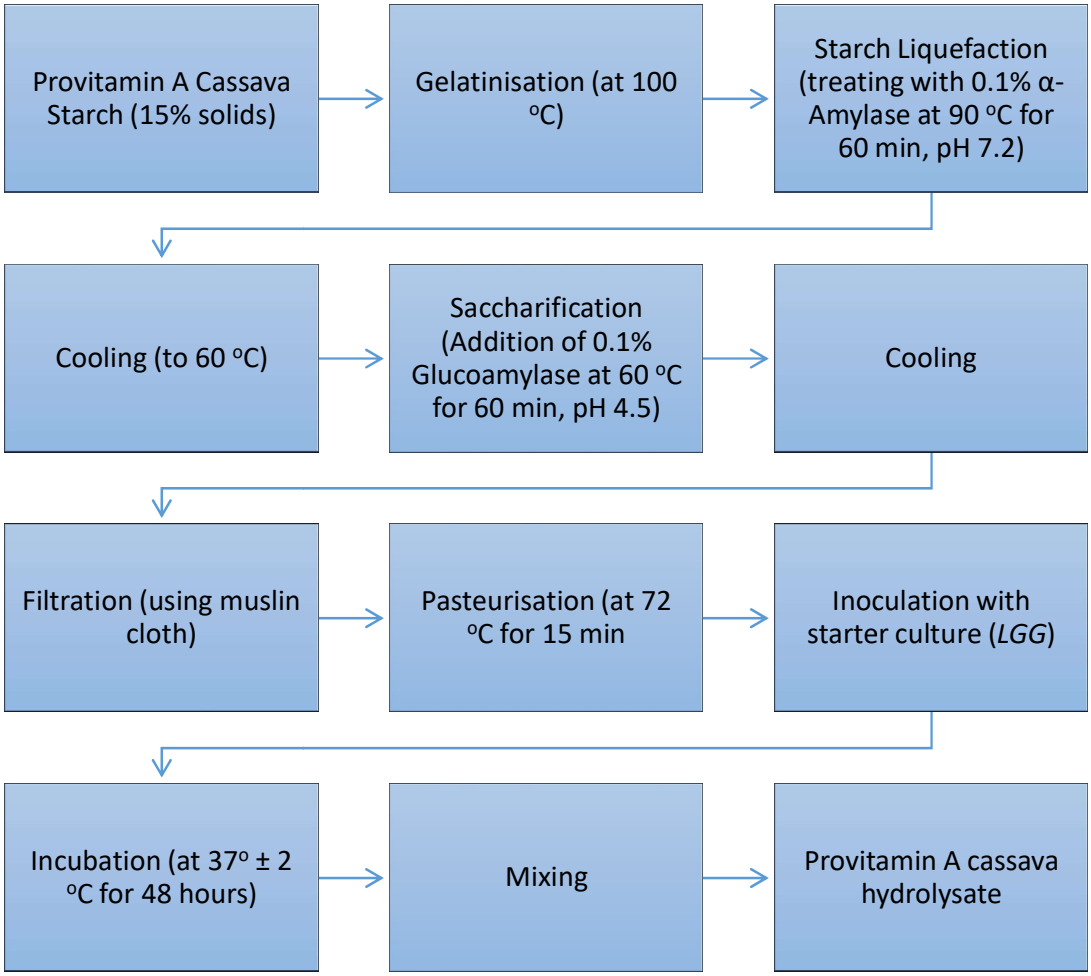


Figure 3.2: Provitamin A cassava hydrolysate production

Source: (Adapted from Ding & Shah, 2008).

Three replicates were maintained for each treatment (Panda and Ray, 2007). The fermented hydrolysate was mixed and kept for further analysis.

3.4 Microencapsulation of probiotics

3.4.1 Microencapsulation of *L. rhamnosus GG* in alginate beads by emulsion technique

Probiotic *L. rhamnosus GG* cells were microencapsulated in alginate beads using a modified method of Rayment *et al.* (2009). 100 mL of 3% w/v sodium alginate (Ajax chemicals Ltd., Melbourne, Australia) was sterilised at 121^{o}C for 15 min, cooled to about 45^{o}C and mixed with 25 mL of *L. rhamnosus GG* cells. The suspension containing alginate and *L. rhamnosus GG* was then dispensed slowly using a syringe (21G x 1½") and needle (0.8x 40mm) into a beaker which already contained 1 g of tween 80 in 600 ml of vegetable oil, to form an emulsion which was thoroughly mixed with a magnetic stirrer at 200 rpm. Calcium chloride solution (0.1M) was slowly added to break the emulsion and form soft beads which were left to harden for 30 min. When bead formation was complete, the calcium-alginate beads were separated from the aqueous phase and stored for 10 hoursat 4^{o}C,for further hardening of the beads.

3.4.2 Microencapsulation of *L. rhamnosus GG* in alginate beads by extrusion method

This was carried out according to the method of Krasaekoopt and Kitsawad (2010). Sodium alginate (100 mL, 3% w/v) was sterilised and mixed with 25 mL of *L. rhamnosus GG* cells. The suspension of alginate and *L. rhamnosus GG* was thenextruded through a sterile syringe (21G x 1½") and needle (0.8x 40mm) into sterile 0.1M $CaCl_2$ solution containing tween 80. The beads were double-rinsed with sterile water once hardening was completed.

3.4.3 Optical examination of calcium-alginate beads containing *L. rhamnosus GG*

The structure of calcium-alginate beads containing *L. rhamnosus GG* were observed and photographed at 10–60 fold magnification with a light microscope (Carl Zeiss Axioskop, Ag, Jena, Germany) model no 146126 fitted with Powershot® A640 digital camera for visual characterisation.

3.4.4 Calcium Alginate beads size determination

Ten (10) beads were selected at random, blotted on paper towels to remove the solution on the beads surface. Beads were weighed and mean weight (g) was calculated. The diameter of the beads was also measured using micrometer screw gauge (Capela *et al.*, 2007).

3.4.5 Release of entrapped cells

A suspension of 1% (w/v) sterile sodium citrate solution (99 mL, pH 6) containing 1g *L. rhamnosus GG* beads (freshly prepared) was agitated by shaking gently at room temperaturefor 10min. This facilitated the release of the entrapped cells (Xu *et al.*, 2013).

3.4.6 Enumeration of *L. rhamnosus GG* cells

Enumeration of viable *L. rhamnosus GG* cells was done by pour plate method using MRS agar. Incubation was done under microaerophilic conditions for 48 hoursat 37^{o}C. Viability of *L. rhamnosus GG* cells was expressed as CFUmL^{-1} for free cells and CFUg^{-1} for encapsulated cells (Capela *et al.*, 2007).

$$\text{CFUmL}^{-1} = \frac{(\text{Number of colonies X dilution factor})}{\text{volume of culture plate}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad \text{(Equation 3.1)}$$

3.4.7 Determination of survival rate and encapsulation efficiency

Percentage survival of probiotic organisms during encapsulation was calculated with the following equation:

$$\text{Survival (\%)} = \left(\frac{y+z}{x}\right) \text{X } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{ (Equation 3.2)}$$

Where:

x = the population of *L. rhamnosus GG* cells before encapsulation;

y = the population of *L. rhamnosus GG* cells trapped in the calcium alginate beads;

z = the population of *L. rhamnosus GG* cells in the fluid around calcium alginate beads.

The efficiency of encapsulation was evaluated by the following equation:

$$\text{Efficiency (\%)} = \left(\frac{y}{x}\right) \text{X } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{ (Equation 3.3)}$$

Where:

x = the initial population of *L. rhamnosus GG* cells before encapsulation;

y = the population of *L. rhamnosus GG* cells entrapped in the calcium alginate beads (Chávarri *et al.*, 2010).

3.5 Simulation of In-vitro gastric and intestinal conditions

3.5.1 Preparation of gastric and intestinal juices

Solutions with chemical similarities to the gastro-intestinal tract juices of a fasted human were prepared following the methods of Rayment *et al.*(2009) and Capela *et al.*(2007). The simulated gastric juice (SGJ) without pepsin (Heidebach *et al.*, 2009, Arora *et al.*, 2014) contained 0.08M HCl adjusted to pH 1.5 with HCl or NaOH. Simulated intestinal juice (SIJ) without pancreatin (Zhou *et al.*, 1998) was prepared by dissolving sodium deoxycholate (bile salts) in intestinal solution which was a mixture of 6.5g/L NaCl, 0.22 g/L $CaCl_2$, 0.835 g/L KCl, and 1.386g/L $NaHCO_3$ (Sigma–Aldrich analytical grade chemicals) and adjusted to a final pH 7.5 and concentration of 3.0g/L.

3.5.2 Sequential Incubation of Free and Microencapsulated *Lactobacillus rhamnosus GG* in Simulated Gastric and Intestinal Juices.

Freshly encapsulated *L. rhamnosus GG* cells (1g) or washed free *L. rhamnosus GG* cells (1ml) were mixed in sterilised simulated gastric juice (SGJ) (10 ml) and it was separated into batches which were incubated at 37^{o}C for a period 30, 60, 90, and 120 minrespectively. Beads were then sampled and washed with peptone solution (0.1%) at the various time intervals (Rayment *et al.*, 2009). Samples of free cells were also withdrawn at 30, 60, 90, and 120 min respectively after the addition of the organism. Aliquots (1 mL) from each treatment were serially diluted in 10-folds with peptone solution and viable cell counts was enumerated on MRS agar after 48 hours of microaerophilic incubation at 37°C (Iyer and Kailasapathy, 2005).

After 120 min incubation in simulated gastric juice (SGJ), 1g of the *L. rhamnosus GG* microspheres were removed by filtration while 1ml aliquot of free *L. rhamnosus GG* cells was subsequently placed in separate flasks containing 10 mL of simulated intestinal juice. The pH was adjusted to 7.5 using 0.1 M NaOH, followed by incubation of the flasks under microaerophilic conditions at 37°C for 120min. During incubation, flasks were shaken manually and the pH was readjusted periodically to 7.5 after sampling at 60min interval. After incubation in the simulated intestinal juice. Aliquots (1.0 mL) were removed and enumerated for *Lactobacillus rhamnosus GG*.

3.6 Chemical Analysis

3.6.1 Determination of pH

The pH of provitamin A cassava hydrolysate sample were determined with the pH meter model BA 350 with glass electrode. The meter was standardised with buffer solution of pH 7.0 and 4.0 respectively. Provitamin A cassava hydrolysate sample (50mL) was poured in a beaker and the pH determined (in triplicates) by dipping the electrode into the samples, and readings taken directly from the meter (Pearson, 1976).

3.6.2 Determination of % total titratable acidity (TTA)

Provitamin A hydrolysate sample (5mL) was thoroughly mixed, diluted with distilled water up to 50 mL. Aliquot (5 mL) was then titrated against 0.1M NaOH (standard) to an end point of pH 8.1, determined by monitoring the pH with the electronic pH meter. The % total titratable acidity was recorded as the percentage of lactic acid contained in the provitamin A cassava hydrolysate (AOAC, 2002).

The % TTA was calculated as:

$$\%\ \text{TTA (lactic acid)} = \frac{\text{Titre value x molarity of NaOH x 90.08}}{\text{1000 x volume of sample}} \quad \text{----------} \quad \text{(Equation 3.4)}$$

where the factor 90.08 is the molar mass of lactic acid.

3.6.3 Organic acidsquantification

The organic acids (lactic, acetic and propionic acids) were quantified according to the method described by Tian *et al.* (2017). This was done using a HPLC system coupled with ultraviolet detector (Cecil CE 4200, Agilent, Palo Alto, CA, USA) coupled with a dual pump (Cecil CE 4100) with pump pressure of 7mPa, using gradient elution conditions. Hydrolysate were centrifuged at 12,000 x g at 4°C for 10 min to obtain supernatants. Supernatant was filtered into HPLC sample vials using 0.45 µm membranes (Pall, N.Y., USA). Ion exchange column C18 (Agilent) was used at 20°C for chromatographic analysis. Separation of 20 µl injection volumes was done at a flow rate of 2 ml/min for the first 5 min, then 3 ml/min for 6-10 min at wavelength 210 nm. Organic acids standards used (lactic acid, acetic acid and propionic acid) were chromatographic grade (Sigma Aldrich Co., Germany). Peak area and Peak retention time were the parameters used for quantification of HPLC results. Extraction and injection were done in triplicate for each sample.

3.6.4 Estimation of Total sugars by Anthrone method

Estimation of total sugars was done according to method of Pearson *et al*. (1976). Preparation of Anthrone reagent was done by dissolving 200mg of Anthrone in 100ml of ice cold 95% H_2SO_4 which was freshly prepared prior to use. The standard glucose solution (stock) was prepared at a glucose concentration of 1mg/mL of distilled water. Stock solution (10 mL) was diluted to 100 mL with distilled water to prepare the working solution. Toluene was added in drops and the mixture was stored at 4^oC. Glucose standard curve was plotted by preparing glucose concentrations in the range 0-0.8mL of the working standard diluted to 1mL with distilled water.

Provitamin A cassava hydrolysate (1mL) was diluted in 10mL of distilled water in a 25mL bottle. The content was mixed vigorously before the addition of 15mL of 52% Perchloric acid. This was continuously stirred for a period of 30min. Filtration of the mixture was done using Whatman no1 filter paper. Filtrate (1 mL) was mixed in a test-tube with 4mL of Anthrone reagent. Absorbance was measured using spectrophotometer at 630nm. The total soluble sugars was then estimated using Glucose standard curve (Pearson *et al*., 1976).

3.6.5 Determination of Reducing Sugar (Dinitrosalicylic Acid method)

For the estimation of reducing sugar (Miller, 1959), dinitrosalicylic acid reagent (DNS reagents) was prepared by dissolving 1g Dinitrosalisylic acid, crystalline phenol (200mg) and sodium sulphite (50mg) in 1% NaOH (100 mL) and stored at 4^oC.

Standard solution (dextrose 2mg/mL) from 0.2-1mL was made up to 1mL with the sample solution in different test tubes. DNS reagent (2mL) was added in each of the test tube, followed by heating of the content in a boiling water bath for 5min. 40% Rochelle salt solution (1 mL) was added to each of the test tubes while contents were still warm. Test tubes were cooled to room temperature. Volume was made up to 11mL in each of the test tube after cooling, dark red colour was allowed to develop and absorbance was measured at 560nm by spectrophotometer (Systronic, model no: 2202). Standard curve was prepared with 0-2 mg dextrose solutions absorbance. Calculationwas done from the standard graph equation (Miller, 1959).

3.6.6 Total soluble sugars determination

The total soluble sugars concentration (^{o}Brix) of the hydrolysates was determined using a refractometer.

3.6.7 Elemental Mineral Analysis

Provitamin A cassava hydrolysate samples (200 mL) was digested by 20mL of concentrated HNO_3 in a Kjeldahl flask and the mixture was left to stand overnight. 5mL of concentrated $HClO_4$ and 0.5mL of H_2SO_4 were added to the flask and then heated until it stopped emitting any white smoke. The digests were then dissolved in 100 mL of 2% HCl and filtered with a filter paper. Mineral analysis was carried out using Atomic Absorption Spectroscopy (AAS, Perkin-Elmer, Optima 2000, USA), using an inductively coupled plasma multi element reference standard solution viii (product no. 1.09492.0100, Merck, Germany) with a concentration of 10 mg/L for each of the elements analysed (Kumar *et al.*, 2010).

3.7 Phytochemical, carotenoid and antinutrient analysis

Some preliminary analysis were carried out to determine the phytochemicals present in the provitamin A cassava hydrolysate samples qualitatively prior to quantification.

3.7.1 Estimation of total phenolic Content

Each sample (0.5 mL) was mixed with 50 mL methanol to be extracted for 1 h after which distilled water was used to dilute the methanolic extract at a ratio 5:1 (v/v). Diluted extract (1.25 mL) was then mixed with distilled water (0.5 mL) and 1.25mL of Folin–Ciocalteu reagent (FCR) consecutively and allowed to stand for 6 min prior to the addition of 7% sodium carbonate solution (1.25 mL). The final volume was made up to 3 mL with distilled water and incubated for 90 min at room temperature after which absorbance was measured at 760 nm. Standard curve was prepared from the concentration of 0-600µg of Gallic acid per ml (Chang *et al.*, 2005). The total phenolic content was calculated and expressed as mg GAE/100mL.

3.7.2 Determination of Total flavonoids

Analysis of the total flavonoids was carried out as described by Mbaebie *et al.* (2012).Sample (0.5 mL)was mixed with 0.5 mL of 2% $AlCl_3$-Ethanol solution. This was incubated for 1h to allow the presence of flavonoid which is shown by development of yellow colour. The absorbance was measured by a spectrophotometer at 420nm using uv-vis. Total flavonoid content was calculated by the equation obtained from the standard curve and expressed as mg QE/100mL (Mbaebie *et al.*, 2012).

3.7.3 Antioxidant capacity [2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay]

The radical scavenging activity of antioxidant compounds was measured by DPPH assay (Wong and Chye, 2008). It is based on the principle that DPPH solution has a deep violet colour which turns to pale yellow as the non-radical form is produced. This can be measured spectrophotometrically at 517 nm.

DPPH radical (0.5 mL of 0.1M) (Sigma) was mixed with 1 mL of methanolic extract of the sample in a test-tube. The test tube was shaken vigorously at room temperature and the absorbance of the sample was immediately read at 517nm using spectrophotometer (Cecil, CE 1000). Methanol was used as blank while butyl hydroxytoluene (BHT, sigma) was used as a standard. This was used to plot the calibration curve.

Calculation of the radical scavenging activities was done using the equation below:

$$Radical\ scavenging\ activity\ (\%) = \frac{(Astandard - Asample)}{(Astandar\ )} \times 100$$

---------- (Equation 3.5)

Where $A_{standard}$ is the absorbance of the DPPH standard and A_{sample} is the absorbance of the hydrolysate.

3.7.4 Determination of carotenoid content

3.7.4.1 Extraction of carotenoids

The extraction of carotenoids was done according to the method described by Carvalho *et al.* (2012). Provitamin A cassava hydrolysate (15 mL) was mixed with 3g of Celite 454 to form a paste which was further mixed with 25 mL of acetone and transferred to a separatory funnel connected to a 250-mL flask. Filtration was done under vacuumthrice till a colourless sample was achieved. The extract was then transferred into 40 mL of petroleum ether contained in a separation funnel. Ultrapure water (millipore) was slowly added to remove the acetone. The aqueous phase was discarded. This extraction procedure was carried out four times to remove all residual solvent. The extract was transferred in aflask containing anhydrous sodium sulfate (15 g) and petroleum ether (50 mL) was used to make up the volume. The absorbance was read at a wavelength of 450nm. The calculation of the total carotenoid content was done as follows:

$$\text{Carotenoid contents } (\mu g/g) = \frac{\text{A X V (ml)X } 10^4}{A_1^{1\%}\text{cm X P (g)}}$$

---------- (Equation 3.6)

Where A=absorbance at 450nm

v= total volume of extract

p= weight of sample,

$A_1^{1\%}$cm =2592 (extinction coefficient of β-carotene in petroleum ether) (Carvalho *et al.*, 2012).

3.7.4.2 β-carotene content determination

β-carotene content of the samples was determined according to the method described by White & Broadley (2009). Sample (2 mL) was measured into a 250mLbeaker, 50mL of petroleum ether: acetone (2:1v/v) mixture was added to extract the ß-carotene. Flask containing the mixture was allowed to shake at 200rpm for 20min to ensure uniform mixing at room temperature. This was later centrifuged at 4000rpm for 10min, supernatant was collected and made up to 50mL with solvent mixture. The supernatant was removed to a 250mL funnel to separate the organic layer (upper layer). Aqueous layer was discarded, the organic layer was transferred into 50mLmeasuring cylinder and made up with solvent mixture. Working standard of ß-carotene in the range 0-50ppm was prepared from stock beta carotene solution of 100ppm concentration. The absorbance of samples as well as working standard solutions were read on a Cecil 2483 UV spectrophotometer at a wavelength of 450nm against blank (White and Broadley, 2009).

$$\beta - \text{carotene } (\mu g/100g) = \frac{\text{Absorbance of sample x average gradient x dilution factor}}{10000} \quad \text{---------- (Equation 3.7)}$$

$$\text{Dilution factor} = \frac{\text{Volume of solvent}}{\text{Sample weight}} \quad \text{---------- (Equation 3.8)}$$

3.7.5 Determination of Cyanide Content

Cyanide in provitamin A cassava hydrolysate was determined using alkaline picrate method(Onwuka and Ogbogu, 2007). Provitamin A cassava hydrolysate (2 mL) was mixed in 20mL distilled water and left in a corked conical flask overnight. Filtration was done where necessary. Alkaline picrate solution (4mL) was added to 1mL of the filtrate. Colour was developed by incubating the test-tube in water bath at 90^{o}C for 5 min. Absorbance was read at 490nm. Blank reagent prepared by diluting 4mL of

alkaline picrate with 1mL of distilled water, was used to standardise the spectrophotometer prior to reading the absorbance of the samples.

3.8 Colour measurement

The colour of fermented and unfermented provitamin A cassava hydrolysate was measured using a Konica Minolta CR-410 Chroma meter with data processor DP400 (Konica Minolta Sensing Inc., Osaka, Japan). Calibration of the equipment was done using a calibration plate before each measurement. Colour measurements were performed on provitamin A cassava hydrolysate samples and values were averaged from three consecutive measurements. The colour was described based on the values of Hunter L*, a* and b*, where L* represents lightness (taking values between 0-100, where 0 = black and 100 = white), a* = red–green components, while b* = yellow-blue components(Suda *et al.*, 2003, MaiaCosta *et al.*, 2013).

Calculation of ΔC (Chroma), ΔE*(total colour difference) and hue angle (h^o) were made from L*, a*and b* values using the following equations. Colour measurements were taken in triplicates,

$\Delta E = \sqrt{(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2}$ ---------- (Equation 3.9)

$C = \sqrt{(a^*)^2 + (b^*)^2}$ ---------- (Equation 3.10)

$h° = \tan\text{-}1(b^*/a^*)$ ---------- (Equation 3.11)

3.9 Microbiological Analysis

3.9.1 Determination of presence of pathogenic organisms in provitamin A cassava hydrolysate samples

These tests were carried out on fresh and stored samples of provitamin A cassava hydrolysate to determine the presence of pathogenic organisms such as *E. coli*, *Salmonella spp., Shigella spp.,*and spoilage organisms such as fungi (yeast and mould) in the samples. MacConkey agar was the growth medium used for the enumeration of total Enterobacteriaceae while potato dextrose agar was used for the enumeration of fungi. *Salmonella-Shigella* agarwas used to culture *Salmonella spp.* and *Shigella spp.* Pour plate method was used for the culturing of all the organisms.

3.9.1.1 Media Preparation

MacConkey agar (Oxoid), *Salmonella-Shigella* agar and Potato Dextrose agar were prepared with the instruction of the manufacturer. Each media (500mL) was

homogenised and sterilised at 121^{o}C for 15 min, after which they were allowed to cool before use. All glass wares were also washed and sterilised in an air-oven at 180^{o}C for 15 min.

3.9.1.2 Coliform count

Provitamin A cassava hydrolysate (1 mL) was diluted with 9mL of sterile distilled water to make a ten-fold dilution. It was serially diluted up to 10^{-2}. 1mL of 10^{-1} and 10^{-2} dilutionsof the samples were plated out in triplicates. Sterilised MacConkey agar (10mL) was cooled to about 47^{o}Cand aseptically poured into sterile petri dishes each containing the suitable dilutions of samples. The plates were gently swirled and allowed to set. All inoculated plates were inverted after solidification and incubated at 25 ^{o}C in an aerobic condition for 24 hours before they were observed.

3.9.1.3 Total fungal (yeast and mould) counts

Provitamin A cassava hydrolysate (1 mL) was diluted with 9 mL of sterile distilled water to make a ten-fold dilution. It was then serially diluted up to 10^{-2}. 1mL of the dilutions 10^{-1} and 10^{-2} of the samples were plated out in triplicates. Sterilised Potato dextrose agar (10mL) was cooled to about 47^{o}C and aseptically poured into petri dishes each containing the suitable dilution of samples. The plates were gently swirled and allowed to set. All inoculated plates were inverted after solidification and incubated in an aerobic condition at 25^{o}C for 5 days. The number of microorganisms was calculated as the number of colonies in relation to the dilution factor.

3.9.1.4 Enumeration of *Salmonella* and *Shigella spp.*

Provitamin A cassava hydrolysate (1mL) diluted with 9mL of sterile distilled water to make a ten-fold dilution was serially diluted up to 10^{-2}. 1mL of the dilutions 10^{-1} and 10^{-2} of the samples were plated out in triplicates. Sterilised *Salmonella-Shigella* agar (10mL) was cooled to about 47^{o}Cand aseptically poured into sterile petri dishes each containing the suitable dilution of samples. The plates were gently swirled and allowed to set. All the inoculated plates were inverted after solidification and incubated at 25 ^{o}C in an aerobic condition for 24 hours before they were observed.

3.9.2 Total viable counts of *Lactobacillus rhamnosus GG*

Enumeration of *L. rhamnosus GG* was done using Mann Rogosa Sharpe (MRS) (Himedia). MRS agar was prepared with the instruction of the manufacturer, sterilised

at 121^{o}C for 15min and allowed to cool. The pour plate method was used and samples (1mL) were diluted serially with sterile distilled water to carry out a tenfold serial dilution up to 10^{-10}. Sterilised MRS agar was cooled to about 47 ^{o}Cand poured aseptically into petri dishes containing 1mL of introduced inoculum withdrawn from dilutions 10^{-6}, 10^{-8} and 10^{-10} were plated in triplicates. The plates were gently swirled and allowed to set. All the inoculated plates were inverted after solidification and incubated under microaerophilic conditions at 37^{o}C for 24 hours before they were observed.

3.10 Evaluation of microbiological safety of provitamin A cassava hydrolysate during storage

The Provitamin A cassava hydrolysates with free or encapsulated *L. rhamnosus GG* cells were sealed in 250 mL amber and transparent glass bottles. They were stored at 4ºC for 90 days. The fresh, un-inoculated samples served as the control. All the samples were monitored for changes in microbial (total viable counts of *L. rhamnosus GG, E. coli*, *Salmonella, Shigella,* fungal counts, chemical (pH and organic acid) and physical (colour) properties at various time interval for 90 days. Samples were stored without the addition of any preservative.

3.11 Determination of viscosity

The viscosity of the provitamin A cassava hydrolysate was determined at 20^{o}C using an Ostwald viscometer, which is a capillary viscometer. Provitamin A cassava hydrolysate (20ml) was filled into the Ostwald capillary viscometer, 200Cst. By suction, the sample was pulled up into the upper reservoir bulb and by gravity, it was allowed to flow back into the lower bulb. The time of flow which the sample took to pass between the upper and the lower marks was monitored using a stop watch and recorded in seconds. All measurements were taken in triplicates. The time of flow was then multiplied by the viscometer constant, 0.1 and expressed as mm^2/s.

3.12 Animal study

3.12.1 Ethical Statement

Strict compliance with the guidelines set out by the University of Ibadan Animal Care and Use Research Ethics Committee in handling of experimental animals and in accordance with internationally acceptable best practices were ensured in this study.

Valid approval and ethical clearance with assigned number UI-ACUREC/17/0080 were obtained from the ethics committee of the University of Ibadan for the experiment.

TMS-I011368 hydrolysate only was selected and used as the hydrolysate of choice throughout the animal study.

In order to avoid ambiguity in the nomenclature of the hydrolysate samples, sample codes used are as follows:

PH = TMS-I011368 hydrolysate without *L. rhamnosus GG*

PHE = TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*

PHF = TMS-I011368 hydrolysate with free *L. rhamnosus GG*

PHE1 = TMS-I011368 hydrolysate with $1X10^{10}$ CFU/g encapsulated *L. rhamnosus GG*

PHE2 = TMS-I011368 hydrolysate with $2X10^{10}$ CFU/g encapsulated *L. rhamnosus GG*

PHE4 = TMS-I011368 hydrolysate with $4X10^{10}$ CFU/g encapsulated *L. rhamnosus GG*

PHF1 = TMS-I011368 hydrolysate with $1X10^{10}$ CFU/mL free *L. rhamnosus GG*

PHF2 = TMS-I011368 hydrolysate with $2X10^{10}$ CFU/mL free *L. rhamnosus GG*

PHF4 = TMS-I011368 hydrolysate with $4X10^{10}$ CFU/mL free *L. rhamnosus GG*

W = Distilled Water (control)

3.12.2 Experimental Animals

The rats were housed at the Experimental House of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan. The rats were fed with commercial rat pellets and provided with clean potable water *ad libitum*. The rats were also humanely handled through the experiment. The rats were placed in individual cages in a room with 12:12h light-dark cycle, temperature of 22-24℃and a relative humidity of 77 ± 5%. Provitamin A cassava hydrolysate samples were administered to the Wistar rats using oral gavage.

3.12.3 Evaluation of *In vivo* effect of administration of provitamin A cassava hydrolysate with or without *L. rhamnosus GG* onfaecal microbial ecology of Wistar rats

The microbial population on day 15 after the commencement of the administration of provitamin A cassava hydrolysate without or with *L. rhamnosus GG* to the rats, was

taken as the basal population. Rat faecal samples were collected immediately after being dropped onto a clean paper placed under the cage and collected prior to feeding on days 15 and 30. Fresh faecal samples were dispersed in sterile phosphate-buffered saline (PBS; pH 7.0 and 9 g L^{-1}NaCl) and homogenised for 5 min in a manual glass homogeniser. The quantities of prominent cultivable microflora were enumerated as colony-forming units (CFU). Standard pour-plating technique was employed for culturing of total Lactic acid bacteria and aerobic faecal bacteria. Microaerophilic jar was used for culturing total lactic acid bacteria, which was enumerated using MRS agar (Himedia, Mumbai, India). Enumeration of *Escherichia coli* wascarried out using Macconkey agar (Himedia)and total aerobic organisms were determined using Plate Count Agar (PCA) (Maity *et al.*, 2009, Podolak *et al.*, 2010).

3.12.3.1 Evaluation of Growth Direction Index (GDI).

GDI gives the expansion (increase) or contraction (decrease) of microbial populations in a particular biological system(Samanta *et al.*, 2014). The actual number of bacteria present in faecal samples was expressed as CFU and converted into the corresponding logarithmic values. The difference between log values of test groups and that of control group was calculated and designated as GDI, which is negative when the log of control group is higher than the log of test group, and positive when the log value of control group is lower than the log value of test group (Adak *et al.*, 2013).

GDI was calculated was follows:

$$GDI = \frac{Log\ (Experimental\ group)}{Log\ (Control\ group)}$$ ---------- (Equation 3.12)

3.12.4 Toxicological evaluation of Provitamin A cassava hydrolysate

3.12.4.1 Study design

Adult male and female Wistar rats (120-150 g) were used in the study. Provitamin A cassava hydrolysate was inoculated with probiotic *L. rhamnosus GG* and administered to the rats using oral gavage. Acute toxicity study (LD_{50}) was carried out within 24 hours where the animals were dosed using the up and down procedure(Bruce, 1985). The animals were dosed one at a time. Two separate studies were conducted during the acute toxicity study- the effect of the provitamin A cassava hydrolysate without probiotics and the effect of inclusion of probiotics (*L. rhamnosus GG)*. Three groups of test animals, comprising of 5 animals each was used.

The sub-chronic toxicity study comprised of 8 groups- 2 control groups and 6 experimental groups.

3.12.4.2 Acute toxicity study (LD_{50})

The acute toxicity study (LD_{50}) was determined within 24 hours where the adult female Wister rats (n=65) were administered the provitamin A cassava hydrolysate (vehicle) and or test doses using the up and down procedure. The animals were dosed one at a time(Bruce, 1985). The dose for the next animal would be decreasedif the last animal died, otherwise, i.e. if the animal survived, the dose for the next animal was increased(Tabilo-Munizaga and Barbosa-Ca´novas, 2005). Two separate studies were conducted during the acute toxicity study. In group 1, three (3) groups of test animals, comprising of 5 animals each were administered with provitamin A cassava hydrolysate only, in doses of 0.041mg/mL/kg body weight, 0.082mg/mL/kg body weight, 0.123mg/mL/kg body weight. For group 2, five (5) groups of 5 rats each was administered free *L. rhamnosus GG* cells (probiotics) suspended in the provitamin A cassava hydrolysate (0.041mg/mL) in 5 different inoculum concentrations: 5 x 10^{10} colony forming units/mL, 10 x10^{10} CFU/mL, 20 x 10^{10} CFU/mL, 40 x 10^{10} CFU/mL and 80 x 10^{10} CFU/mL. Group 3was administered encapsulated *L. rhamnosus GG* cells (probiotics) suspended in the provitamin A cassava hydrolysate in 5 different inoculum concentrations as mentioned above. The test sample for each group was administered orally by oral gavage to one rat at a time per group. The rat was then observed for signs of toxicity for the first 30 min followed by hourly observation for 8hours for the first 24 hours. If there were no signs of toxicity or mortality observed in the rat within 24 hours, the rat was monitored for a further 24 hours before termination of the experiment.

3.12.4.3 Sub-Chronic toxicity study

Forty (40) male Wistar rats were used for the sub-chronic toxicity study. Rats were randomly and equally divided into 8 groups. Group 1 was administered distilled water, group 2 was administered provitamin A cassava hydrolysate without *L. rhamnosus GG*, groups 3, 4 and 5 were administered provitamin A cassava hydrolysate with 1, 2 or 4 x 10^{10} CFU/mL of free *L. rhamnosus GG* respectively, while groups 6, 7 and 8 were administered provitamin A cassava hydrolysate with 1, 2 or 4 x 10^{10} CFU/g of encapsulated *L. rhamnosus GG* respectively. All rats were dosed orally once daily for 30 days.

3.12.4.3.1 Physical observation and mortality

Clinical observation was made once daily for ill-health, death or evidence of adverse response to the test samples, e.g. changes in the eyes, fur or skin. Changes in the patterns of behavior or sleep, changes in salivation, mucus membranes or diarrhea, observation of tremors or coma.

3.12.4.3.2 Body weight, feed and water consumption

Body weight of each rat was taken once every week and the changes in the body weights were recorded as percentage change in body weight. The amounts of test sample to be given was re-calculated every week based on the changes in the rats' body weight, to be sure that the dose per kg body weight of the test sample given to the rats remained constant. The rats were allowed to have access to feed and water *ad libitum*.

3.12.5 Sample collection

On day 30, 2mL of blood was drawn from the cephalic vein of each animal. This was done after the area had been thoroughly cleaned with 70% ethanol. The blood for biochemistry assays was collected into plain test tubes without an anticoagulant. The test tube containing the blood was kept in a slanting position on a bench for about 3 hours to enable the blood to clot. The coagulated blood was thereafter centrifuged at 4000 rpm for 10 minat 4°C to obtain serum. The serum was then kept at -20 °C until it was used for clinical biochemistry analyses, using various standard protocols (Ezeokonkwo *et al.*, 2012). All control and treatment rats were afterwards humanely sacrificed by decapitation. A complete inspection was performed to expose the abdominal organs.

3.12.5.1 Relative Organ Weight

A comprehensive gross observation for signs of lesions and abnormality was done on internal organs viz: lung, kidneys, heart, liver, spleen, stomach, gastro-intestinal tract (GIT), ovaries, adrenals and urinary bladder. The kidney, heart, liver and spleen were then selectively dissected out,cleaned and weighed. The equation below was used to calculate the relative organ weight (ROW) of each organ:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100 \qquad \text{---------- (Equation 3.15)}$$

3.12.5.2 Preparation of tissues homogenates for lipid peroxidationassay

Kidney, liver and heart tissues were harvested from rats and placed on ice. Tissues were homogenised in Phosphate buffer (0.1M, pH 7.4) at 1200 rpm in a Teflon glass homogeniser (Mexxcare, MC14362, Aayu-shi Design Pvt. Ltd., India). Homogenate was centrifuged at 10,000 rpm using a cold (4^{o}C) centrifuge (KX3400C Kenxin Intl. Co. Hong Kong) for 10 min. Supernatant was preserved for lipid peroxidation assays while the post mitochondrial fraction (PMF) was discarded (Ogunmefun *et al.*, 2015).

3.12.6 Haematological and biochemical analysis

3.12.6.1 Determination of haematological parameters.

Lithium heparinised bottles was used to collect Blood samples which was immediately analysed using a hematological analyser (KX-21n Sysmex cooperation, Japan). Parameters such as hematocrit [or packed cell volume (PCV)], red blood cell (RBC), hemoglobin (HB), RBC indices - mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the relationship between hematocrit, hemoglobin and erythrocyte count(Santos *et al.*, 2016). White blood cell (WBC), neutrophil, lymphocyte and platelets (PLT) were measured as described by Dacie and Lewis, (2008).

3.12.6.2 Determination of serum biochemical parameters

3.12.6.2.1 Determination of lipid profile

Serum total cholesterol and serum triglyceride were estimated by the enzymatic colorimetric test – Cholesterol oxidase peroxidase (CHOD/PAP) and glycerol phosphate oxidase (GPO/PAP) methods respectively, as explained in the manufacturer's instruction in the commercial laboratory kit obtained from Fortress Diagnostic Ltd, UK. HDL-C was measured by the polyethylene glycol (PEG) precipitation method outlined in the manufacturer's manual in the commercial laboratory kit obtained from Randox laboratories Ltd, UK. Glucose oxidase peroxidase (GOD/POD) method was used to estimate the serum glucose concentration using the laboratory kit obtained from Fortress Diagnostic Ltd, UK (Mahmood and Hamad, 2017).

3.12.6.2.2 Determination of serum creatinine and blood urea nitrogen

3.12.6.2.2.1 Estimation of Serum creatinine

Concentrations of serum creatinine was determined by Jaffe reaction method as explained by Bonsnes and Tausslay (1945) using the kit obtained from Fortress

diagnosis Ltd, UK. For de-proteinisation of serum, picric acid reagent (2 mL)and serum (0.2 mL) were added together. This was properly mixed and centrifuged at 3000 rpm. Buffer reagent (100 µL) was added to supernatant (1.1 mL) to prepare the test sample, buffer reagent (100 µL) and standard creatinine (0.1 mL) to prepare the standard while buffer reagent (100 µL) was also added to distilled water (0.1 mL) to prepare the blank. Picric acid (1.0 mL) was added to standard and blank. The test tubes were mixed thoroughly and left at room temperature for 20 min for orange-coloured complex that is formed when creatinine reacts with alkaline picrate. Absorbance was taken at 520 nm.

$$\text{Serum Creatinine Concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X 2 mg/dL} \quad \text{(Equation 3.16)}$$

Where 2mg/dL = Concentration of creatinine standard

3.12.6.2.2.2 Estimation of blood urea nitrogen

The determination of blood urea was done using Berthelot method as described by Fawcett and Scott (1960). For this procedure, commercial kit (fortress diagnostics Ltd, UK) was used. Serum (10 µL), purified water and standard urea (40 mg/dL) were held in different test tubes and labelled as test sample, blank and standard respectively. To each of these test tubes, 1000 µL of working reagent-I was added, thoroughly mixed and incubated for 5 min at 37 °C (Reagent-I was composed of a mixture of urease reagent, nitroprusside, hypochlorite and salicylate). This was then followed by the addition of 1000 µL of reagent-II to all the test tubes. The intensity of a blue-green coloured compound (indophenol) formed, was measured at wavelength 578nm. (Reagent-II is composed of alkaline buffer).

Urea is converted by the catalytic action of Urease, to carbon dioxide and ammonia which therefore reacts with salicylate, nitroprusside and hypochlorite mixture to produce indophenol.

The calculation of blood urea was done using the formula below:

$$\text{Blood Urea (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \text{X 40} \quad \text{---------- (Equation 3.17)}$$

$$\text{Blood Urea Nitrogen (mg/dL)} = \text{Serum Urea X 0.467} \quad \text{---------- (Equation 3.18)}$$

3.12.6.2.2.3 Assessment of liver function parameters

The clinical biochemistry determinations include total protein, albumin, liver profile parameters such as alkaline phosphatase (ALP),aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Urea and uric acid are the renal profile parameters that were measured (Zhou *et al.*, 1998). All measurements were carried out using commercially available kits with strict adherence to manufacturer's instructions.

3.12.7 Assessment of markers of oxidative stress on the kidney, heart and liver tissues

3.12.7.1 Determination of total protein content in serum and tissue samples

The concentrations of total protein was determined using Biuret method described by Al-Harbi (2016). Biuret reagent was prepared by dissolving CuSO4.5H2O (3 g) (BDH Chemicals, England) in NaOH (0.2M, 500 mL). This was followed by the addition of 9g of Sodium tartarate and 5g of potassium iodide (BDH Chemicals, England). The solution was made to 1 L with 0.2M NaOH.

4 mL of biuret reagent was added to standard stock Bovine Serum Albumin solution (10mg/mL of distilled water) to prepare the standard curve in the range 2-10mg protein/mL already made up to 1mL in different test tubes. The mixture was left at room temperature for 30 min for violet colour to develop. Absorbance was read in a spectrophotometer (H Gumption Medical & Scientific, England) at 540nm. The equation of plot of absorbance against concentration was then used for further calculations.

Suitable dilutions of the serum and tissue supernatants were made with distilled water. Triplicate samples containing 1mL of each diluted sample and 3mL of Biuret reagent were incubated for 30 min at room temperature. Absorbance was measured at 540 nm,with distilled water as blank. Protein content of samples were calculated from the standard curve, keeping the dilution factor in consideration.

3.12.7.2 Estimation of Nitric Oxide (NO) in tissue samples

This assay involves the measurement of the concentration of Nitrite (which is an indication of Nitric oxide production) in the liver, heart and kidney tissues using Greiss reagent as described by Olaleye *et al.* (2007). Greiss reagent was made by mixing 5% phosphoric acid (5 mL)and 0.1% N- (1-Naphthyl) Ethylenediaminedihydrochloride (0.1g) in 100mL of distilled water, prior tothe addition of 1g of Sulfanilic acid.

200 µL Griess reagent was added to 20 µL of tissue supernatants, then incubated for 20 min at room temperature. Absorbance at 490 nm was measured by micro plate reader. Nitrite concentration in the sample was calculated from standard curve of Sodium nitrite ($NaNO_2$) and expressed as µmol nitrite/mg protein.

3.12.7.3 Hydrogen Peroxide Generation

Hydrogen peroxide generation was measured based on FOX 1 method as described by (Ktari *et al.*, 2017).

FOX 1 reagent was prepared by the addition of 100µmol/L Xylenol Orange (SureChem Products Ltd; Suffolk, England) to 250mMol/L Ammonium Ferrous Sulphate (HAAFCO Scientific supplies Ltd), followed by the addition of 100 mMol/L Sorbitol (BDH Chemicals Ltd, England) and 25 mMol/L of 1M H_2SO_4. 50µL of sample and 950 µL of FOX 1 reagent were mixed and vortexed. Incubation for 30 min at room temperature was done to allow colour to develop. Absorbance was read at 560 nm.

3.12.7.4 Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase activity in the homogenates was quantified by measuring the inhibition of auto-oxidation of epinephrine (Adrenaline) at pH 10.2 and 30^{o}C (Marineli *et al.*, 2015). 50 mg of Adrenaline was dissolved in 100mL distilled water and acidified using 0.5mL concentrated HCl. This was to preserve the stability, by preventing the oxidation of epinephrine for up to four weeks. 30 µL of tissue homogenate, 2.5mL of 0.05M carbonate buffer (pH 10.2) and 300µL of 0.3mM Epinephrine were sequentially mixed. Absorbance at 480 nm was readat 30 sec interval for 150 sec. Changes were monitored and calculated as follows:

$$Change\ in\ absorbance\ per\ minute\ = \frac{A3 - A0}{2.5} \quad \text{--------- (Equation 3.19)}$$

Where, A_0 = absorbance after 0 seconds

A_3 = absorbance after 150 seconds

$$\%\ Inhibition\ = \frac{Increase\ in\ absorbance\ for\ substrate}{Increase\ in\ absorbance\ of\ blank}\ x\ 100 \quad \text{---------- (Equation 3.20)}$$

1 unit of SOD activity = the amount of SOD needed to achieve 50% inhibition of the oxidation of adrenaline to adreno-chrome within 1minute.

3.12.7.5 Determination of reduced glutathione (GSH) level

Reduced glutathione was determined according to the method described by Samarghandia *et al.* (2015). 0.5 mL of sample was mixed with 0.5 mL of 4%

Sulphosalicyclic acid (precipitating solution) (Rem Light Laboratories, Pvt Ltd, India). This mixture was centrifuged for 5 min at 4,000rpm. 4.5mL of Ellman's reagent (5′, 5′-Dithiobis- (2-nitrobenzoate) (Sigma-Aldrich Chemical, USA) was added to the supernatant in a test tube. Absorbance was read at 412 nm against blank which was prepared with 2mL of the 0.1M phosphate buffer, 3mL of diluted precipitating solution. 1mL of blank was added to 4.5mL Ellman's reagent. Absorbance was read within 5 minat 412 nm (Samarghandia *et al.,* 2015).

3.12.7.6 Determinaton of Glutathione-S-Transferase (GST) activity in tissues of rats

10 µL of sample and 140µL of 0.1 M Potassium phosphate buffer (pH 6.5) were mixed together, then 10 µL of GSH (Sigma chemical Co., London) and 50 µL of CDNB (1-Chloro,2,4-dinitrobenzene) (Sigma Aldrich chemical Co., London) were added respectively. Half plate is read first at 340nm then the other half at 405nm, setting blanks for comparison. The extinction coefficient of CDBN = 9.6 mm^{-1} cm^{-1}(Samarghandian *et al.*, 2015).

3.12.7.7 Glutathione Peroxidase (GPX) Determination

The following reagents were added sequentially in the same order: 0.05mL of phosphate buffer, 0.1mL of $NaNO_3$ (0.0325g $NaNO_3$ in 15mL of distilled water), 0.2 mL of GSH (0.123g GSH (Sigma-Aldrich Chemical, USA) dissolved in 10mL in phosphate buffer), 0.1mL of H_2O_2 (28uL H_2O_2 (Surechem Products Ltd.) in 100 mL of distilled water), 0.5 mL of sample, and 0.6mL of distilled water. Incubation of the entire mixture was done at 37^oC for 3min before the addition of 0.5mL. It was then centrifuged at 3,000rpm for 5min. 1mL of supernatant was mixed with 2mL of K_2PHO_4 (5.23g K_2HPO_4(Oxford Laboratory Unit, India) in 100mL of distilled water) and 1mL of DTNB (0.04g DTNB (Sigma-Aldrich Chemical, USA) in 100mL of phosphate buffer). Absorbance was read at 412nm(Al-Harbi, 2016).

1 unit of GPx activity = the amount of enzyme necessary to utilise 1nmol of NADPH/min at 25^oC.

3.12.7.8 Assessment of Lipid Peroxidation

This was assessed as described by Ogunmefun *et al.* (2015). 30 µl Tris-HCl buffer 0.1 M, pH 7.4, sample supernatant (0-100 µL) and 30 µL of 250 µM freshly prepared FeSO4 was mixed, followed by the addition of 100 µL of supernatant. This mixture was made up to 300 µL with distilled water and subsequently incubated for 2 hours at

37°C. 300 µL 8.1% Sodium dodecyl sulphate (SDS) was added for colour development.Then, 600 µL of acetic acid/HCl (pH 3.4) mixture and 600 µL of 0.8% Thiobarbituric acid (TBA) were further added. This mixture was then incubated for 1 hour at 100°C. Samples were cooled in ice for 10 min and subsequently centrifuged at 10,000 g for 10 min at 4 °C. The absorbance was read at 532 nm using a 96-well microplate against a reference blank of distilled water.

Working solution was prepared with 0.75% TBA, 30% Trichloroacetic acid (TCA) and NaOH (5%) at ratio of 1:100:100 (w:v:v).

Standard curve was obtained using malondialdehyde standard (MDA)(0.625–85 nmol MDA/mL). Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of 1.56 x 10^5 $M^{-1}Cm^{-1}$.

$$MDA\ (units\ per\ mg\ protein) = \frac{Absorbance\ x\ volume\ of mixture}{E532nm\ x\ volume\ of\ sample\ x\ mg\ protein}$$

(Equation 3.21)

3.13 Determination of glycemic index (GI) using Wistar rats

Glycemic index of provitamin A cassava hydrolysate was determined using twenty five male Wistar rats (120-150g) randomly and equally divided into 5 groups. The rats were acclimatised for 14days before commencement of the study. All the rats were administered with glucose (Sigma Aldrich, purity 96%) at a dose of 2g/kg body weight. Group 1 rats were administered the provitamin A cassava hydrolysates only, while groups 2 and 3 rats were administered provitamin A cassava hydrolysates with $2X10^{10}$ (PHF2) and $4X10^{10}$ CFU/ml (PHF4) of free *L. rhamnosus GG* respectively. Groups 4 and 5 rats were administered Provitamin A cassava hydrolysates with $2X10^{10}$ (PHE2) and $4X10^{10}$ CFU/g (PHE4) of encapsulated *L. rhamnosus GG* respectively.

3.13.1 Blood collection and determination of blood glucose level

The blood samples were collected by cutting the tip of the rat tail with the use of surgical blade. Blood glucose levels (BGL) were measured using Accu-Chek® glucometer before administration of glucose (0 min) and at 15, 30, 45, 60, 90 and 120 min post-administration of glucose for the Oral Glucose Tolerance Test (OGTT). The rats were administered Provitamin A cassava hydrolysates with or without *L. rhamnosus GG* respectively as described above. All five groups were monitored for further 120minutes at the intervals earlier mentioned.

3.13.2 Calculation of glycemic index

The Glycemic Index of the provitamin A cassava hydrolysates was calculated by plotting the blood glucose values of subject against time. The area under 2hours blood glucose response curves for glucose standard (glucose AUC) and for the test sample (provitamin A cassava hydrolysates) was then measured. Blood glucose curves were plotted from OGTT values for each individual subject. The incremental area under the curves (IAUC) were obtained for glucose standard and Provitamin A cassava hydrolysates in the same manner post-administration of each to reflect the total increase in blood glucose concentration. In the measurement of the incremental area under curve (IAUC), the peak areas below the baseline were ignored. This was obtained using GraphPad prisms® 5.0. The glycemic index value for the test sample was then calculated by dividing the incremental IAUC for the test sample by the incremental IAUC for glucose. The mean glycemic index value for the animals in each group was the final glycemic index value for each test sample.

GI was calculated by the formula:

$$\text{Glycemic Index} = \frac{\text{IAUC of test food}}{\text{IAUC of glucose standard}} \text{X } 100 \text{ ---------- (Equation 3.14)}$$

(Jenkins *et al.*, 1981).

3.14 Studies involving Human subjects

3.14.1 Ethical statement

Valid approval and ethical clearance with assigned number UI/EC/18/0158 were obtained from the UI/UCH Ethics Committee of the University of the University of Ibadan before the commencement of the experiment.

3.14.2 Determination of Glycemic Index using human subjects

10 healthy volunteers, within the age range of 15-35 years participated (5 males, 5 females). The volunteers were within the average range of body weight and size. The participants were tested for their fasting blood glucose levels after which they were administered a 50g available carbohydrate portion of white bread after an overnight fast. This was done in order to determine their oral glucose tolerance. The white bread served as the control with a classified glycemic index of 100. The provitamin A cassava hydrolysate was then administered and taken over 15 min. The time before oral administration commenced was designated as zero time. Blood samples were taken from warmed fingers by pricks using manual lancets at an interval of 0, 15, 30,

45, 60, 90 and 120min. The blood glucose concentrations were monitored by taking direct readings using an Accu-check glucometer and the glycemic index was calculated by the method of Jenkins *et al*. (1981). The values of blood glucose was plotted against time. The area under 2hours blood glucose response curve (AUC), for glucose and for the provitamin A hydrolysate were then measured.

GI wascalculated by the formula:

$$\text{Glycemic Index} = \frac{\text{AUC of test food}}{\text{AUC of reference glucose}} \text{X } 100$$

(Jenkins *et al.*, 1981).

3.15 Sensory analysis

3.15.1 Development of descriptors for product attributes

Panelists (50) comprising of students of the faculty of technology, University of Ibadan were recruited. Consensus vocabulary for taste, appearance, aroma,texture and flavor attributes for provitamin A cassava hydrolysates with and without *L. rhamnosus GG* beads were developed. The product attributes, their definitions and corresponding reference standards were developed according to the modified procedure of Krasaekoopt and Kitsawad (2010). The consensus terms used for the probiotic hydrolysates are shown in table 3.1.

Questionnaires on which the meaning of each product descriptor was explained was distributed and panelists were asked to read through the questionnaires to avoid any misinterpretation. Prior to evaluation, a session was held to familiarise panelists with the product. Water for mouth-rinsing in between tasting was also provided. The hydrolysates presented for evaluation did not contain sugar or any other artificial sweetener.

3.17.2 Evaluation of sensory attributes of provitamin A cassava hydrolysate with and without *L. rhamnosus GG*

Forty (40mL) of provitamin A cassava hydrolysate was served in polypropylene transparent cups which had been labeled with 3-lettered random alphabets. The prepared samples were evaluated for the specified attributes and overall acceptability using a 9-point hedonic scale where 9 represents “like extremely” and 1 represents “dislike extremely” (Larmond, 1977).

3.16 Statistical Analysis

Statistical analyses were performed using IBM SPSS 23.0 statistical package for windows (SPSS Inc., Chicago, USA) and GraphPad Prisms 5.0. Descriptive statistics (mean and standard deviation (sd) were computed. A test of one-way anova (analysis of variance) was employed for comparison of means difference among test groups. Means were separated, when necessary using Duncan's multiple range test and significance was accepted at probability $p < 0.05$. Any two means not marked by the same superscript (e.g. ^{a} and ^{b} or ^{b} and ^{c} within rows) are significantly different ($\alpha_{0.05}$). Any two means marked by the same superscript (e.g. ^{a} and ^{a} or ^{b} and ^{b} within rows) are not significantly different ($\alpha_{0.05}$) (Li *et al.*, 2009).

Two-tailed Pearson correlation test was conducted to determine the correlations among variables. All results are expressed as mean ±sd and (range) (Oloyo, 2001).

Table 3.1: Product descriptors, meaning and imaginary reference used forsensory evaluation.

Product descriptors	Meaning	Imaginary reference
Yellow colour (9=extremely yellow; 1= extremely colourless)	Degree to which the sample is visually deep yellow	Ripe mango
Sweetness	Fundamental sweet taste	Sugar
Sourness	Fundamental sour taste	Lime

Swallow-ability	Ease of swallow of sample	Water
Texture/ mouth feel	Thickness in the mouth	
Off flavor	Degree of closeness to a strongly fermented drink	Strongly fermented palm wine
After taste/feel	Particles remaining in the mouth after swallowing	Particles
Cloudiness (1= extremely clear; 9= extremely turbid)	Degree of clarity of the sample	Milk
Overall acceptability	General likeness for the sample	Based on individual preference

Source: Adapted from Rao (2014).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Encapsulation of *L. rhamnosus GG.*

4.1.1 Properties of calcium-alginate beads

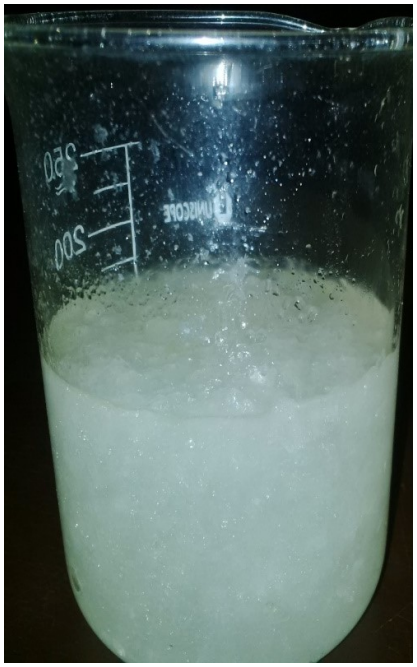
The sizes of microspheres are shown in Table 4.1. The microspheres produced by extrusion had a mean diameter of 2.37±0.33 mm and a mean weight of 0.021±0.007 g. Microspheres produced using the emulsion technique had a mean diameter of 2.41±0.33 mm and a mean weight of 0.032±0.014 g. Encapsulation efficiency in microspheres produced by extrusion was 92.15% while microspheres produced by emulsion had encapsulation efficiency of 89.14%.

The images of shape and surface morphologies of microspheres captured using light microscope are presented in Figures 4.1-4.2. The microspheres produced using emulsion method were spherical in shape and the surface of microspheres had cracks giving a rough appearance. Surface morphology of microspheres produced by extrusion showed a dense and more uniform surface layer compared with microspheres produced by emulsion. The visible cracks that were observed could be as a result of syneresis which is essential to loss of water (Paques *et al.*, 2014, Puguan *et al.*, 2014) that occurs during bead maturation. Patel *et al.* (2017) stated that syneresis could occur as an effect of cross linking cation as well as concentration of alginate on the de-swelling of particles, leading to loss of water.

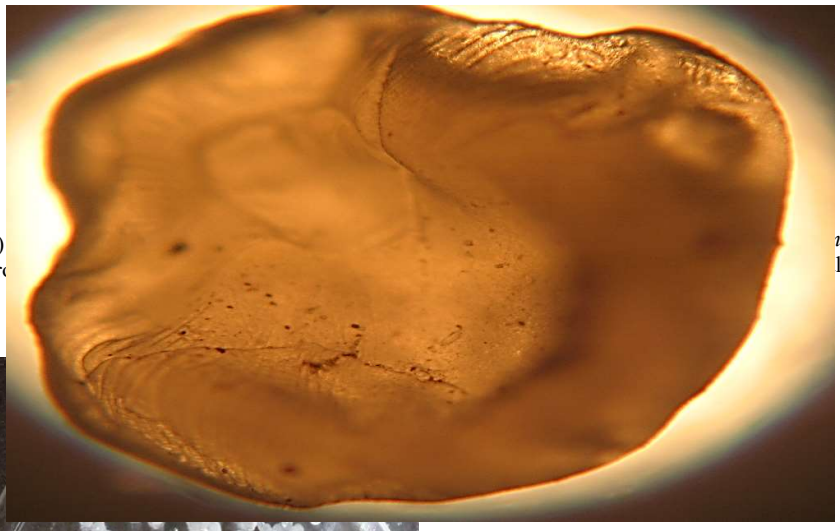
The shape of microspheres produced using emulsion technique was also spherical but it had a wrinkled layer on the surface and centre structure appeared loose. It was observed that a wider particle size was produced by the emulsion technique in contrast to the extrusion technique where the alginate-cell mixture was dropped directly into the hardening agent with a nozzle. The spherical shape of microspheres produced using emulsion technique were irregular and they were relatively larger in sizes than beads produced by extrusion technique. The larger size of microspheres produced by emulsion could be due to the effect of homogenisation on the size of beads

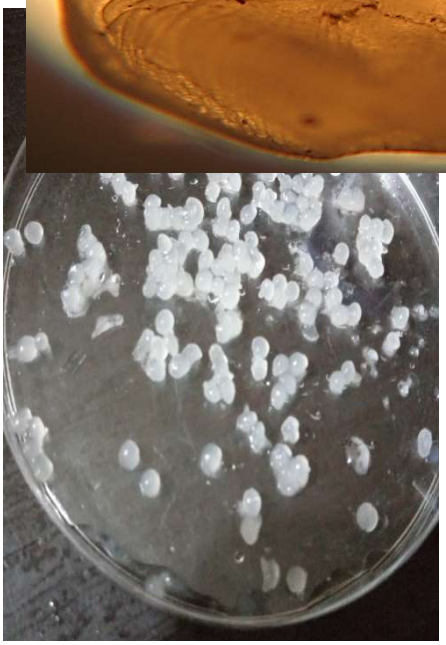
Table 4.1: Properties of *L. rhamnosus GG* microspheres

Properties	Microspheres by Emulsion	Microspheres by Extrusion
Diameter of Microspheres (mm)	2.41±0.33	2.37±0.38
Weight of Microspheres(g)	0.03±0.014	0.02±0.007
Recovered viable cells (Log CFU/g)	8.91	9.21
Initial Loading capacity (Log CFU/ml)	10	10
Encapsulation Efficiency (%)	89.14	92.15



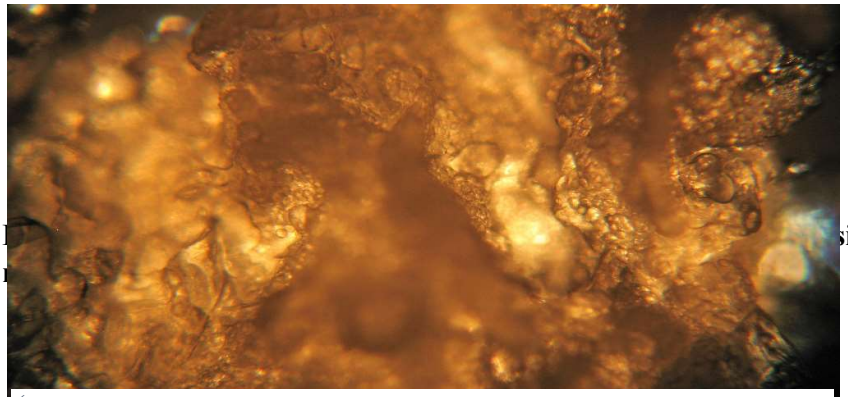
(i) *nosus GG* microspheres
pr lsion method





-

(iii). Ca-alginate microspheres of *L. rhamnosus GG* cells



sion and emulsion

(i). External structure of Ca-alginate encapsulated *L. rhamnosus GG* produced by

Figure 4.2:External structure of Ca-alginate encapsulated *L. rhamnosus GG* produced by extrusion and emulsion methods as viewed under the Light microscope.

(Heidebach *et al.*, 2009). Capela *et al.* (2007) studied the effect of homogenisation on the sizes of bead produced as well as the viability of encapsulated probiotic bacteria. Various strains of probiotics were encapsulated by emulsion technique using 3% alginate. The report was an unacceptably wide distribution in sizes of calcium alginate

beads lacking uniformity in their diameters (Capela *et al.*, 2007, Heidebach *et al.*, 2009). In food system applications, one of the most important characteristics of probiotic beads is their average diameter (Ding and Shah, 2008). Since it is expected to remain in the food product and be consumed with it, sizes should be well controlled as this may influence the consumer acceptability of samples into which the probiotic beads are incorporated. While larger capsule sizes may negatively affect consumer acceptability, capsule sizes that are too low may not be able to withstand the mechanical effects of expanding cell biomass during a prolonged fermentation process (Heidebach *et al.*, 2012). Heidebach *et al.* (2012) stated that microspheres in the range of 1-3mm are preferable since particle size influences the texture of foods as it determines the dispersion in foods. Hence, the sensory properties. The sizes of microspheres also have an indirect impact on the viability of probiotics as it determines the resistance of organisms to harsh environmental stresses (El-Salam and El-Shibiny, 2015). The shape of capsules determines their flow properties, which is important in industrial processes (Rokka and Rantamäki, 2010).

Encapsulation efficiency in microspheres produced by extrusion was higher than those produced by emulsion technique. The process of encapsulation of *L. rhamnosus GG* conferred protection around the live microorganisms by creating a microenvironment which offered a barrier which physically protects them against adverse conditions of the environment. The use of calcium chloride was employed as hardening agent in order to achieve a stronger cross-linking of the structure of the microcapsules produced (Patel *et al.*, 2017).

Encapsulation efficiency seems to be directly dependent upon the abilityof microspheres to entrap a good percentage of probiotic cells and prevention of leakage of cells into the medium surrounding the cells which could result into cell loss. This leakage could either be due to expanding biomass within the wall material or mechanical instability of the microsphere due to an inadequate barrier formation (Heidebach *et al.*, 2012). However, both types of microspheres produced in this study had remarkable encapsulation efficiencies. A high initial encapsulation efficiency of microspheres is necessary to attain the required therapeutic minimum of probiotics in the food product to which the microspheres are added (Heidebach *et al.*, 2012).

4.1.2 Effect of microspheres stored at 4°C on the viability of *L. rhamnosus GG*

The viability of *L. rhamnosus GG* in microspheres stored at 4°C for 7 days is presented in Figure 4.3. The initial viable cell counts of *L. rhamnosus GG* cells in the extruded and emulsion microspheres were 9.34 Log CFU/g and 9.27 Log CFU/g respectively. The viable cell counts of *L. rhamnosus GG* reduced to 8.35 Log CFU/g and 7.24 Log CFU/g respectively by the end of the 7days storage at 4°C. This translated to 10.60 % and 21.90% reduction in viable counts of *L. rhamnosus GG* cells in the microspheres produced by extrusion and emulsion respectively. This observed viability is directly related to the stability of the microspheres (El-Salam and El-Shibiny, 2015). This observation showed that microspheres produced by extrusion had higher stability at 4°C compared to those produced by emulsion technique.

4.2 Effect of microencapsulation on the survival of probiotic cells in simulated gastric and intestinal conditions

Figure 4.4 presents the rate of survival of *L. rhamnosus GG* cells incubated sequentially in simulated gastric juice and simulated intestinal juice. The rates of survival of free *L. rhamnosus GG* cells after 30, 60, 90 and 120 minutes resident times in simulated gastric juice were 79.6, 53.6, 33.6 and 6.53% respectively, while the survival rates of the encapsulated *L. rhamnosus GG* cells were 95.42%, 93.26, 83.56 and 77.36% respectively at the same resident times. After 60 minutes resident time in Simulated Intestinal Juice, the rate of survival of encapsulated *L. rhamnosus GG* cells reduced to 31.21% while the viable free *L. rhamnosus GG* cells was 1.22%. However, by the end of 120 minutes resident time in SIJ there was no observed viable free cell at all while encapsulated cells had 8.53% viable cells.

The rate of release of encapsulated *L. rhamnosus GG* cells from microspheres during incubation in simulated gastric and intestinal conditions is presented in Figure 4.5. The *L. rhamnosus GG* cells released in SGJ was 1.21, 3.93, 7.99 and 13.55% after 30, 60 and 120 minutes resident time respectively in SGJ while 2.37, 8.03, 13.95 and 23.95% of *L. rhamnosus GG* cells were released from microspheres after 30, 60 and 120 minutes resident time respectively during incubation in SIJ.

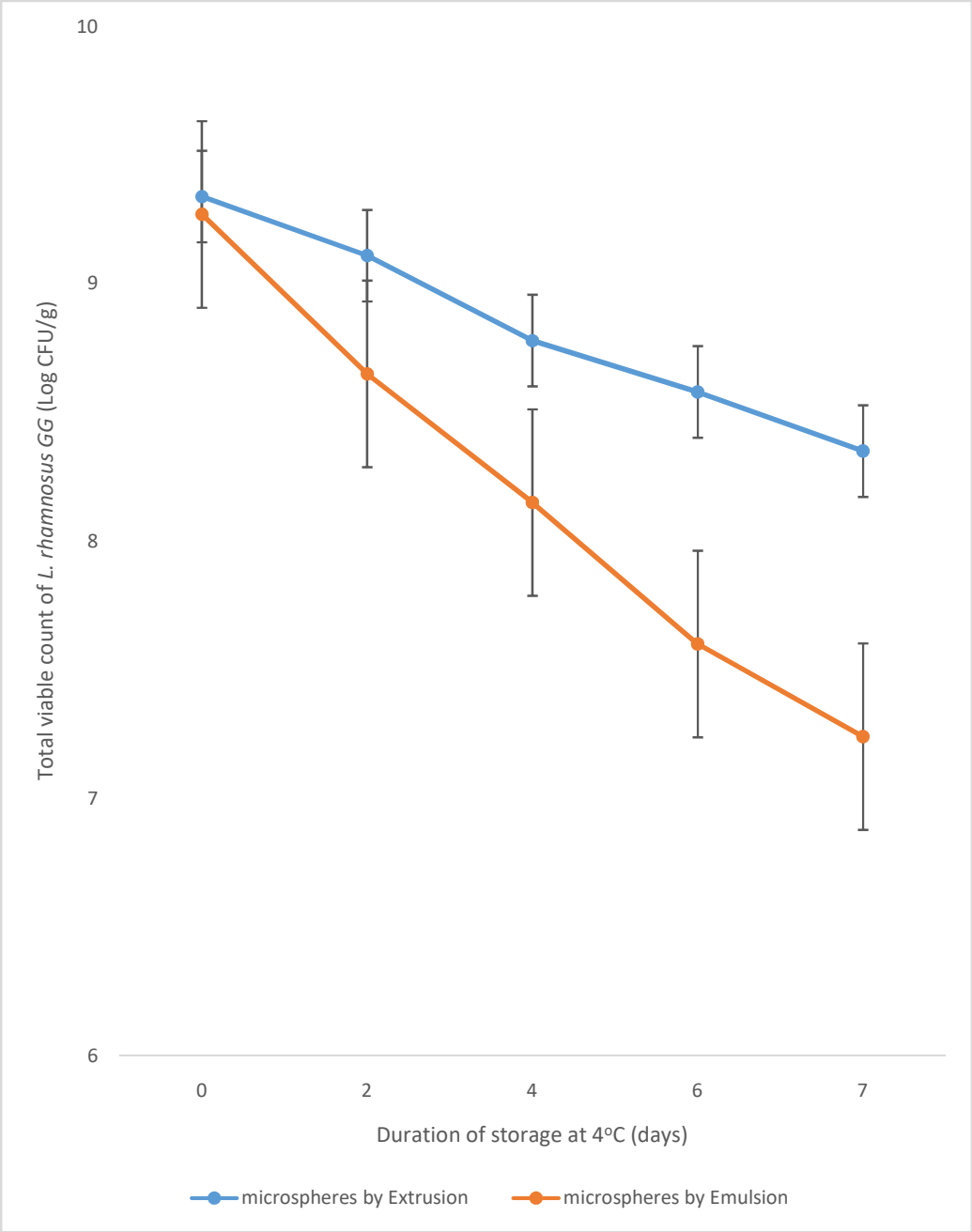


Figure 4.3: Stability of *L. rhamnosus GG*microspheres stored at 4°C for 7days

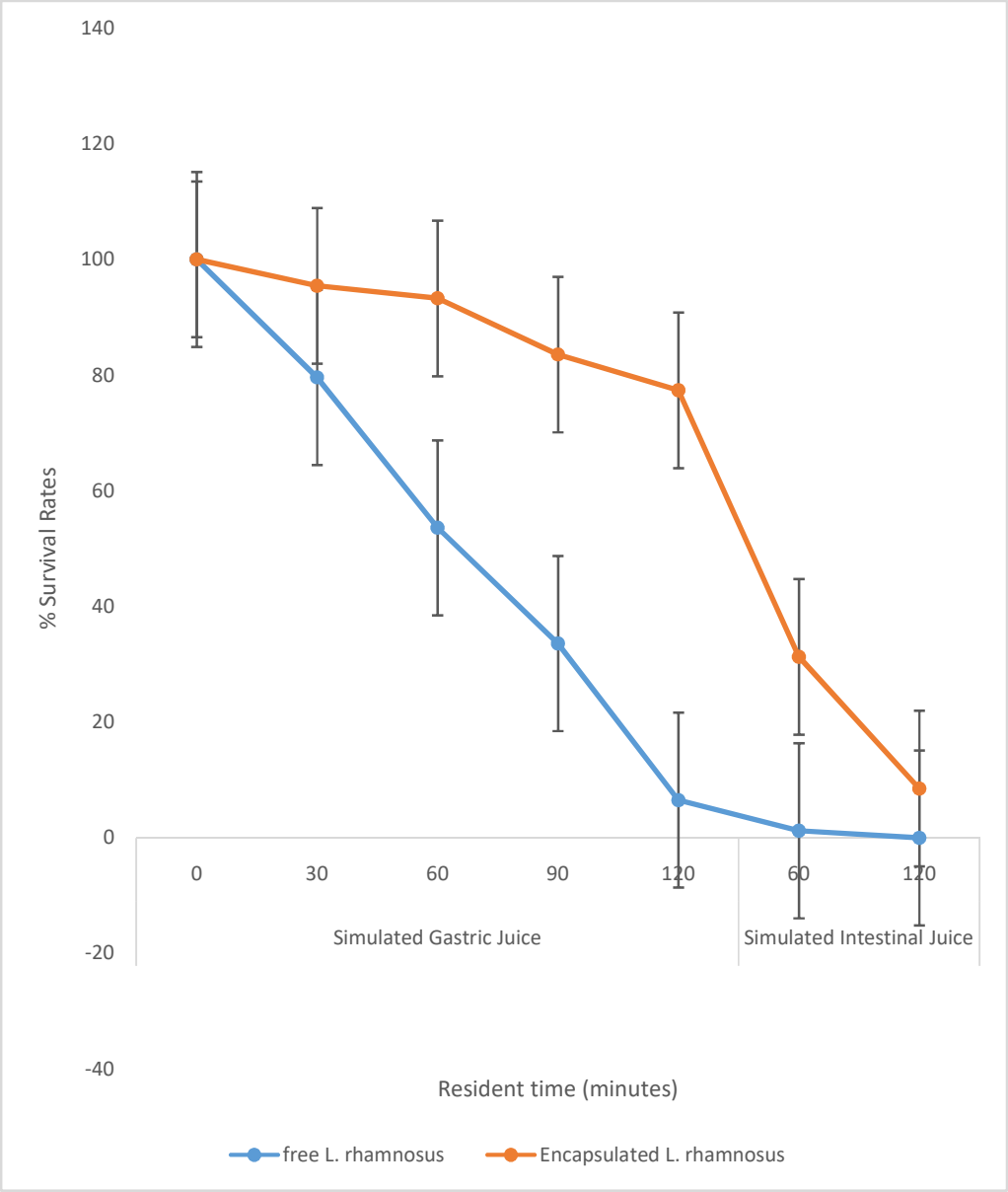


Figure 4.4: Rate of survival of *L. rhamnosus GG*cells during sequential incubation in simulated gastric and intestinal juice

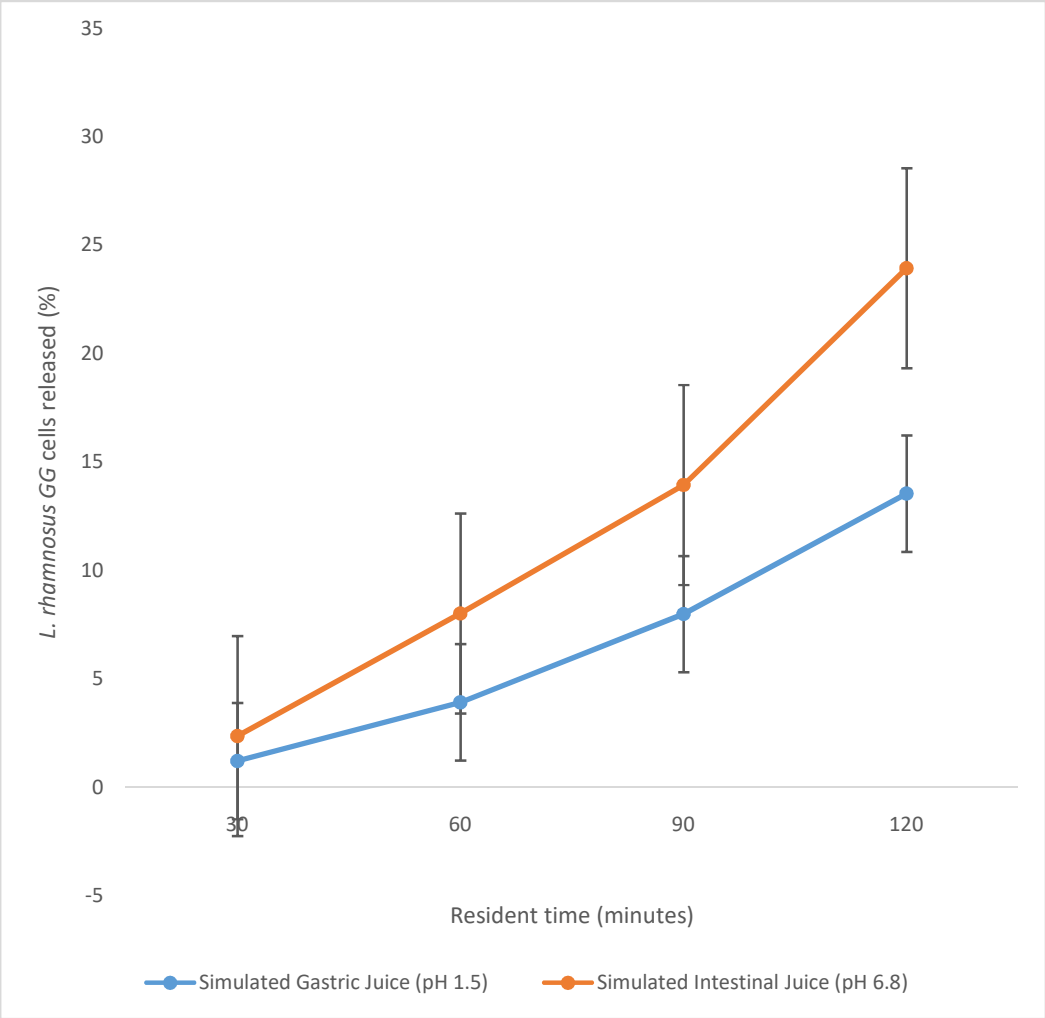


Figure 4.5: Rate of cell release of encapsulated*L. rhamnosus GG* cells during simultaneous incubation in simulated Gastro-intestinal conditions.

The ability of the free and encapsulated *L. rhamnosus GG* cells to maintain viability while passing through the gastro-intestinal tract after oral administration is deduced from the viability maintained during exposure of the free and encapsulated *L. rhamnosus GG*cells to simulated gastric and intestinal juices (Cook *et al.*, 2012). Free *L. rhamnosus GG* exhibited a more rapid decrease in viability compared to the encapsulated cells. The lower reduction in the survival rates observed between 0 and 120 minutes of exposure of the encapsulated cells to simulated gastric juice at pH 1.5 in comparison with the free cells means that the rates of survival of *L. rhamnosus GG* observed in encapsulated cells were higher than that of free *L. rhamnosus GG* cells. This could be due to the fact that encapsulation of *L. rhamnosus GG* protected the cells sufficiently and prevented cell destruction during exposure to low pH (Chávarri *et al.*, 2010). The human gastric environment is characterised by a very low pH which could lead to a considerable reduction of *L. rhamnosus GG* cell viability during transit through the human gastrointestinal environment. Encapsulation of *L. rhamnosus GG* thus enhanced the viability of the organisms compared to the free *L. rhamnosus GG* cells under similar conditions at pH 1.5. This observation is consistent with the findings of Yazhini *et al.*(2017). Therefore, for microencapsulation of probiotics to be meaningful, the probiotics must be able to withstand the strong acidic conditions during transit through the human gastric environment (Chávarri *et al.*, 2010). Encapsulated *L. rhamnosus GG* cells resisted the simulated gastric conditions better than the free cells. This was evident in the higher percentage of viable cells after 120 minutes of exposure to simulated gastric juice. Free *L. rhamnosus GG* cells could not withstand the harsh acidic condition (pH 1.5) due to the absence of protective barriers hence the lower survival rate. The fact that a higher survival rate was maintained by the encapsulated cells during exposure to gastric conditions is an advantage which means that high number of the probiotic cells would be able to reach the colon (Cook *et al.*, 2012). The rate of cell loss was observed to be more rapid after the cells were transferred to simulated intestinal juice (SIJ).

The rate of cell release of *L. rhamnosus GG* cells from microspheres during gastro-intestinal transit showed that the amount of cells released from the microspheres (indicated by the decline in enumerated cells) during their exposure to SGJ were minimal. Hence the higher survival rates previously observed. However, after the samples were transferred from SGJ to SIJ, *L. rhamnosus GG* cells were observed to be

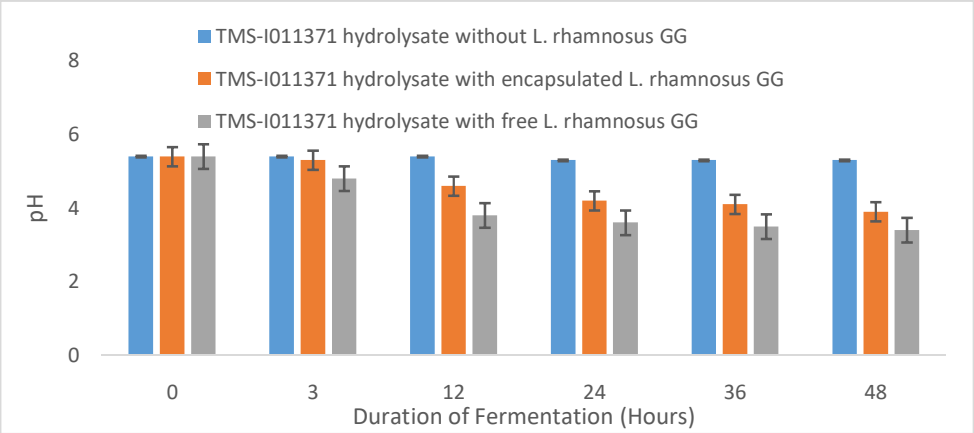
released at a faster rate, thus a larger amount of cells were released into the harsh environment and this resulted in higher rate of cell loss. Bajpai and Sharma, (2004) treated beads with simulated gastric and intestinal juices and also observed a higher rate of cell release with the explanation that the alginate component tends to undergo acid catalyzed hydrolysis when beads were treated with SIJ. This hydrolysis was attributed to the conversion of $–COO^-$ groups into –COOH groups, leading to the disappearance of the electrostatic attraction between the Ca^{2+} and –COO groups (Bajpai and Sharma, 2004, Mazumder and Bera, 2013) and a rapid disintegration of the beads start to occur. This offers explanation to why the release rate and amount of *L. rhamnosus GG* cells were higher in SIJ than in SGJ suggesting that *L. rhamnosus GG* cells could be released continuously from the beads in the gastro-intestinal tract.

The survival of cells within the period when they were exposed to simulated intestinal conditions in the presence of 0.3% bile salt showed that the loss of viability was not due solely to bile salt. Champagne and Gardner (2008) did not attribute the reported minimal loss of viability *L. rhamnosus* (and other probiotic strains tested) during a 2-hrincubation at 37^oC in the presence of 0.3% bile salt, to the effect of bile salt. It was explained that the loss of viability could be due to the prior pH stress experienced by the cells. The loss in viability of encapsulated cells could also be as a result of the disintegration of the protective beads around the *L. rhamnosus GG* cells thus exposing the cells to the unfavourable environmental conditions. Rayment *et al.* (2009) reported swelling and an eventual disintegration of Ca-alginate microspheres placed in intestinal solution and attributed the observation to an increase in the electrostatic forces of repulsion that could occur at pH above the acid dissociation constant (pKa) of the uronic acid group present in alginate.

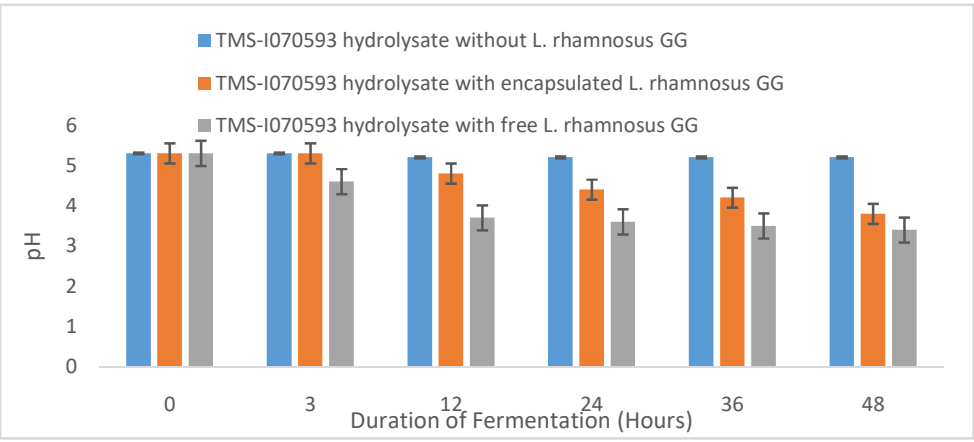
4.3 Changes in chemical attributes of provitamin A cassava hydrolysates containing free and encapsulated *L. rhamnosus GG*during fermentation

4.3.1 Changes in pH of probiotic provitamin A cassava hydrolysate

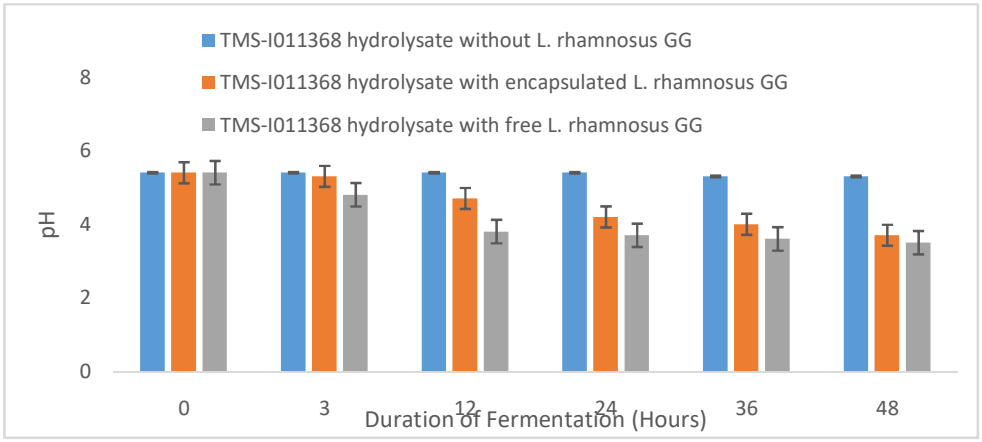
The changes in pH of provitamin A cassava hydrolysates are presented in Figure 4.6. The pH of provitamin A cassava hydrolysates with free *L. rhamnosus GG* reduced rapidly from an initial pH of 5.40±0.00 to 4.70±0.09, while that of the hydrolysate with encapsulated *L. rhamnosus GG* did not significantly change within the first 3 hours of fermentation. Further pH decrease to 3.70±0.10 was observed in the hydrolysates with



free *L. rhamnosus GG*, while that of the hydrolysates with encapsulated *L. rhamnosus GG* reduced from 5.40±0.00 to 4.70±0.10 between 3-12 hours of fermentation.



(b). Change in pH during fermentation of TMS-I070593 hydrolysate containing free and encapsulated *L. rhamnosus GG*.



(c). Change in pH during fermentation of TMS-I011368 hydrolysate containing free and encapsulated *L. rhamnosus GG*.

The reason for a more rapid decrease in the pH of the hydrolysate with encapsulated *L. rhamnosus GG* between 24 and 48

After 48 hours, the pH of the provitamin A hydrolysate maintained a constant value of 3.5.

A rapid reduction was observed in the pH of the provitamin A cassava hydrolysates with free *L. rhamnosus GG* contrary to the gradual but slower rate of pH reduction in the hydrolysate with encapsulated *L. rhamnosus GG* within the first 12 hours of fermentation. This could be attributed to the delayed interaction of the *L. rhamnosus GG* cells with the surrounding environment as a result of the organisms being in an immobilised state while the free cells were able to act on the hydrolysates immediately. However fermentation still progressed in the hydrolysates containing encapsulated cells, although at a gradual rate within the first 12 hours. The observed rapid drop in pH indicates a quick acidification rate owing to the production of lactic acid. A quick acidification rate is essential in preventing the growth of undesirable microorganism in the fermentation medium (Charalampopoulos *et al.*, 2002). Adebayo-Tayo and Stephanie (2016) stated that the activity of butyric acid bacteria can suppress the activity of lactic acid bacteria in a slowly acidified medium, resulting in spoilage. The decrease in pH was due to the metabolic activity of the probiotic *L. rhamnosus GG* (Adebayo-Tayo and Stephanie, 2016). This showed that *L. rhamnosus GG* was capable of producing lactic acid in the provitamin A cassava hydrolysate, thereby acidifying the medium. Further pH decrease observed was due to increased acidity owing to the continuous build-up of lactic acid in the provitamin A cassava hydrolysate. The rate of acidification which was indicated by the rate of pH reduction was slower in the free *L. rhamnosus* GG, while the encapsulated *L. rhamnosus GG* exhibited a more rapid rate of pH reduction between 36-48 hours. After the 48th hour, there was no further decrease below pH 3.5 in all the hydrolysate. This observation was accompanied by a minimal increase in lactic acid showing a diminished metabolic activity of the *L. rhamnosus GG* as a result of large amounts of lactic acid. This shows that the limiting pH of *L. rhamnosus GG* was achieved around pH 3.5 (Sivudu *et al.*, 2014). The growth limiting pH is the pH at which there is an imbalance between the intracellular pH and the pH outside the cells of the organism (extracellular pH) as a result of large amount of lactate, thus limiting cell growth and metabolic activities (Wang *et al.*, 2017, Chen *et al.*, 2019). This observation is consistent with Sivudu *et al*.(2014) who observed that *L. fermentum*

and *L. casei* maintained a stable pH in mixed watermelon and tomato juice during prolonged incubation up to 72 hours at 37^{o}C. Ostlie et al, 2005 also reported that *L.*

rhamnosus GG and L. reuteri CD2112 inoculated into milk maintained a stable pH during prolonged incubation at 37^{o}C from 24-48 hours (Sivudu *et al.*, 2014, Ostlie *et al.*, 2005). The same trend was observed for all the provitamin A cassava hydrolysate samples from the three varieties of provitamin A cassava used. Yoon *et al*. (2006) fermented cabbage juice with *L. casei, L. plantarum and L. delbreueckii* for up to 72hours and recorded no significant change from pH 3.4 in all the samples.

4.3.2 Changes in the rate of lactic acid production during fermentation

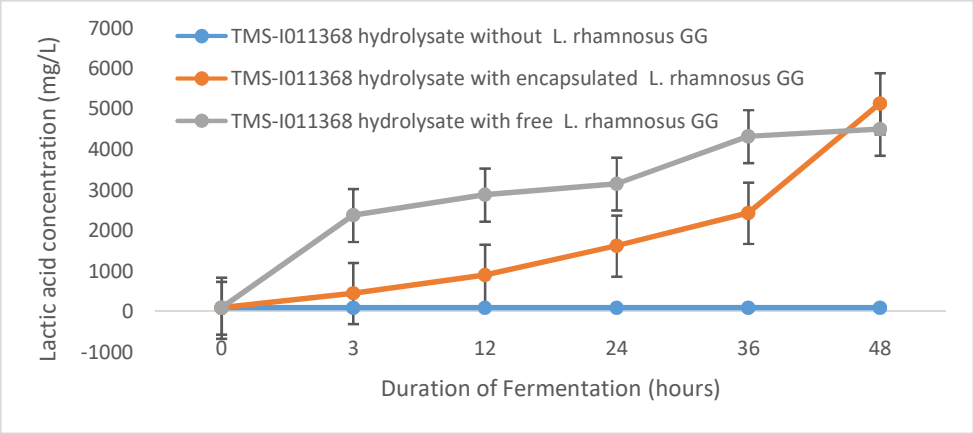
Figure 4.7 presents the rate of lactic acid production by free and encapsulated *L. rhamnosus GG* in TMS-I011368, TMS-I070593, and TMS-I011371 hydrolysates during fermentation. The concentration of lactic acid produced by the free *L. rhamnosus GG* in the TMS-I011371, TMS-I070593 and TMS-I011368 hydrolysates ranged between 1350±18.14 and 2376±20.01 mg/L respectively by the 3rd hour of fermentation. The encapsulated *L. rhamnosus GG* produced only 450 mg/L lactic acid within the same period in the hydrolysates. The lactic acid produced by the free cells by the 12th hour was in the range of 2880-3150 mg/L while the encapsulated cells produced lactic acid ranging from 720-900 mg/L within the same period. The lactic acid produced by the free *L. rhamnosus GG* increased to 3150-3330 mg/L while encapsulated *L. rhamnosus GG* produced only 1440 mg/L by the end of 24 hours. The lactic acid produced by the encapsulated *L. rhamnosus GG* between 36-48 hours ranged from 1980 to 5490 mg/L, while that of free cells increased at a slower rate in the range of 3420-4500 mg/L.

There was a significant increase in lactic acid production by both free and encapsulated cells during fermentation. However, the rate at which the free *L. rhamnosus GG* cells produced lactic acid was more rapid than that of the encapsulated cells. There was a slow but gradual increase in the lactic acid produced in the samples containing encapsulated cells during the first 36 hours. However, a notable increase in the lactic acid produced by the encapsulated cells was observed from 36-48 hours. The encapsulated cells exhibited a delayed activity in their ability to act on the fermentable sugars in the hydrolysate. This delayed activity of the *L. rhamnosus GG* cells could be explained by the immobilised state. A different pattern was observed in the samples containing the free cells. The free cells exhibited a consistent increase in lactic acid production up till 36 hours, after which the production began to decline. After 48 hours, there was a minimal and insignificant increase in lactic acid production for both

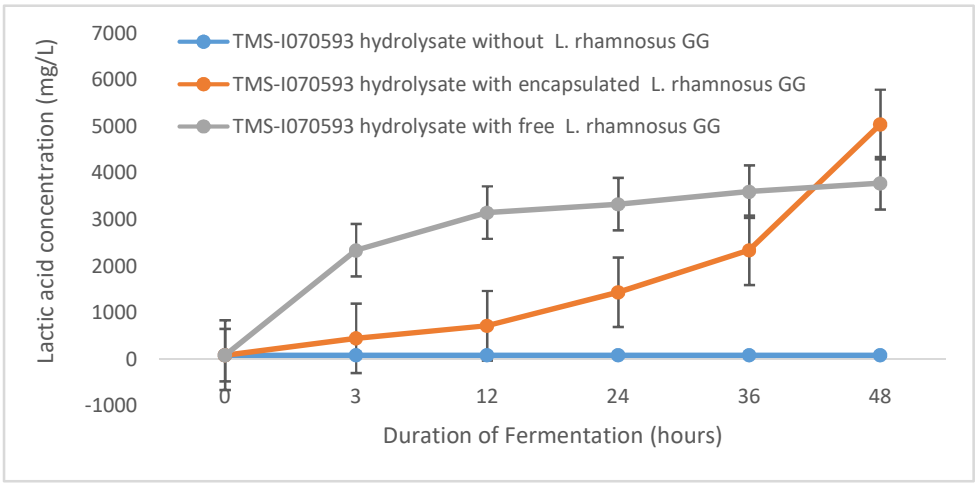
free cells andencapsulated cells. This coincided with an insignificant change in pH. The observed increase in lactic acid during the period of fermentation was accompanied by a simultaneous decrease in pH and reducing sugars (section 4.3.1 and 4.3.3) respectively.

The simultaneous decrease in reducing sugar content with increased production of lactic acid shows that the *L. rhamnosus GG* cells utilised the available sugars in the samples to produce lactic acid (MaiaCosta *et al.*, 2013, Tian *et al.*, 2017). This corroborates Panda *et al*.(2007) who also reported a reduction in pH with a simultaneous increase in lactic acid during fermentation of lacto juice produced from sweet potatoes using *L. plantarum.* The lactic acid continued to accumulate slowly until the time the available sugars in the samples were exhausted, then the rate at which lactic acid was being produced also reduced. This coincided with the 48hour of fermentation, after which the rate of lactic acid production by both free and encapsulated *L. rhamnosus GG* was minimal and insignificant. This is consistent with several existing literatures (Mousavi *et al.*, 2010, Chen *et al.*, 2019).

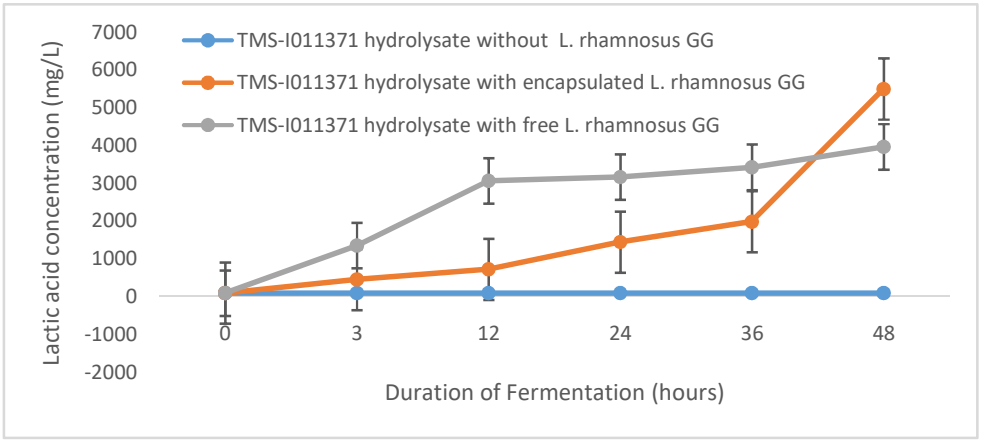
The optimum metabolic activities of *Lactobacilli*- which includes lactic acid production, depends on the presence of glucose in the fermenting medium, and a consistent gradient between the intracellular and extracellular pH (Corcoran *et al.*, 2005). The metabolic activities of *L. rhamnosus GG* could be inhibited at high concentrations of lactic acid. The presence of high lactate concentrations could induce a low intracellular pH in *Lactobacilli*(Ai *et al.*, 2017) as undissociated lactic and acetic acids can diffuse across the cell membrane thus lowering cytoplasmic pH (Amund, 2016). Glucose plays a role in homeostasis. When glucose is present in a low extracellular pH medium, the intracellular pH of *Lactobacilli* remains higher. However, when the intracellular pH reduces below the extracellular pH, cellular functions are inhibited and cell death may even occur (Corcoran *et al.*, 2005). This is why there was no significant production of lactic acid after 48 hours of fermentation. This observation suggests that the rate of lactic acid production was only significant at pH > 3.5.



(a). Changes in lactic acid content of TMS-I011368 hydrolysate containing encapsulated and free *L. rhamnosus GG* cells during fermentation



(b). Changes in lactic acid content of TMS-I070593 hydrolysates containing encapsulated and free *L. rhamnosus GG* cells during fermentation



(c). Changes in lactic acid production of TMS-I011371 hydrolysate containingencapsulated and free *L. rhamnosus GG* cells during fermentation

4.3.3 Effect of fermentation on the total sugar, reducing sugar total and soluble sugars (brix) content of provitamin A cassava hydrolysate

The effect of fermentation on the total sugar, reducing sugar and total soluble sugars (brix) content of provitamin A cassava hydrolysate are presented in Table 4.2.The concentration of reducing sugar in the hydrolysates with free *L. rhamnosus GG* reduced from the range of 17.61±0.15 and 21.20±0.12 g/100mL at the start of the fermentation period, to the range of 8.66±0.03 and 9.02±0.02 g/100mL at the end of 48 hours fermentation period. The reducing sugar of the hydrolysates with encapsulated *L. rhamnosus GG* reduced from the range of 17.61±0.15 - 21.20±0.12 g/100mL at the start of fermentation to 8.65±0.02 - 9.02±0.01 mg/100mL by the end of fermentation. Total sugar in all the samples generally decreased from between 52.88±1.00 and 58.20±1.21g/100mL before fermentation to between 51.68±1.09 and 52.28±1.10 g/100mL in the hydrolysate with free *L. rhamnosus GG* and between 42.24±2.22 and 51.68±1.09 g/100mL in the hydrolysate with encapsulated *L. rhamnosus GG* respectively after fermentation. Total soluble sugar also reduced from between 5.98±0.02 and 6.00±0.01 ^{o}Brix before fermentation to 5.62±0.01 - 5.71±0.02 in the hydrolysate with free *L. rhamnosus GG*, and 5.51±0.02 - 5.52±0.03 ^{o}Brix in the hydrolysate with encapsulated *L. rhamnosus GG* after fermentation.

The observed decrease in the reducing sugar, total sugar and total soluble sugar concentrations of provitamin A hydrolysate samples occurred along with reduction in pH and an increase in lactic acid production. All the reductions observed in the sugar content of the provitamin A cassava hydrolysate were due to their bioconversion by *L. rhamnosus GG* to lactic acid and their utilisation during cell synthesis (Okereke *et al.*, 2016). The simple sugars are utilised by *L. rhamnosus GG* for their metabolism. This is evident in the increase in cell numbers of *L. rhamnosus GG* cells up to the 36th hour of the fermentation period. Production of organic acids, which is mainly lactic acid is the main feature of fermentation by lactic acid bacteria. The organic acids are produced as a result of the degradation of sugars contained in the raw material thereby causing the pH to decrease. These observations of decreased pH, decreased reducing sugars and on the chemical increased acidity are corroborated by several authors who studied the effect

Table 4.2: The effect of fermentation on the total sugar, reducing sugar and total soluble sugars (^{o}Brix) content of provitamin A cassava hydrolysatecontaining free and encapsulated *L. rhamnosus GG*

	Stage of Determination	TMS-I070593 Hydrolysate	TMS-I011371 Hydrolysate	TMS-I011368 Hydrolysate
Reducing Sugar (g/100mL)				
	During hydrolysis	5.60±0.02	2.74±0.10	4.78±0.05
	Before Fermentation	21.20±0.12	17.61±0.15	18.80±0.11
	After Fermentation (with free *L. rhamnosus GG*)	8.66±0.03	8.69±0.01	9.02±0.02
	After Fermentation (with encapsulated *L. rhamnosus GG*)	8.65±0.02	8.68±0.01	9.02±0.01
Total Sugar (g/100mL)				
	During hydrolysis	32.91±0.21	33.45±0.42	34.21±0.02
	Before Fermentation	55.84±1.02	58.20±1.21	52.88±1.00
	After Fermentation (with free *L. rhamnosus GG*)	51.68±2.02	52.28±1.10	51.68±1.09
	After Fermentation (with encapsulated *L. rhamnosus GG*)	52.88±0.02	51.68±0.56	42.24±2.22
Total Soluble Sugar (^{o}Brix)				
	During hydrolysis	2.98±0.02	3.00±0.01	2.98±0.02
	Before Fermentation	6.00±0.01	5.98±0.02	5.98±0.02
	After Fermentation (with free *L. rhamnosus GG*)	5.62±0.01	5.70±0.02	5.71±0.02
	After Fermentation (with encapsulated cell)	5.52±0.03	5.51±0.02	5.52±0.02

of probiotics on the chemical properties of beverages from plant sources during fermentation (Panda *et al.*, 2007, Panda and Ray, 2007, Mousavi *et al.*, 2010). *L. rhamnosus GG* is a facultative heterofermentative bacterium that is capable of converting hexoses to L(+)-lactic acid and pentoses to a mixture of lactic acid and acetic acid (Valik *et al.*, 2008). This implies that *L. rhamnosus GG* utilises sugars to produce lactic acid in a fermentation medium; and as the amount of organic acids increase, the amounts of sugars in the medium decrease. Therefore, the amounts of organic acids in a fermentation medium is inversely proportional to the amount of sugars. By the time the available sugars are exhausted, metabolic activities of *L. rhamnosus GG* reduced, which was reflected in the reduced rate of lactic acid production. Corcoran *et al.*(2005) explained that *Lactobacilli* could exhibit reduced cellular activities and survival at lower pH, in the absence of glucose.

4.3.4 Dextrose equivalents of provitamin A cassava hydrolysates

The Dextrose equivalents (DE) of the provitamin A cassava hydrolysates is presented in Figure 4.8. The DE of TMS-I011371 hydrolysate increased from DE 7 after liquefaction to DE 34 after saccharification, while that of TMS-I070593 hydrolysate increased from 13 to 36 and TMS-I011368 increased from 12 to 36 after liquefaction and saccharification respectively.

Dextrose equivalent (DE) is the term which defines the degree of starch hydrolysis. The provitamin A cassava starch had a reduced dextrose equivalent (DE) before liquefaction. However, the DE of the hydrolysates increased significantly after saccharification which marks the end of complete starch hydrolysis process. This change in dextrose equivalent is directly dependent on the change in reducing sugar content as a proportion of the total sugar. The increase in reducing sugar content after hydrolysis/before fermentation (Table 4.2) therefore explains the increase in dextrose equivalent. The increase in dextrose equivalent was observed to influence the viscosity of the hydrolysates as well as the sensory properties. This is because the hydrolysates contain a higher percentage of saccharides (Dokic *et al.*, 2004).

4.4 Kinematic viscosity (mm^2/s) of provitamin A cassava hydrolysates

The kinematic viscosities of the provitamin A cassava hydrolysates are represented in figure 4.9. The kinematic viscosity of water was 1.00mm^2/s. The kinematic viscosities of the hydrolysates without *L. rhamnosus GG* ranged between 1.12 mm^2/s

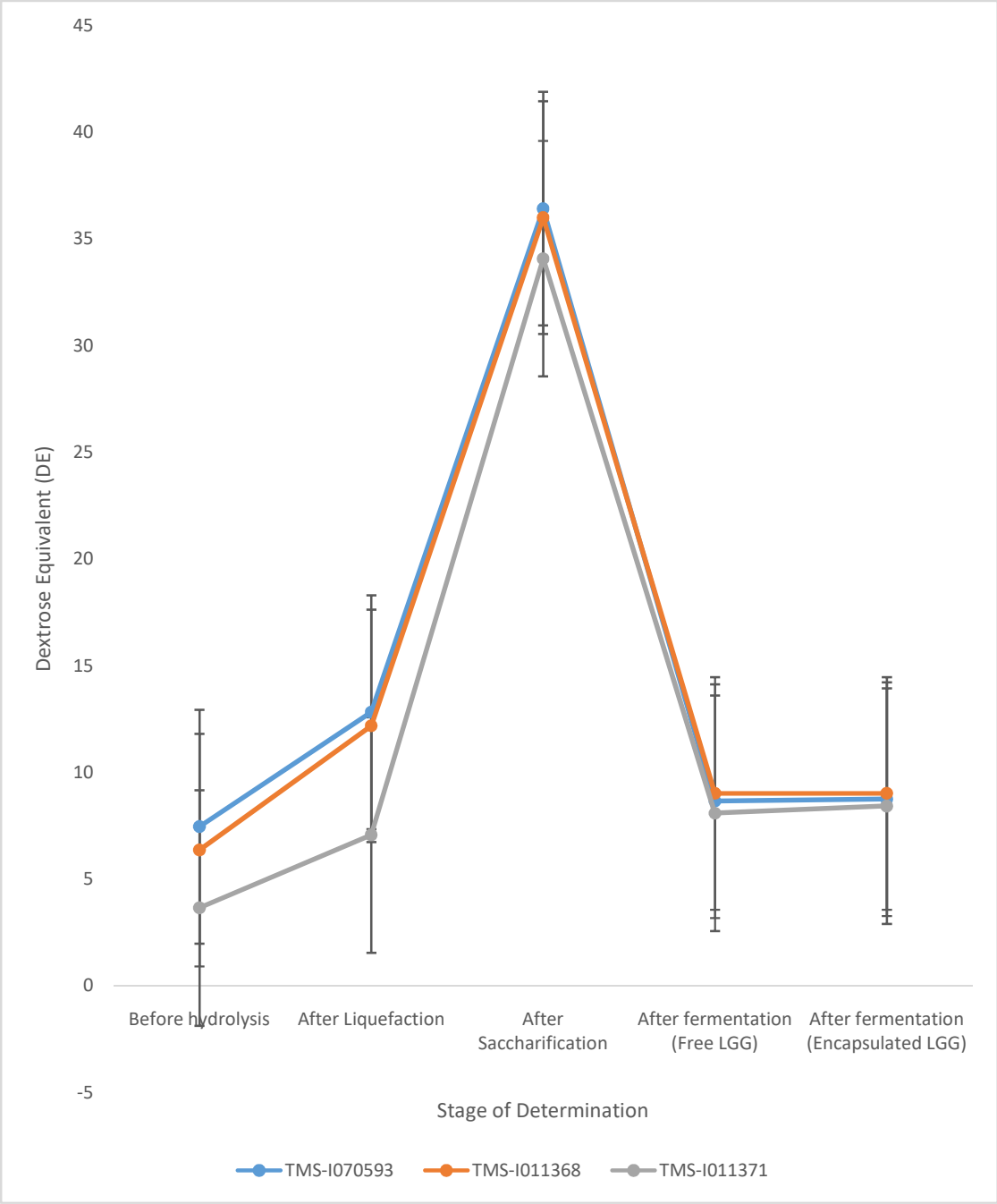


Figure 4.8: Dextrose Equivalent of Provitamin A cassava hydrolysatescontaining free and encapsulated *L. rhamnosus GG*

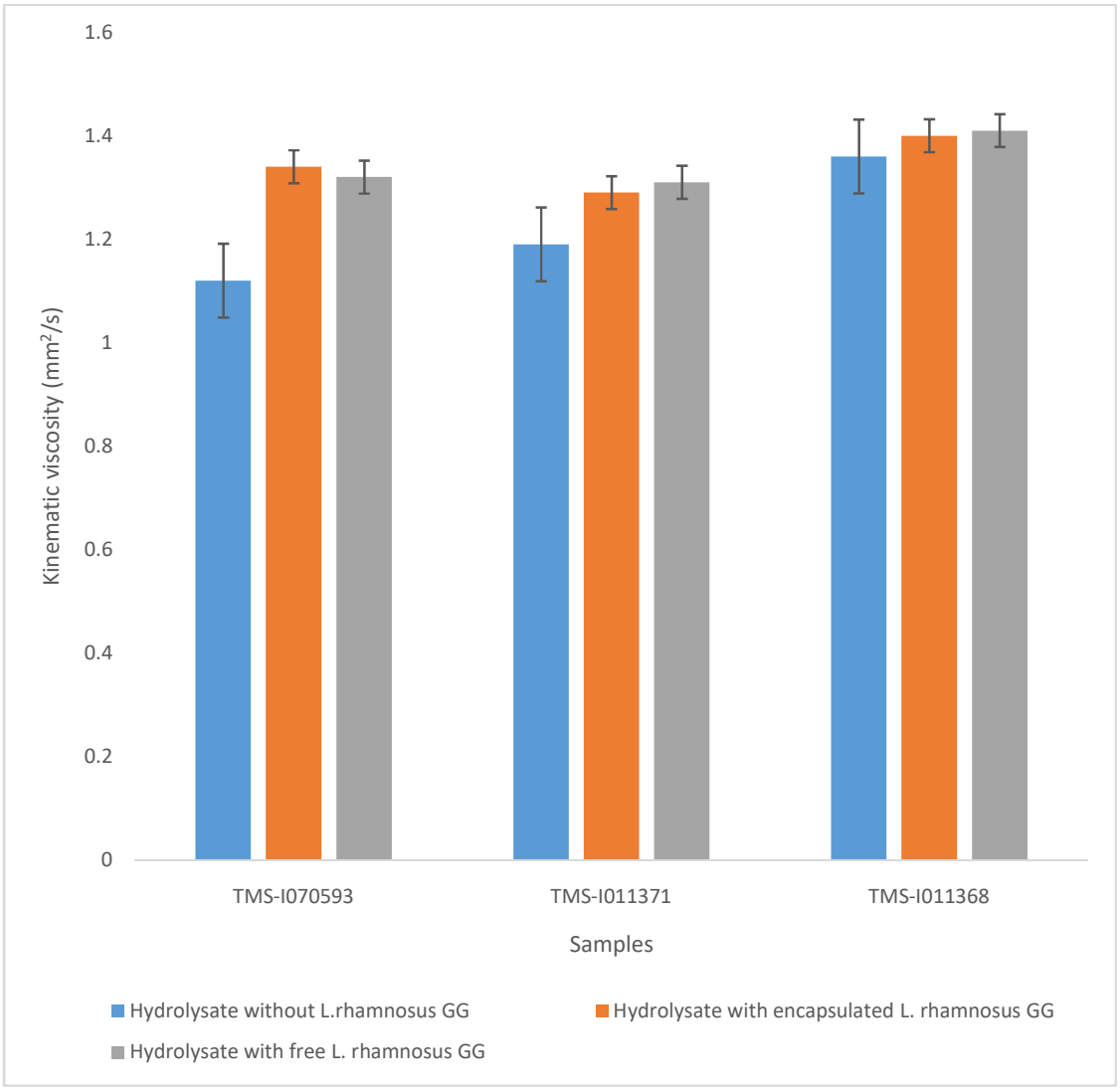


Figure 4.9: Kinematic viscosity (mm^2/s) of provitamin A cassava hydrolysatescontaining free and encapsulated *L. rhamnosus GG*

(TMS- I070593), and 1.36 mm^2/s (TMS-I011368). TMS-I011368 hydrolysates with free *L. rhamnosus GG* also had the highest kinematic viscosity (1.41 mm^2/s), followed by TMS-I011368 hydrolysates with encapsulated *L. rhamnosus GG* (1.40 mm^2/s), while the lowest kinematic viscosity was that of TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG.* The TMS-I011368 hydrolysate had viscosity that was higher than that of water in the range of 0.36-0.41mm^2/s while TMS-I011371 hydrolysates showed viscosities higher than that of water in the range of 0.19-0.31mm^2/s and TMS-I070593 hydrolysates were 0.12-0.34mm^2/s higher in kinematic viscosity than water at 20^oC.

The provitamin A hydrolysates had viscosities similar to that of water. Provitamin A cassava hydrolysates can be classified as a Newtonian fluid. This nearness in viscosity of the hydrolysates to that of water is attributable to the degree of hydrolysis of the samples as shown by the dextrose equivalents of the hydrolysates presented in Figure 4.8. Sun *et al.* (2010) stated that when the DE of starch hydrolysates is > 22, the viscosity of the sample is similar to distilled water since the relationship between DE and viscosity is linear or curvilinear. This is because the hydrolysates contain a higher percentage of saccharides that have a reduced degree of polymerisation and also a narrower distribution of the saccharides fraction (Dokic *et al.*, 2004). However, the varietal differences appears to influence the kinematic viscosities of the hydrolysates. Fermentation seemed to marginally increase the viscosity of the hydrolysate regardless of the variety. It was also observed that the samples containing free *L. rhamnosus GG* cells appeared to have the highest viscosity. This could be due to the increase in the biomass of the *L. rhamnosus GG* cells in the hydrolysates.

4.5 Mineral analysis

The results of mineral composition of provitamin A cassava hydrolysates are presented in Table 4.3. The provitamin A hydrolysates without *L. rhamnosus GG* had the lowest values of Fe, Na, K and Ca with values ranging from 1.19±0.07 mg/kg (TMS–I070593) to 1.36±0.02 mg/kg (TMS-I011368) for Fe, 46.63±0.56 mg/kg (TMS-I011368) to 56.93±0.12 mg/kg (TMS-I011371) for Na, 93.22±1.12 mg/kg (TMS-I070593) to 95.83±1.27 mg/kg (TMS-I011371) for K and 3.95±0.01 mg/kg (TMS-

I070593) to 5.27±0.26 mg/kg (TMS-I011371) for Ca. The highest values of Fe, Na, K and Ca

Table 4.3: Mineral elements composition of provitamin A cassava hydrolysates with and without *L. rhamnosus GG* (mg/kg)

	TMS-I011368 hydrolysate without *L. rhamnosus GG*	TMS-I011368 hydrolysate with free *L. rhamnosus GG*	TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*	TMS-I011371 hydrolysate without *L. rhamnosus GG*	TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*	TMS-I011371 hydrolysate with free *L. rhamnosus GG*	TMS-I070593 hydrolysate without *L. rhamnosus GG*	TMS-I070593 hydrolysate with encapsulated *L. rhamnosus GG*	TMS-I070593 hydrolysate with free *L. rhamnosus GG*	US Recommended daily Intake (mg/kg)	US Tolerable Upper Intake Limit (mg/kg)
Pb	0.35 ± 0.00^{a}	0.19 ± 0.00^{e}	0.31 ± 0.03^{abc}	0.32 ± 0.01^{abc}	0.27 ± 0.04^{cd}	0.24 ± 0.01^{d}	0.32 ± 0.02^{abc}	0.29 ± 0.03^{bcd}	0.33 ± 0.00^{ab}	NS	NS
Mn	0.43 ± 0.00^{b}	0.34 ± 0.00^{f}	0.42 ± 0.00^{b}	0.39 ± 0.00^{d}	0.36 ± 0.00^{e}	0.37 ± 0.00^{e}	0.52 ± 0.00^{a}	0.51 ± 0.01^{a}	0.41 ± 0.00^{c}	1.8-2.3	11**
Cu	0.03 ± 0.00^{d}	0.03 ± 0.01^{d}	0.11 ± 0.04^{a}	0.05 ± 0.00^{cd}	0.09 ± 0.00^{ab}	0.03 ± 0.03^{d}	0.08 ± 0.00^{abc}	0.06 ± 0.01^{bcd}	0.02 ± 0.00^{d}	0.9**	10**
Ni	9.97 ± 0.08^{c}	9.35 ± 0.01^{d}	5.70 ± 0.00^{f}	9.87 ± 0.00^{c}	8.05 ± 0.01^{e}	4.50 ± 0.01^{g}	13.04 ± 0.23^{b}	13.80 ± 0.01^{a}	4.50 ± 0.01^{g}	Not Specified	1000
Cd	0.08 ± 0.00^{bc}	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}	0.08 ± 0.00^{bc}	0.09 ± 0.00^{b}	0.02 ± 0.00^{d}	0.09 ± 0.01^{bc}	0.13 ± 0.00^{a}	0.07 ± 0.00^{c}	NS	NS
Zn	0.65 ± 0.01^{cd}	0.92 ± 0.03^{a}	0.72 ± 0.01^{b}	0.54 ± 0.01^{f}	0.66 ± 0.00^{c}	0.59 ± 0.03^{ef}	0.59 ± 0.03^{ef}	0.62 ± 0.04^{cde}	0.60 ± 0.01^{de}	8-11**	40**
Fe	1.36 ± 0.02^{c}	1.93 ± 0.07^{b}	1.43 ± 0.00^{c}	1.21 ± 0.07^{d}	2.29 ± 0.01^{a}	1.23 ± 0.02^{d}	1.19 ± 0.07^{d}	2.01 ± 0.00^{b}	1.38 ± 0.01^{d}	8-18**	45**
Na	46.63 ± 0.56^{g}	54.29 ± 0.01^{e}	61.39 ± 0.62^{c}	56.93 ± 0.12^{d}	64.99 ± 0.08^{b}	68.33 ± 0.07^{a}	48.15 ± 0.07^{f}	64.50 ± 0.73^{b}	56.53 ± 0.11^{d}	500-2400	<2400
K	93.90 ± 0.28^{g}	94.90 ± 0.81^{fg}	99.91 ± 0.63^{e}	95.83 ± 1.27^{f}	134.49 ± 0.87^{a}	125.86 ± 0.69^{b}	93.22 ± 1.12^{g}	110.01 ± 0.30^{c}	106.08 ± 0.23^{d}	1600-3500	3700
Ca	5.51 ± 0.10^{f}	8.30 ± 0.03^{b}	14.05 ± 0.01^{a}	5.27 ± 0.26^{f}	6.15 ± 0.01^{d}	7.57 ± 0.07^{c}	3.95 ± 0.01^{g}	8.35 ± 0.01^{b}	5.28 ± 0.06^{f}	700-1300*	2500*

*Source: (Ross *et al.*, 2011)

**Source: (Goldhaber, 2003)

*Means ±sd with the same alphabet superscripts in the same column are not significantly different $_{(\alpha 0.05)}$

*NS = Not Specified

observed were 1.43±0.00 mg/kg (TMS-I011368 hydrolysates with encapsulated *L. rhamnosus GG*), 68.33±0.07mg/kg (TMS-I011371 hydrolysate with encapsulated *L.rhamnosus GG*), 134.49±0.87mg/kg (TMS-I011371 with encapsulated *L. rhamnosus GG*) and 14.05±0.01 (TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*) respectively.

The hydrolysates with *L. rhamnosus GG* (fermented) were observed to have higher amounts of macro-elements than the hydrolysates without *L. rhamnosus GG* (unfermented). This could suggest that fermentation enhanced the available microelement content of provitamin A cassava hydrolysate. Adewusi *et al.* (1999) reported that fermentation improved the available mineral content of fermented cassava products in comparison with the unfermented. There was also an observed influence of encapsulation on the amount of macro-elements (sodium, potassium and calcium) present in the samples as there were higher amount present in the hydrolysates containing encapsulated *L. rhamnosus GG* than in the hydrolysates containing free *L. rhamnosus GG*. The addition of *L. rhamnosus GG* is responsible for the observed improvement in macro-elements. This could be due to the fact that macro-elements (such as Na, K, Mg, Ca and Fe) constitute 95% of the cellular mass of microorganisms and are responsible for cell synthesis and normal functioning of cellular structure (de Melo Pereira *et al.*, 2019). Therefore, fermentation with *L. rhamnosus GG* may have improved the available major elements. There was an observed variation in the amounts of trace elements in the provitamin A cassava hydrolysates, with no particular trend in the different varieties. (de Melo Pereira *et al.*, 2019) explained that elements such as Pb, Cu, Mn, Zn, Co, Cd, Mo, Ni, Cu, etc. are present in trace amounts in microbial cells, to function as cofactors for certain enzymes and in the regulation of certain metabolic pathways.

All the trace elements determined showed no particular pattern in the variation of their concentration relative to mode of fermentation or differences in variety. However, all these trace elements are present in all the hydrolysate samples within the safe range specified by the US tolerable upper intake limit (Table 4.3). Soetan *et al.* (2010) explained that the physiological responses that define toxicity or deficiencies of these mineral elements are broadly influenced by the different interferences and interrelationships in the metabolism and absorption of the different mineral elements.

The provitamin A cassava hydrolysates have low contents of mineral elements. The results further confirm that the amount of micronutrients in cassava and cassava products is low. This is because cassava naturally adapts easily to poor soils and growth conditions. The concentration of micronutrients in the soil determines the concentration of micronutrients in the cassava plants, cassava roots and hence the products (White and Broadley, 2009). However, consumption of a reasonable quantity of the provitamin A cassava hydrolysates will have a significant contribution to the recommended daily intake of these mineral elements.

4.6 Phytochemical and anti-nutrients of provitamin A cassava hydrolysates

4.6.1 Total flavonoid content, total phenolic content and total antioxidant capacity as measured by DPPH free radical scavenging activity

The total flavonoid, total phenolic and free radical scavenging activity of provitamin A cassava hydrolysate is presented in Table 4.4.

The highest total flavonoid and total phenolic content were 0.44±0.33 mg GAE/100mL and 2.23±0.06 mg QE/100mL observed in TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG* while the lowest were 0.25±0.02 mg GAE/100mL and 1.92±0.05 mg QE/100mL observed in TMS-I070593 hydrolysate with free *L. rhamnosus GG* and TMS-I011368 hydrolysate with free *L. rhamnosus GG* respectively. The total flavonoid and total phenolic content of the hydrolysate without *L. rhamnosus GG* rangedbetween 0.60±0.03 (TMS-I070593) and 0.78±0.01 (TMS-I011371) mg QE/100mL, and between 0.22±0.01 mg GAE/100mL (TMS-I070593) and 0.23±0.03 mg GAE/100mL (TMS-I011371) respectively.

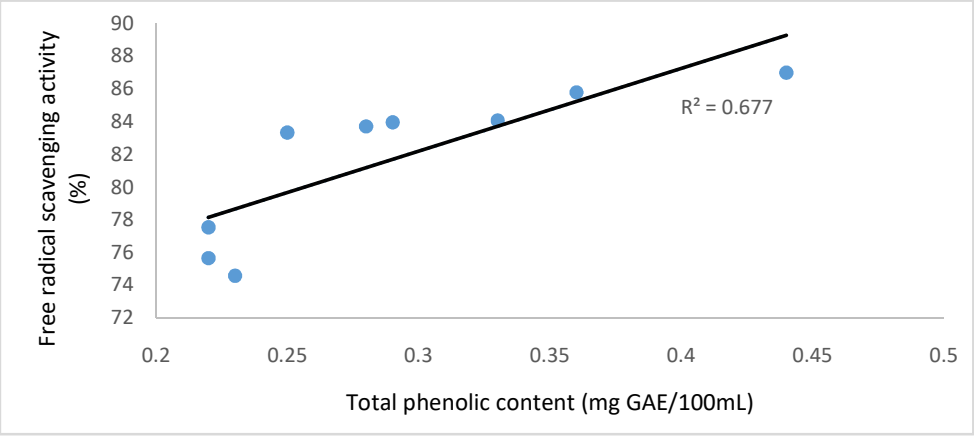
Free radical scavenging activity of the unfermented provitamin A cassava hydrolysate ranged from 74.56±2.44% (TMS-I011371) to 77.51±1.74% (TMS-I011368), while that of the fermented hydrolysate ranged from 83.30±1.75 (TMS-I011371 hydrolysate with free *L. rhamnosus GG*) to 86.97±1.77% (TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*).

The relationship between phenolic content and the free radical scavenging activity of provitamin A cassava hydrolysate is shown in Figure 4.10. The regression analysis shows that phenolic content contributes about 68% ($R^2 = 0.6776$), while total flavonoid contributes about 95% ($R^2 = 0.9462$) of the free radical scavenging activity measured in

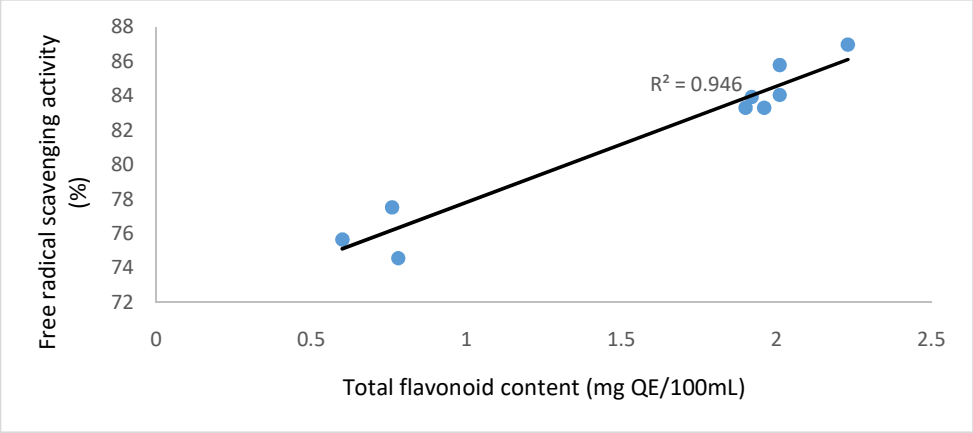
Table 4.4: Phytochemical, antioxidant capacity and carotenoid content of provitamin A cassava hydrolysates with and without *L. rhamnosus GG*

Samples	Total Phenolic content (mg GAE/100mL)	Total Flavonoids content (QE/100mL)	Free Radical Scavenging Activity (%)	Total Carotenoids (mg/g)	Beta Carotene (mg/g)	Beta carotene as % Total carotenoid
TMS-I070593 Hydrolysate without *L. rhamnosus GG*	0.22±0.02^{c}	0.60±0.03^{c}	75.65±3.10^{b}	0.19±0.01^{a}	0.05±0.01^{e}	26.26
TMS-I070593 Hydrolysate with Encapsulated *L. rhamnosus*	0.28±0.03^{bc}	1.96±0.02^{b}	83.70±3.13^{a}	0.11±0.04^{c}	0.07±0.01^{d}	63.63
TMS-I070593 Hydrolysate with Free *L. rhamnosus GG*	0.25±0.02^{c}	1.90±0.22^{b}	83.30±1.75^{a}	0.13±0.03^{b}	0.07±0.00^{d}	53.84
TMS-I011368 Hydrolysate without *L. rhamnosus GG*	0.22±0.01^{c}	0.76±0.03^{c}	77.51±1.74^{b}	0.20±0.08^{a}	0.08±0.01^{cd}	40
TMS-I011368 Hydrolysate with Encapsulated *L. rhamnosus GG*	0.36±0.10^{ab}	2.01±0.22^{ab}	85.78±4.25^{a}	0.18±0.00^{a}	0.17±0.01^{a}	94.44
TMS-I011368 Hydrolysate with Free *L. rhamnosus GG*	0.29±0.10^{bc}	1.92±0.05^{b}	83.93±3.10^{a}	0.19±0.00^{a}	0.11±0.00^{b}	57.89
TMS-I011371 Hydrolysate without *L. rhamnosus GG*	0.23±0.03^{c}	0.78±0.01^{c}	74.56±2.44^{b}	0.21±0.07^{a}	0.08±0.01^{d}	38.10
TMS-I011371 Hydrolysate with Encapsulated *L. rhamnosus GG*	0.44±0.03^{a}	2.23±0.06^{a}	86.97±1.77^{a}	0.20±0.01^{a}	0.11±0.00^{b}	55
TMS-I011371 Hydrolysate with Free *L. rhamnosus GG*	0.33±0.08^{bc}	2.01±0.24^{ab}	84.04±2.25^{a}	0.19±0.08^{a}	0.09±0.00^{c}	47.37

*Means ±sd with the same alphabet superscripts in the same column are not significantly different $_{(\alpha 0.05)}$



(a). Relationship between total phenolic content and free radical scavenging activity of provitamin A cassava hydrolysate



(b). Relationship between total flavonoid content and free radical scavenging activity of provitamin A cassava hydrolysate

the provitamin A cassava hydrolysates. There was an increase in the total flavonoid and total phenolic compounds in the hydrolysates containing *L. rhamnosus GG* (fermented)compared to the unfermented hydrolysate after fermentation. This increase was accompanied by an increase in the free radical scavenging activity as measured by DPPH. This suggests that lactic acid fermentation improved the phytochemical and bioactive compounds in the hydrolysate. This observation is consistent with other reports from the literature. Mantzourani *et al.* (2019) and Mousavi *et al.*(2013) reported an increase in the total phenolic content of pomegranate juice fermented with probiotics, and stated that lactic acid fermentation enhances the total phenolics content of juices from fruits, vegetables or other plant sources. This could be due to the fact that microorganisms can utilise the available carbon source for biosynthesis of alkaloids, terpenoids, flavonoids, etc. during fermentation (de Melo Pereira *et al.*, 2019). Kachouri *et al.* (2015) reported an increase in phenolic compounds in olive oil fermented with *L. plantarum,* attributing the increase to reductive depolymerisation of phenols to volatile phenol derivatives that are more soluble in the product. Filannino *et al.* (2015) also explained several mechanisms and pathways through which *Lactobacilli* can metabolise phenolic acids by decarboxylase or reductase activities, to corresponding derivatives leading to the production of multiple reduced phenolic compounds during fermentation. It was also noted that the samples fermented with encapsulated *L. rhamnosus GG* cellshad a more pronounced increase in phenolic content across all varieties. This could be due to protection of polyphenol compounds from degradation by physiological activities of the encapsulated *L. rhamnosus GG* (Li *et al.*, 2019).

Fermentation by *L. rhamnosus GG* was observed to improve the free radical scavenging activity in the samples. This is because *L. rhamnosus GG* has the ability to increase antioxidant levels and neutralise the effects initiated by reactive oxygen species (Wang *et al.*, 2017). Lactic acid bacteria has the ability to synthesise NADH oxidase/NADH peroxidase system which balances the NAD^+/NADH ratio and catalyzes the reduction of O_2 to H_2O_2 which is further decomposed to H_2O (Kachouri *et al.*, 2015). Thus, inhibiting the formation of oxygen radical that could be responsible for autoxidation of phenolic compounds. Nazzaro *et al.* (2008) reported an increase in the antioxidant activity of carrot juice inoculated with *L. rhamnosus* and *L. bulgaricus* but noted that better results were achieved with *L. rhamnosus.*Dordevic *et al.* (2010)

also reported an increase in the antioxidant activity and total phenolics upon fermentation of cereals with *L. rhamnosus* andyeast *Saccharomyces cerevisiae.* Hur *et al.* (2014) explained that an increase in antioxidant activity of plant-based foods can be due to the fact that the process of fermentation helps in the release or synthesis of antioxidant compounds such as phenolics and flavonoids. Probiotics can also synthesise various metabolites such as butyrate, glutathione and folate which have antioxidant capacity (Bujna *et al.*, 2018). Evidence has shown that selected strains of probiotics, including some strains of *Lactobacillus* and *Bifidobacteria* possess significant antioxidant properties both *in vitro* and *in vivo* (Amaretti *et al.*, 2013).

The regression analysis in this study shows a strong positive correlation between the total flavonoid and total phenolic content, and the free radical scavenging activity of provitamin A cassava hydrolysate as measured by DPPH. Yao *et al.* (2010) also reported an extremely significant positive correlation between the total flavonoid and total phenolic contents, and the antioxidant activity of celery as measured by DPPH.

4.6.2 Effect of fermentation on beta carotene content

The data showing the effect of fermentation on beta carotene is presented in Table 4.4.The total carotenoid content ranged between 0.19±0.05 and 0.21±0.07 mg/g, with beta carotene making up about 26 to 40% of the carotenoids in all the hydrolysate without *L. rhamnosus GG*. The total carotenoid content reduced to the range of 0.11±0.04 and 0.20±0.01 mg/g in all the provitamin A cassava hydrolysate with *L. rhamnosus GG.* Percentageβ-carotene retention ranged from 47.37% (TMS-I011371 hydrolysate with free *L. rhamnosus GG*) to 94.44% (TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*). TMS-I070593hydrolysate with free *L. rhamnosus GG* was observed to have the lowest concentration of beta carotene and total carotenoid among all the fermented samples. Beta carotene retention in the hydrolysates with respect to the beta carotene content of the raw cassava roots is presented in Figure 4.11. TMS-I011368 hydrolysate was observed to have the highest ($\alpha_{0.05}$) beta carotene retention ranging between 43.13% (hydrolysate without *L. rhamnosus GG*) and 91.64% (hydrolysate with encapsulated *L. rhamnosus GG*), followed by the TMS-I011371 hydrolysate (58.10 – 79.88%) and TMS-I070593 (36.39 ─ 39.85%) respectively. Although, the TMS-I070593 variety had the highest beta carotene content of 177mg/kg and a deeper yellow tone in the fresh root, it showed the least beta retention.

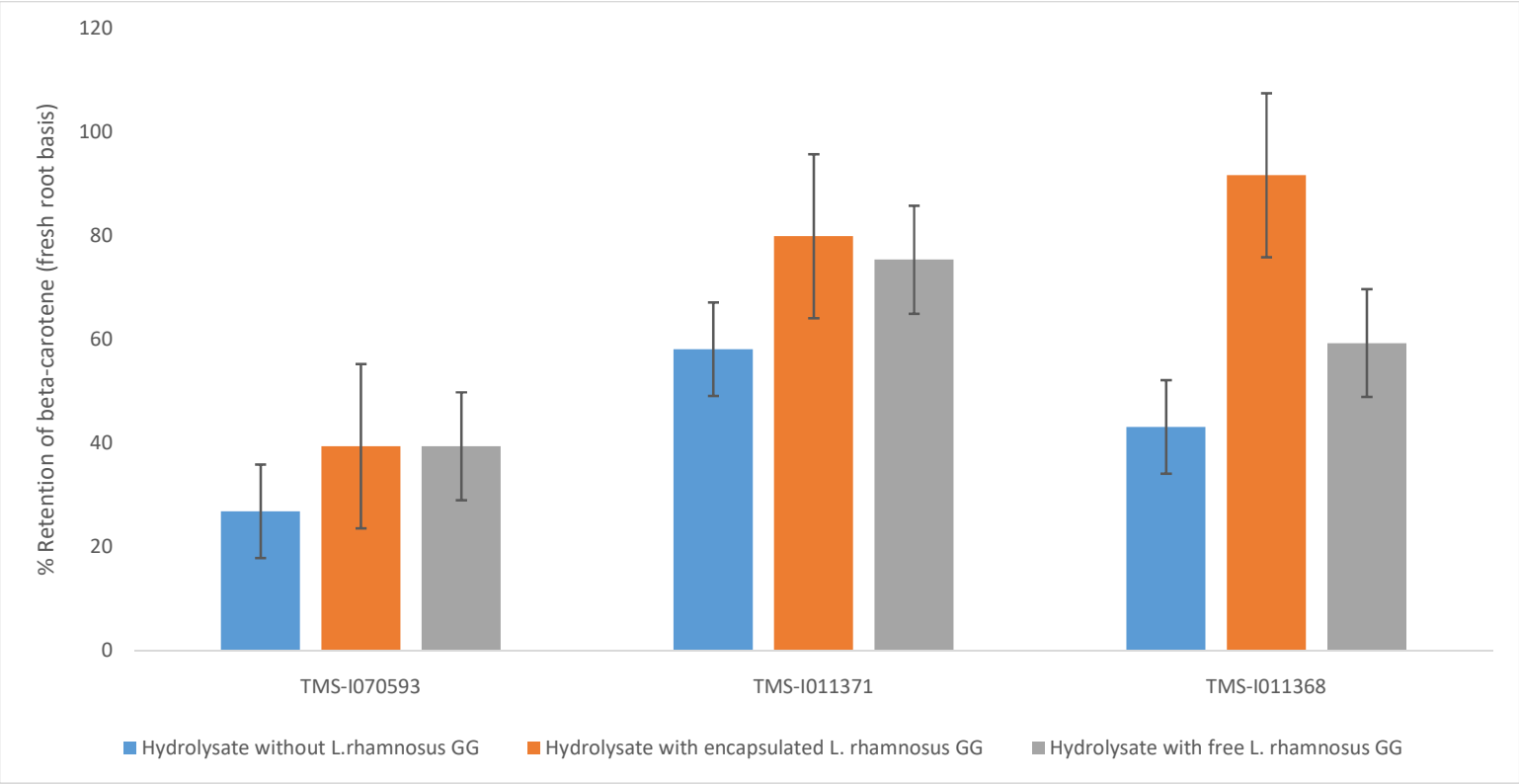


Figure 4.11: Rate of Beta carotene retention in the provitamin A cassava hydrolysates containing free and encapsulated*L. rhamnosus GG*with respect to fresh cassava roots

Variations due to differences in cultivars were observed in the retention of beta carotene content in the provitamin A cassava hydrolysate. Oloo *et al.*(2014) reported very large variability in the values of β-carotene for different varieties of orange-fleshed sweet potato grown at the same or different locations.

Fermentation was observed to marginally reduce the total carotenoid content and contrastingly, increased the beta carotene content of the provitamin A hydrolysate. However, there was no significant ($\alpha_{0.05}$) differences in the total carotenoids content across all the samples. This implies that fermentation did not significantly affect the levels of the total carotenoids. This observation was corroborated by the work of Panda and Ray (2007) who observed that the carotenoids content of Orange-fleshed sweet potato lacto juice remained unchanged by fermentation. Carotenoids are unsaturated and unstable in nature thus are easily degraded by oxygen, ultraviolet light and heating during food processing which may lead to significant losses (White and Broadley, 2009). Reduction in the carotenoid content has been attributed to a decrease in the all-E-β-carotene with an increase in the 13-Z-β-carotene as a result of E-Z isomerisation (Carvalho *et al.*, 2012). The fermented samples were observed to have higher beta carotene contents than their unfermented counterpart. TMS-I011368 hydrolysate with the encapsulated *L. rhamnosus GG* cells showed the highest ($\alpha_{0.05}$) beta-carotene content. This shows that values of beta carotene was significantly enhanced by lactic fermentation. This result is consistent with existing literatures (Panda and Ray, 2007, Oloo *et al.*, 2014) which reported that lactic fermentation significantly preserved beta-carotene content. The retained β–carotene of provitamin A cassava is observed to be dependent on variety and it may be difficult to compare results with literatures because the literature on the percentage retention of total carotenoids and total β-carotene in sweet yellow cassava roots is scarce (Carvalho *et al.*, 2012). However, a significant amount of β-carotene (91.64%) was retained in the in the TMS-I011368 hydrolysate even after fermentation.

4.6.3 Residual Hydrogen cyanide content of provitamin A cassava hydrolysates

Figure 4.12 shows the progressive reduction in hydrogen cyanide content (% fresh weight basis). The initial hydrogen cyanide content in the fresh provitamin A cassava roots ranged from 25.06±0.01 to 38.70±0.01 mg/kg. This reduced to 23.00-23.30 mg/kg in the starch with notable reduction in the range of 1.00±0.02 and 1.80±0.00mg/kg

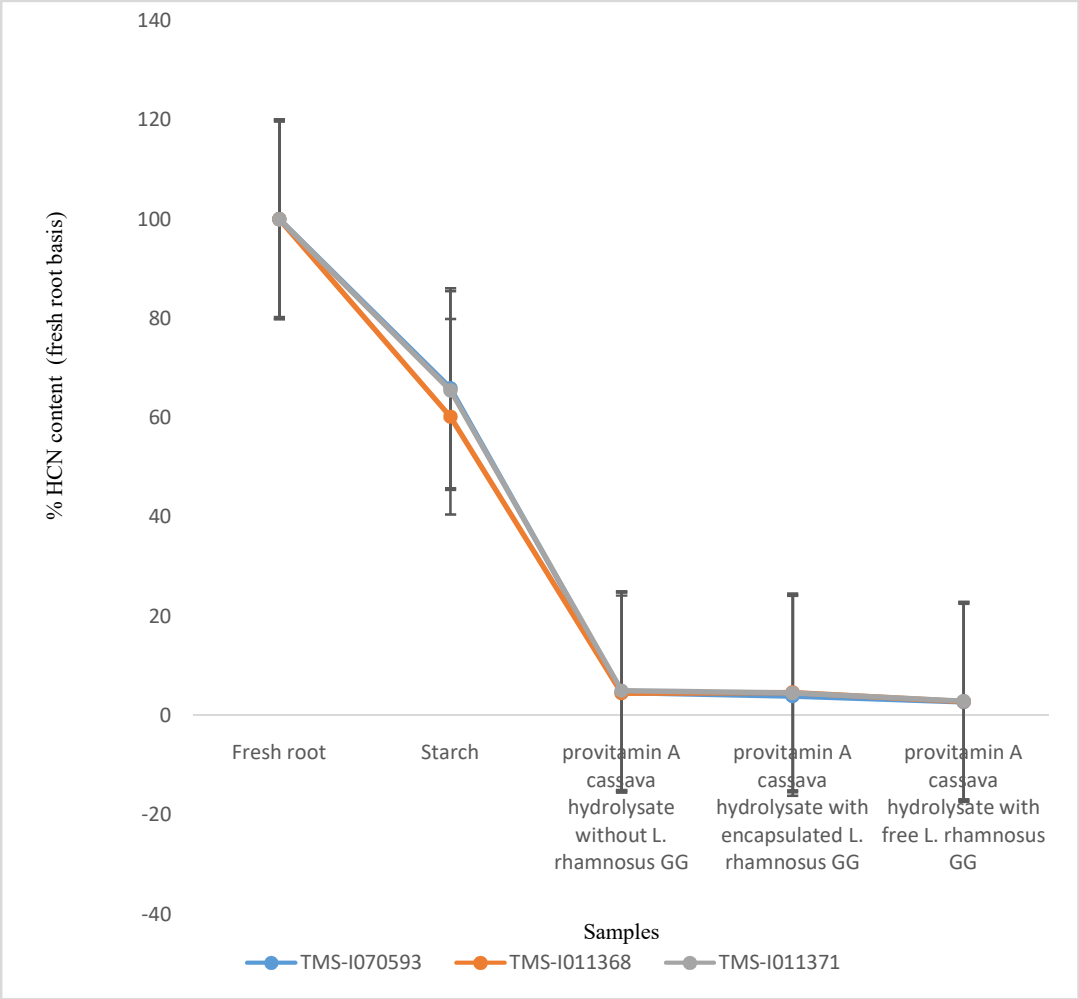


Figure 4.12: Percentage (%) reduction in cyanide contents during processing of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* in comparison with fresh roots.

observed in the probiotic provitamin A cassava hydrolysates fermented with free and encapsulated *L. rhamnosus GG*.This translated to a reduction of 60.21-65.95, 4.50-5.02, 3.99-4.64 and 2.76-2.93% in the starch, hydrolysate without *L. rhamnosus GG*, hydrolysate with encapsulated *L. rhamnosus GG* and the hydrolysate with free *L. rhamnosus GG* respectively.The residual HCN of provitamin A cassava hydrolysate is presented in Table 4.5. Residual HCN content in the provitamin A hydrolysate ranged between 1.00±0.02 and 1.80±0.002 mg/kg.

Processing of provitamin A cassava hydrolysate in combination with fermentation was observed to significantly reduce the HCN content in the provitamin A cassava hydrolysates relative to the HCN content of the fresh root. Falade and Akingbala (2010) showed that fermentation reduced cyanide content to as low as 9.5mg/kg in fermented cassava flour and further lowered to 9.1mg/kg in fermented *gari* while the unfermented counterparts were 21.3mg/kg and 14.6mg/kg respectively. Talsma *et al.* (2016) also reported marked reduction in cyanide levels of cassava by fermentation. The lower residual cyanide contents reported in these cassava products further shows that processes that combine fermentation with the application of heat helps to achieve better detoxification. However, the values of residual HCN content in the provitamin A cassava hydrolysates with or without *L. rhamnosus GG* (Table 4.5) was significantly lower than the maximum limits of 10mg/kg specified by WHO (Cardoso *et al.*, 2005). This means that the provitamin A cassava hydrolysates with or without probiotics is safe and will not impart cyanide toxicity to consumers.

Residual cyanide can be detoxified in the body by the enzyme Rhodanese, which has the highest activity in the kidney, liver, brain, lung, muscle and stomach (Aminlari *et al.*, 2007). Rhodanese hydrolyses cyanide to a non-toxic compound thiocyanate (Chaudhary and Gupta, 2012).

4.7 Hunter L, a, b colour attributes of provitamin A cassava hydrolysates

Table 4.6 shows the colour attributes of the provitamin A cassava hydrolysates. The L* (lightness) values of all the hydrolysates ranged between 37.80±0.80 (TMS-I011371 hydrolysate with free *L. rhamnosus GG*) and 40.11±0.90 (TMS-I070593 hydrolysate with free *L. rhamnosus GG*). The highest Hunter a* value (-0.81±0.01) was observed in TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*.

TMS-I011368 hydrolysate with free *L. rhamnosus GG* had the lowest Hunter a* (-1.74±0.06), highest

Table 4.5: Residual hydrogen cyanide content (mg/100g fresh root weight) of provitamin A cassava hydrolysates with and without *L. rhamnosus GG*

Samples	Residual HCN (mg/kg)
TMS-I070593 Hydrolysate without *L. rhamnosus GG*	1.70±0.00[a]
TMS-I070593 Hydrolysate + encapsulated *L. rhamnosus GG*	1.50±0.009[ab]
TMS-I070593 Hydrolysate + free *L. rhamnosus GG*	1.00±0.02[c]
TMS-I011368 Hydrolysate without *L. rhamnosus GG*	1.80±0.00[a]
TMS-I011368 Hydrolysate + encapsulated *L. rhamnosus GG*	1.70±0.002[a]
TMS-I011368 Hydrolysate + free *L. rhamnosus GG*	1.70±0.00[a]
TMS-I011371 Hydrolysate without *L. rhamnosus GG*	1.80±0.002[a]
TMS-I011371 Hydrolysate + encapsulated *L. rhamnosus GG*	1.60±0.01[ab]
TMS 1371 Hydrolysate + free *L. rhamnosus GG*	1.70±0.003[a]

*Means ± sd with the same alphabet superscripts in the same column are not significantly different $_{(\alpha 0.05)}$

Table 4.6: Hunter L, a, b colour attributes of provitamin A cassava hydrolysate with and without *L. rhamnosus GG*

Samples	L*	a* (red-green)	b* (blue-yellow)	Chroma	Hue
TMS-I011368 hydrolysate without *L. rhamnosus GG*	39.26±0.79^{abc}	-1.25±0.05^{d}	3.69±0.17^{b}	3.90±0.17^{b}	71.23±0.52^{ab}
TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*	39.34±0.96^{abc}	-1.57±0.06^{e}	5.20±0.60^{a}	5.43±0.58^{a}	73.12±1.65^{a}
TMS-I011368 hydrolysate with free *L. rhamnosus GG*	39.89±0.85^{a}	-1.74±0.06^{f}	5.78±0.61^{a}	6.04±0.60^{a}	73.15±1.22^{a}
TMS-I011371 hydrolysate without *L. rhamnosus GG*	38.37±0.98^{bc}	-1.28±0.04^{d}	3.15±0.13^{bc}	3.40±0.12^{bc}	67.92±1.04^{bc}
TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*	38.54±0.89^{abc}	-0.81±0.01^{a}	1.92±0.25^{de}	2.09±0.23^{ef}	67.05±2.66^{bc}
TMS-I011371 hydrolysate with free *L. rhamnosus GG*	37.80±0.80^{c}	-0.90±0.16^{ab}	1.45±0.30^{e}	1.71±0.33^{f}	58.12±2.60^{d}
TMS-I070593 hydrolysate without *L. rhamnosus GG*	39.98±0.99^{a}	-1.09±0.06^{c}	2.58±0.61^{cd}	2.80±0.56^{cde}	66.51±4.77^{bc}
TMS-I070593 hydrolysate with encapsulated *L. rhamnosus GG*	38.67±0.36^{abc}	-0.98±0.06^{bc}	2.00±0.16^{d}	2.23±0.14^{def}	63.88±2.62^{c}
TMS-I070593 hydrolysate with free *L. rhamnosus GG*	40.11±0.90^{a}	-1.29±0.07^{d}	2.57±0.47^{cd}	2.88±0.45^{cd}	63.09±3.39^{c}

*Means ± sd with the same alphabet superscripts in the same column are not significantly different $_{(\alpha 0.05)}$

b* (5.78±0.61), and the highest Chroma (6.04±0.60) values. The lowest b* (1.45±0.30) and Chroma (1.71±0.33) values were observed in the TMS-I011371 hydrolysate with free *L. rhamnosus GG*.The results of the correlation analysis (Table 4.7) shows that beta carotene concentration correlated positively by a factor of 0.5765 and 0.5753 with b* and chroma respectively. Beta carotene concentration has a negative correlation of -0.4944 with a*, and a weak, negative correlation of -0.1380 with L*. b*, which indicates the strength of the yellow attribute, had a strong, positive correlation with chroma by a value of 0.9997. There was a strong negative correlation of -0.9498 between a* and b*. There was also a strong negative correlation of -0.9574 between greenness, a* and chroma.

The values of the L attribute indicates that the provitamin A cassava hydrolysates are not dark in colour but they are also not white. These values are lower than the L* values of greater than 70 reported for cassava roots and starches reported by Eke *et al.* (2009) and Ayetigbo *et al.* (2018). Although the TMS-I011368 hydrolysate with encapsulated or free *L. rhamnosus GG* cells were observed to have the highest significant ($\alpha_{0.05}$) Hunter a* values among all the hydrolysates, the a* attribute for all the samples tended towards light green. There is no minimum and maximum limits for a* values. However, the attribute a* on the HunterLab colour space can take positive (red) or negative (green) values. This is in line with the report of Ladeira *et al.* (2013).

The TMS-I011368 hydrolysates with encapsulated or free *L. rhamnosus GG* cells tended more towards green than all the other samples. None of the hydrolysates assumed positive a* values which is within the red-blue coordinates on the HunterLab colour space.The TMS-I011368 hydrolysates with encapsulated or free *L. rhamnosus GG* cells were observed to have the highest significant ($\alpha_{0.05}$) values of b* which is the attribute that indicates the degree of blue-yellow of a particular sample. Positive b* values indicate yellow while negative b* value indicate blue (Pathare *et al.*, 2013). All the provitamin A cassava hydrolysate were yellowish. Fermentation was not observed to have significant ($\alpha_{0.05}$) effect on the b* values of all the samples. However, there was an observed influence of variety among all the samples. Variety TMS-I011368 was the most yellowish, followed by the hydrolysates from TMS-I011371 then TMS-I070593 showed the least yellowish attribute. This could be explained by their varyingrate of beta carotene retention which is the major carotenoidresponsible for the

Table 4.7: Pearson correlation between beta carotene and colour attributes of provitamin A cassava hydrolysates.

	beta carotene concentration (μg/g)	*L* (Lightness)*	*a* (red-green)*	*b* (blue-yellow)*	*Chroma (colourfulness)*
beta carotene	1				
L* (lightness)	-0.13802	1			
a* (greenness)	-0.49439	-0.58974	1		
b* (yellowness)	0.576501	0.516569	-0.94984	1	
Chroma (colourfulness)	0.575322	0.523176	-0.95741	0.999664	1

yellow colour.

The stronger correlation observed between beta carotene concentration, chroma, and b* respectively, showed that there was a higher degree of association or dependence of the yellow attribute and the colourfulness of the hydrolysates on the concentration of the beta carotene. There was a lesser correlation between beta carotene concentration and L* which is the degree of lightness of the provitamin A cassava hydrolysates. This is similar to the findings of Itle and Kabelka (2009) who reported a strong correlation between b* and chroma, although these attributes were also strongly correlated with lutein.The strong positive correlation of 0.9997 observed between b* and chroma also showed strong dependence, i.e. most of the colourfulness of the provitamin A cassava hydrolysate was derived from its yellow carotenoids. Negative correlation was observed between beta carotene and a* of the samples which showed that there was no association of beta carotene with red-green attribute.

The chroma was significantly higher for all the fermented TMS-I011368 and TMS-I011371 hydrolysates than the unfermented ones. This could be as a result of the increase in total phenolic and flavonoid (section 4.6.1), and carotenoid contents (section 4.6.2) of provitamin A cassava hydrolysates during fermentation (discussed earlier). Significant ($\alpha_{0.05}$) effect of variety was also observed on the chroma of provitamin A hydrolysate.

4.8 Microbial analysis

4.8.1 Total viable count of *L. rhamnosus GG* in Provitamin A cassava hydrolysates during fermentation

The viability of encapsulated and free *L. rhamnosus GG* in provitamin A cassava hydrolysate during fermentation is presented in Figure 4.13. Total viable counts of encapsulated *L. rhamnosus GG* increased from the range 7.73-7.60 log CFU/g at the beginning of the fermentation period to 8.23-8.37 Log CFU/g after the first 12 hours of fermentation. The viability of free *L. rhamnosus GG* in Provitamin A cassava hydrolysate increased from 7.32-7.45 Log CFU/ml to 7.80-7.96 Log CFU/ml after 12hours. These values increased to 9.12-9.32 Log CFU/g and 9.12-9.23 Log CFU/mL

for the encapsulated and free *L. rhamnosus GG* cells respectively by the end of the 48hours fermentation period.

The total viable counts of encapsulated and free *L. rhamnosus GG* cells increased continuously throughout the fermentation period. This increase in cell numbers was due to the ability of *L. rhamnosus GG* to utilise the available sugars in the provitamin A cassava hydrolysate for their metabolismwith the resultant production of lactic acid (as discussed earlier in section 4.3.1 and 4.3.2). The Provitamin A cassava hydrolysates supported the growth of the *L. rhamnosus GG* effectively. *L. rhamnosus GG* (encapsulated or free) maintained significant viability in provitamin A cassava hydrolysate, with viable counts above 9 log CFU.

4.8.2 Microbiological safety of provitamin A cassava hydrolysate after fermentation.

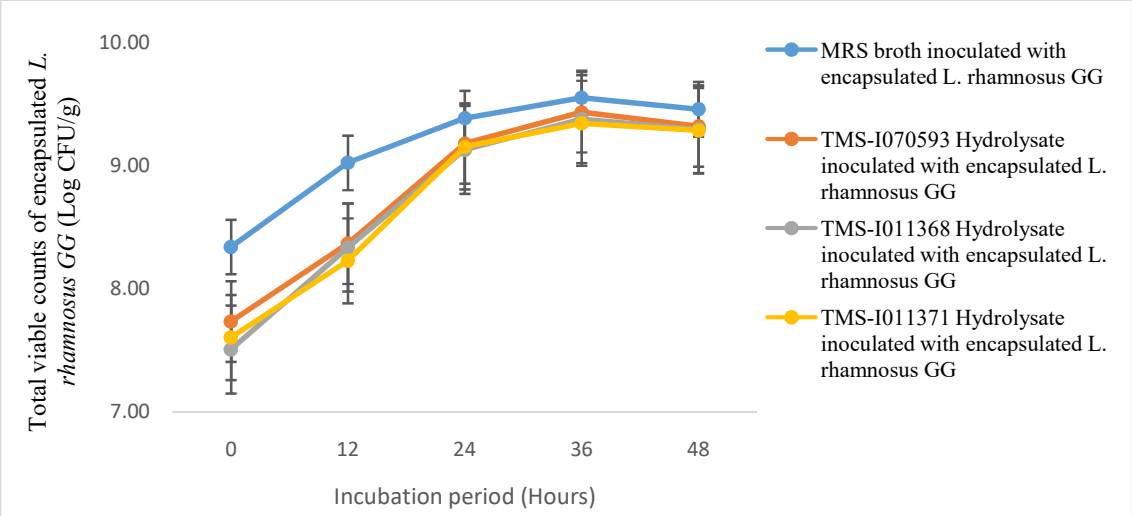
The results showed that there was no observed growth of *E. coli*,*Salmonella sp., Shigella sp.,*and fungi in the samples of fresh provitamin A cassava hydrolysates. Fungal contaminants such as yeast and mould, if present in any food sample have the potential of producing mycotoxins. Adegoke and Letuma (2013)stated that it is important to investigate the presence of fungal contaminants in foods since mycotoxins are produced by the proliferation of yeast and mould. The absence of fungal growth was due to the ability of *Lactobacilli* to inhibit the proliferation of pathogenic organisms (Nath *et al.*, 2014). The product is therefore considered to be safe and might not constitute microbiological hazard if consumed within 30 days. Specifically,*L. rhamnosus GG* has been indicated to have a strong antimicrobial activity against *Salmonella enterica* and other pathogens due to the accumulation of lactic acid (Zhang *et al.*, 2011).

4.9 Storage studies

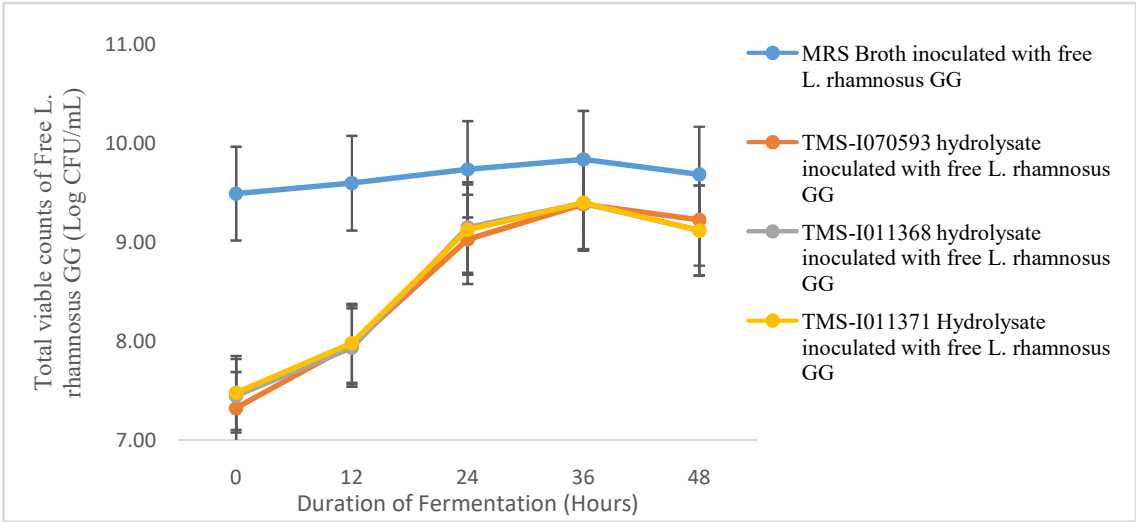
4.9.1 Effect of probiotic on the pH of provitamin A cassava hydrolysates during storage at 4^{o}C

Changes in the pH of provitamin A cassava hydrolysates with free or encapsulated *L. rhamnosus GG* during refrigerated (4^{o}C) storage for 60 days are shown in Figure 4.14. The pH of provitamin A cassava hydrolysates reduced from 3.7 - 3.8 at the day 0 of storage to 3.66 - 3.71 (hydrolysates with encapsulated *L. rhamnosus GG*), and from 3.4 - 3.5 to 3.36 - 3.43 (hydrolysates with free *L. rhamnosus GG*) at the end of the 60 days

of storage. The hydrolysates without *L. rhamnosus GG* reduced from 5.2 - 5.3 at the day

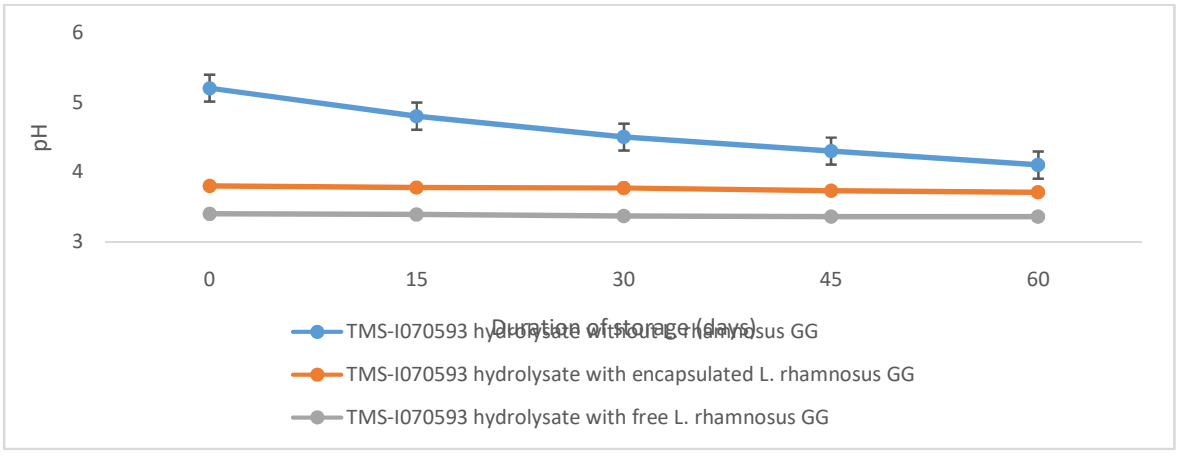


(a). Total viable count of encapsulated *L. rhamnosus GG* in Provitamin A cassava hydrolysate.



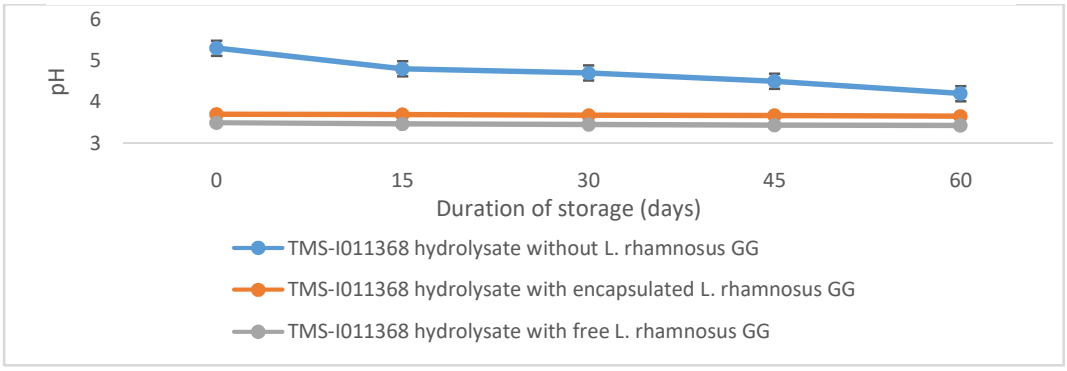
(b). Total viable count of free *L. rhamnosus GG* in Provitamin A cassava hydrolysates

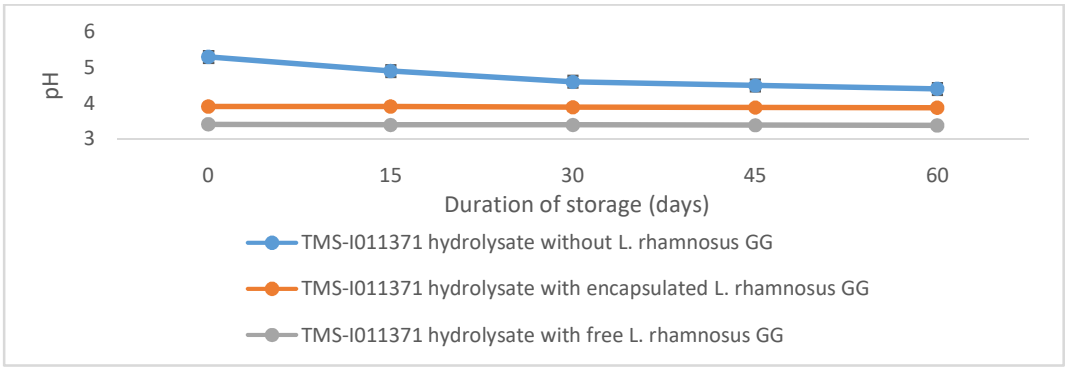
(a). Changes in pH of TMS-I070593 hydrolysate during 60 days of storage at 4°C



(c). Changes in pH of TMS-I011371 hydrolysate during 60 days storage at 4°C

(b). Changes in pH of TMS-I011368 hydrolysate during 60 days storage at 4°C





0 of storage to 4.1 - 4.2 by the end of the storage period.

A decline in pH was observed in all the hydrolysates with either free or encapsulated *L. rhamnosus GG* cells by the end of the storage period. This is due to post-acidification of provitamin A cassava during refrigerated storage showing that minimal fermentation still continued during refrigerated storage, although at a slow rate. It suggests that the *L. rhamnosus GG* cells were viable, although with a reduced activity due to the low temperature storage. Champagne and Gardner (2008) also recorded an unchanged pH of probiotic fruit drink containing *L. rhamnosus* during storage and explained that the unchanged pH indicated a weak metabolic activity of the probiotic organism during the storage period. The final pH of the hydrolysate with free *L. rhamnosus GG* was lower than that with the encapsulated *L. rhamnosus GG.* However, there was no significant difference in final pH between the provitamin A cassava hydrolysate with free or encapsulated *L. rhamnosus GG.* This is in agreement with Sohail *et al.* (2012) who reported an insignificant difference in the pH of orange juice with free or encapsulated *L. rhamnosus GG* and *L. acidophilus*, but concluded that encapsulated *L. rhamnosus GG* has the potential of reducing acidification.

4.9.2 Changes in the organic acid concentration of the provitamin A cassava hydrolysates during storage at 4^{o}C

The changes in the concentration of organic acid content of the provitamin A cassava hydrolysate samples by the end of the 60 days storage period is presented in Table 4.8. The lactic acid content of hydrolysate samples with free *L. rhamnosus GG* increased gradually from the range of 3780.33±0.58 and 4501±1.53 mg/L at the beginning of storage to the range of 9637.04±300.94 and 13676.90±880.38 mg/L by the end of the storage period. The hydrolysates with encapsulated *L. rhamnosus GG* increased in lactic acid concentration in the range of 5040±1.00 and 5490±1.00 mg/L at the beginning of storage to 8194.81±156.65 and 9658.76±175.48 mg/L by the end of the 60 days of storage.

There was a significant increase in lactic acid production in all the samples, with minimal production of acetic acid in all of the samples during the storage period. Only lactic acid was observed to be present in the hydrolysates during fermentation. The

Table 4.8: Changes in Organic acid content of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C

Storage duration	Organic Acid	TMS-I011371 hydrolysate without *L. rhamnosus GG*	TMS-I011368 hydrolysate without *L. rhamnosus GG*	TMS-I070593 hydrolysate without *L. rhamnosus GG*	TMS-I070593 hydrolysate with free *L. rhamnosus GG*	TMS-I011368 hydrolysate with free *L. rhamnosus GG*	TMS-I011371 hydrolysate with free *L. rhamnosus GG*	TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*	TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*	TMS-I070593 hydrolysate with encapsulated *L. rhamnosus GG*
Day 0	Lactic Acid (mg/L)	89.69±0.58^{g}	90.03±0.06^{g}	90.33±1.53^{g}	3780.00±1.00^{f}	4501.33±1.53^{d}	3960.33±1.53^{e}	5130±1.00^{b}	5490±1.00^{a}	5040±1.00^{c}
	Acetic Acid (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Propanoic acid (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Lactate: Acetate yield	-	-	-	-	-	-	-	-	-
Day 30	Lactic Acid (mg/L)	155.23±0.08^{g}	152.01±0.03^{g}	159.33±0.02^{g}	5670.21±0.20^{c}	7052.33±0.89^{a}	6530.13±0.71^{b}	6640.01±0.10^{e}	7180±0.81^{d}	6921±1.10^{f}
	Acetic Acid (mg/L)	107.11±0.23^{g}	98.61±0.08^{h}	112.05±0.07^{g}	321.51±0.51^{f}	362.28±0.20^{a}	344.71±0.04^{c}	337.32±0.01^{d}	325.30±0.05^{e}	352.31±0.25^{b}
	Propanoic acid (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Lactate: Acetate yield	1.45	1.54	1.42	17.64		-	-	-	-
Day 60	Lactic Acid (mg/L)	5777.82±574.64^{f}	7789.96±267.61^{de}	7088.54±16.20^{e}	13676.90±880.38^{a}	10510.79±0.30^{b}	9637.04±300.94^{bc}	8194.81±156.65^{cd}	9658.76±175.48^{bc}	8765.51±249.64^{cd}
	Acetic Acid (mg/L)	2436.37±85.82^{a}	2460.74±31.25^{a}	2199.34±0.94^{ab}	1090.36±9.60	1555.47±0.75^{cd}	1046.27±30.19^{ab}	1305.07±260.43^{de}	1084.06±54.93^{e}	1283.87±390.50^{bc}
	Propanoic acid (mg/L)	ND	296.24	ND	ND	ND	ND	ND	ND	ND
	Lactate: Acetate yield	2.37	3.17	3.22	12.54	6.76	9.21	6.28	8.91	6.83

*Means ± sd with the same alphabet superscripts in the same row are not significantly different $_{(\alpha 0.05)}$

Keys: *ND*= Not detected

presence of acetic acid was only detected after fermentation (during storage). This is because low temperature does not completely inhibit acetic acid production (Schlabitz*et al.*, 2015). This result corroborates Chen *et al.* (2019) which reported an increase in lactic and acetic acids in all the samples of apple juice fermented with four strains of LAB (including *L. rhamnosus GG*). The metabolic activity of the added bacterial cultures is reflected by the organic acid profile of the fermented product (Rokka and Rantamäki, 2010). The unfermented samples (control) were also observed to have lactic acid produced in it during storage, though it recorded the least amount of lactic acid with traces of propanoic acid in one of the samples without probiotics. This is consistent with the findings of Tian *et al.* (2017), which reported an increase in lactic acid content of yoghurt samples with and without probiotics during storage at 4^{o}C. The presence of organic acid in the provitamin A cassava samples without probiotics is attributable to the fact that provitamin A cassava hydrolysate is an acidic medium which could favour the proliferation of some other members of the *LAB* generaapart from *L. rhamnosus GG* during long storage. These other lactic acid bacteria are also capable of fermenting sugars with the primary production of lactic acid.

L. rhamnosus GG is a facultative heterofermentative bacterium that is capable of fermenting hexoses ($C_6H_{12}O_6$) such as lactose and fructose to lactic acid. They can also ferment pentoses ($C_5H_{10}O_5$) such as ribose and deoxyribose to a mixture of lactic acid and acetic acid (Daneshi *et al.*, 2013). Zalan *et al.* (2010) explained that some strains of lactic acid bacteria could show changes in their fermentation profile from homofermentative to mixed acid. This result is in agreement with the work of Adhikari *et al.* (2002) who reported the concomitant production of lactic acid with acetic acid during storage at 4^{o}C in yoghurt inoculated with encapsulated and non-encapsulated probiotic (*Bifidobacterium).*Karbasi *et al.*(2015) reported that the production of acetic acid by *L. rhamnosus* was higher than the quantity produced by *L. acidophilus* in fermented Date Palm syrup. This shows that *L. rhamnosus GG* is well capable of producing acetic acid in fermented substrates. Charalampopoulos *et al.* (2002) stated that the presence of acetic acid could be attributed to the presence of oxygen or electron acceptors and these could shift the metabolic pathway towards acetic acid production. The encapsulated cells were observed to produce lesser lactic acid during storage compared to the free *L. rhamnosus GG* cells due to the protected state in which the *L. rhamnosus GG* cells were. Hence they were less reactive with the environment

and could be said to be more stable during the storage period. Although, high concentrations of acetic acid in beverages (juices or wines) have been reported to have an adverse effect on the sensory qualities (Karbasi *et al.*, 2015). The presence of acetic acid can impart sourness into a sample. Increase in acetic and propionic acids produced by probiotics may cause reduced consumer acceptability of a product (Rokka and Rantamäki, 2010). However, a high ratio of lactate: acetate yield was observed in the provitamin A cassava hydrolysate samples containing *L. rhamnosus GG* (either free cell or encapsulated) as compared to the samples without probiotics. This observed ratio is expected to be more appropriate in preserving good sensory property of taste and aroma since Bujna *et al.* (2018) stated that higher ratio of lactic acid to acetic acid in a fermented product may contribute to its savouriness while the reverse could make the product unacceptable (Rokka and Rantamäki, 2010).

4.9.3 Changes in the Hunter L, a b (ΔL, Δa and Δb) colour attributes of probiotic provitamin A cassava hydrolysate during storage

The changes in the Hunter L, a b (ΔL, Δa and Δb) and ΔE, change in colour intensity of hydrolysates from TMS-I011368, TMS-I070593, and TMS-I011371varieties are presented in Tables 4.9, 4.10 and 4.11.After 30 days of storage, L attribute increased by factors ranging from 1.17±0.98 to 5.31±0.96 across all the samples, with TMS-I070593 hydrolysate having the least ΔL and TMS-I01171 hydrolysates with the highest ΔL. a* attribute increased from 0.60± 0.02 (TMS-I011371 hydrolysate without *L. rhamnosus GG* stored in transparent containers) to 1.93±0.05 (TMS-I070593 with encapsulated *L. rhamnosus GG* stored in transparent containers). b* attributes reduced across all the hydrolysates with TMS-I011368 hydrolysate with free *L. rhamnosus GG* stored in amber containers having the least value of Δb while TMS-I011368 hydrolysate without *L. rhamnosus GG*,stored in amber containers had the highest change in the b* attribute. ΔE increased in the range of 2.79±0.61 (TMS-I011371 hydrolysate without *L. rhamnosus GG* stored in transparent containers) to 6.57±0.28 (TMS-I070593 with encapsulated *L. rhamnosus GG* stored in transparent containers).

After 60 days of storage, TMS-I011371 hydrolysate without *L. rhamnosus GG* stored in amber containers had the highest change in L attribute (5.91±0.98), while TMS-

I070593 with free *L. rhamnosus GG* stored in transparent containers had the least change in L

Storage period (days)	Colour attributes	TMS-I011368 Hydrolysates without *L. rhamnosus GG* (Amber)	TMS-I011368 Hydrolysates without *L. rhamnosus GG* (Transparent)	TMS-I011368 Hydrolysates with Encapsulated *L. rhamnosus GG* (Amber)	TMS-I011368 Hydrolysates with Encapsulated *L. rhamnosus GG* (Transparent)	TMS-I011368 Hydrolysates with Free *L. rhamnosus GG* (Amber)	TMS-I011368 Hydrolysates with Free *L. rhamnosus GG* (Transparent)
Day 0	L	39.26±0.79^{abc}	39.26±0.79^{abc}	39.34±0.96^{abc}	39.34±0.96^{abc}	39.89±0.85^{a}	39.89±0.85^{a}
	a	-1.25±0.05^{d}	-1.25±0.05^{d}	-1.57±0.06^{e}	-1.57±0.06^{e}	-1.74±0.06^{f}	-1.74±0.06^{f}
	b	3.69±0.17^{b}	3.69±0.17^{b}	5.20±0.60^{a}	5.20±0.60^{a}	5.78±0.61^{a}	5.78±0.61^{a}
	C	3.90±0.17b	3.90±0.17b	5.43±0.58^{a}	5.43±0.58^{a}	6.04±0.60^{a}	6.04±0.60^{a}
Day 30	ΔL	2.25±0.77^{a}	2.14±0.77^{a}	1.97±0.94^{a}	1.66±0.94^{a}	2.17±0.81^{a}	2.24±0.84^{a}
	Δa	1.60±0.05^{c}	1.89±0.05^{d}	1.31±0.07^{c}	1.35±0.06^{c}	1.38±0.06^{c}	1.91±0.08^{a}
	Δb	-4.66±0.17^{c}	-2.33±0.17^{b}	-2.65±0.56^{b}	-2.70±0.60^{b}	-1.22±0.66^{a}	-3.09±0.63^{b}
	ΔE	5.45±0.29^{a}	3.33±0.46^{a}	3.17±0.81^{a}	3.48±0.93^{a}	4.03±0.94^{a}	3.22±0.88^{a}
Day 60	ΔL	1.68±0.79^{a}	2.24±0.79^{a}	1.60±0.96^{a}	2.67±0.96^{a}	1.76±0.85^{a}	1.77±0.85^{a}
	Δa	1.89±0.05^{b}	1.39±0.05^{c}	1.47±0.06^{c}	2.01±0.06^{a}	1.88±0.06^{b}	1.23±0.06^{d}
	Δb	-2.55±0.17^{bc}	-2.61±0.17^{c}	-1.99±0.60^{a}	-2.18±0.60^{bc}	-1.72±0.61^{abc}	-1.61±0.61^{ab}
	ΔE	3.64±0.33^{a}	3.70±0.42^{a}	2.45±0.92^{a}	4.02±0.99^{a}	2.63±0.99^{a}	2.71±0.95^{a}

Table 4.9: Changes in the Hunter L, a, b colour attributes of TMS-I011368 hydrolysate containing free and encapsulated *L. rhamnosus GG*during 60 days of storage at 4^{o}C

*Means ± sd with the same alphabet superscripts in the same column are not significantly different $_{(\alpha 0.05)}$

Table 4.10: Changes in the colour attributes of TMS-I070593 hydrolysates containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C

	Colour attributes	TMS-I070593 Hydrolysates without LGG (Amber)	TMS-I070593 Hydrolysates without *L. rhamnosus GG* (Transparent)	TMS-I070593 Hydrolysates with Encapsulated *L. rhamnosus GG* (Amber)	TMS-I070593 Hydrolysates with Encapsulated *L. rhamnosus GG* (Transparent)	TMS-I070593 Hydrolysates with Free *L. rhamnosus GG* (Amber)	TMS-I070593 Hydrolysates with Free *L. rhamnosus GG* (Transparent)
Day 0	L	39.98±0.99^{a}	39.98±0.99^{a}	38.67±0.36^{abc}	38.67±0.36^{abc}	40.11±0.90^{a}	40.11±0.90^{a}
	a	-1.09±0.06^{c}	-1.09±0.06^{c}	-0.98±0.06^{bc}	-0.98±0.06^{bc}	-1.29±0.07^{d}	-1.29±0.07^{d}
	b	2.58±0.61^{cd}	2.58±0.61^{cd}	2.00±0.16^{d}	2.00±0.16^{d}	2.57±0.47^{cd}	2.57±0.47^{cd}
	C	2.80±0.56^{cde}	2.80±0.56^{cde}	2.23±0.14^{def}	2.23±0.14^{def}	2.88±0.45^{cd}	2.88±0.45^{cd}
Day 30	ΔL	1.17±0.98^{b}	2.44±0.99^{ab}	2.62±0.4^{ab}	3.29±0.37^{a}	1.65±0.94^{b}	2.44±0.9^{0ab}
	Δa	1.68±0.05^{c}	1.76±0.07^{bc}	1.38±0.07^{d}	1.93±0.05^{a}	1.32±0.07^{d}	1.87±0.07^{ab}
	Δb	-4.65±0.62^{ab}	-2.93±0.61^{a}	-4.48±0.16^{b}	-5.35±0.16^{c}	-3.98±0.44^{b}	-4.24±0.45^{b}
	ΔE	5.13±0.71^{b}	4.24±0.92^{b}	5.37±0.28^{ab}	6.57±0.28^{a}	4.54±0.78^{b}	5.26±0.82^{b}
Day 60	ΔL	2.87±0.99^{a}	2.87±0.99^{a}	3.72±0.36^{a}	3.90±0.36^{a}	2.76±0.9^{a}	2.42±0.90^{a}
	Δa	1.72±0.06^{b}	1.88±0.06^{a}	1.89±0.06^{a}	1.92±0.06^{a}	1.67±0.07^{b}	1.87±0.07^{a}

Δb	-4.65±0.61 [a]	-4.84±0.61 [a]	-4.77±0.16 [a]	-4.92±0.16 [a]	-4.17±0.47 [a]	-4.98±0.47 [a]
ΔE	5.76±0.91 [a]	5.97±0.9 [a]	6.34±0.3 [a]	6.56±0.30[a]	5.29±0.88 [a]	5.86±0.81 [a]

*Means within the same row superscripted by the same alphabets are not significantly different $_{(\alpha 0.05)}$

Table 4.11: Changes in the colour attributes of TMS-I011371 hydrolysates containing free and encapsulated *L. rhamnosus GG* during 60 days of storage at 4^{o}C

Storage period	Colour attributes	TMS-I011371 Hydrolysates without *L. rhamnosus GG* (Amber)	TMS-I011371 Hydrolysates without *L. rhamnosus GG* (Transparent)	TMS-I011371 Hydrolysates with Encapsulated *L. rhamnosus GG* (Amber)	TMS-I011371 Hydrolysates with Encapsulated *L. rhamnosus GG* (Transparent)	TMS-I011371 Hydrolysates with Free *L. rhamnosus GG* (Amber)	TMS-I011371 Hydrolysates with Free *L. rhamnosus GG* (Transparent)
Day 0	L	38.37±0.98^{bc}	38.37±0.98^{bc}	38.54±0.89^{abc}	38.54±0.89^{abc}	37.80±0.80 [c]	37.80±0.80 [c]
	a	-1.28±0.04^{d}	-1.28±0.04^{d}	-0.81±0.01 [a]	-0.81±0.01 [a]	-0.90±0.16^{ab}	-0.90±0.16^{ab}
	b	3.15±0.13^{bc}	3.15±0.13^{bc}	1.92±0.25^{de}	1.92±0.25^{de}	1.45±0.30^{e}	1.45±0.30^{e}
	C	3.40±0.12^{bc}	3.40±0.12^{bc}	2.09±0.23^{ef}	2.09±0.23^{ef}	1.71±0.33^{f}	1.71±0.33^{f}
Day 30	ΔL	5.31±0.96^{a}	1.33±1.03^{c}	3.05±0.66^{bc}	3.03±1.66^{bc}	4.29±0.79^{a}	3.67±0.82^{ab}
	Δa	1.19±0.03^{c}	0.60±0.02^{d}	1.41±0.02^{b}	1.45±0.01^{ab}	1.47±0.17^{ab}	1.64±0.19^{a}
	Δb	-2.64±0.62^{ab}	-2.28±0.13^{a}	-3.75±0.25^{d}	-3.09±0.25^{bc}	-4.38±0.29^{e}	-3.56±0.30^{cd}
	ΔE	6.07±0.92^{ab}	2.79±0.61^{c}	4.78±0.71^{b}	5.29±0.83^{ab}	6.32±0.71^{a}	5.39±0.74^{ab}
Day 60	ΔL	5.29±0.98^{ab}	5.91±0.98^{a}	3.65±0.89^{c}	4.03±0.89^{b}	4.29±0.80^{ab}	4.88±0.80^{ab}
	Δa	1.14±0.04^{e}	7.31±0.04^{a}	6.05±0.01^{d}	6.51±0.01^{b}	6.53±0.16^{b}	6.74±0.16^{c}

	Δb	-2.71±0.13[a]	-2.89±0.13[a]	-3.32±0.25[b]	-3.78±0.25[c]	-4.18±0.30[cd]	-4.40±0.30[d]
	ΔE	9.31±0.62[ab]	9.86±0.65[a]	7.83±0.53[c]	8.56±0.54[bc]	8.88±0.45[ab]	9.43±0.48[ab]

*Means ± sd with the same alphabet superscripts in the same column are not significantly different $_{(\alpha 0.05)}$

attribute (2.42±0.90). Δa across all the hydrolysates increased in range from 1.14±0.04 (TMS-I011371 hydrolysate without *L. rhamnosus GG* stored in amber containers) to 7.31±0.04 (TMS-I011371 hydrolysate without *L. rhamnosus GG* stored in transparent containers). Δb reduced in the range of -4.98±0.47 (TMS-I070593 with free *L. rhamnosus GG* stored in transparent containers) to -1.72±0.61 (TMS-I011368 hydrolysate with free *L. rhamnosus GG* stored in amber containers). ΔE ranged from 2.45±0.92 (TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG* stored in amber containers) to 9.43±0.48 (TMS-I011371 hydrolysate with free *L. rhamnosus GG* stored in transparent containers).

The increase in the L attribute of all the samples after 30 days of storage, with further increase at the end of 60 days of storage was accompanied by an increase in the a* attribute, with corresponding decrease in the b* attributes of the hydrolysates. There was no significant influence of *L. rhamnosus GG* inclusion- either free or encapsulated on the L attribute. This observation could suggest oxidation of the carotenoids contained in the hydrolysates.

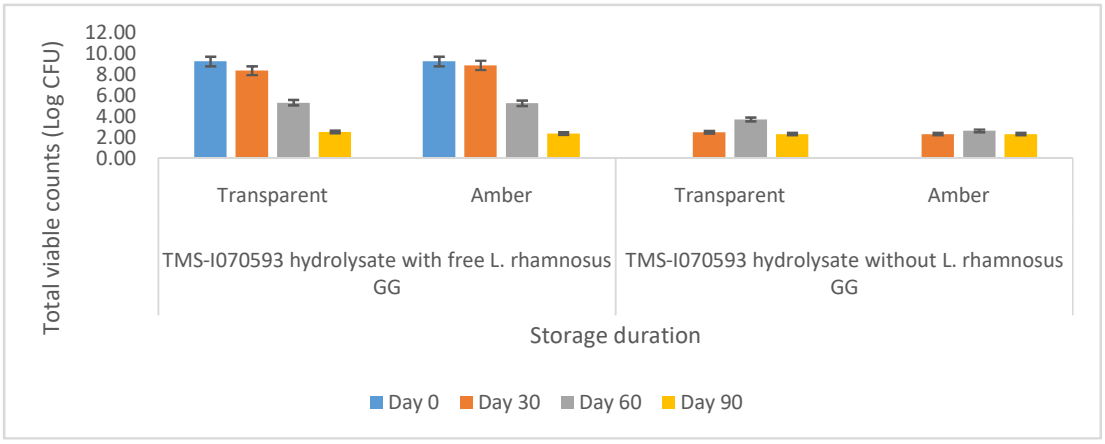
There was a significant influence of packaging material transparency or opacity on the ΔL of the samples during storage for 30 days and up to 60 days of storage. The significant increase in the Hunter L (lightness) can be attributable to autooxidation of the carotenoids contents (Boon *et al.*, 2010). The Hunter a values reduced towards the positive during storage, thereby giving Δa values in the positive range. This shows that the hydrolysates reduced in the green coordinates during storage with no particular trend attributable to the effect of packaging, or either the inclusion probiotics or mode of inoculation of the *L. rhamnosus GG* (i.e. free or encapsulated). The Hunter b values reduced throughout the 60 days of storage, indicating a loss of the yellow colour which is dependent on the β-carotene concentration. This suggests that the carotenoids (β-carotene in particular) was not sufficiently preserved by the refrigerated (4^{o}C) storage. The reduction in the yellow colour of the hydrolysates was more pronounced in the hydrolysates stored in transparent bottles. This shows that the reduction in the incidence of light on the amber bottles preserved the carotenoids better in the hydrolysates, thus the preservation of the yellow colour stored in the amber bottles. The hydrolysates stored in transparent bottles could be prone to photo-degradation (Boon *et al.*, 2010). There was a significant increase in the ΔE values by the 30th day and by the end of storage period but there was no significant ($\alpha_{0.05}$) difference in the

ΔE values of all the hydrolysates stored either in transparent or amber bottles; or those with the inclusion of free or encapsulated *L. rhamnosus GG* when compared with the control samples. Therefore, the significant differences observed in the ΔE of the hydrolysates were not attributable to the effect of inclusion of *L. rhamnosus GG* (free or encapsulated). There was no observed effect of variety on the colour attributes of provitamin A cassava hydrolysates.

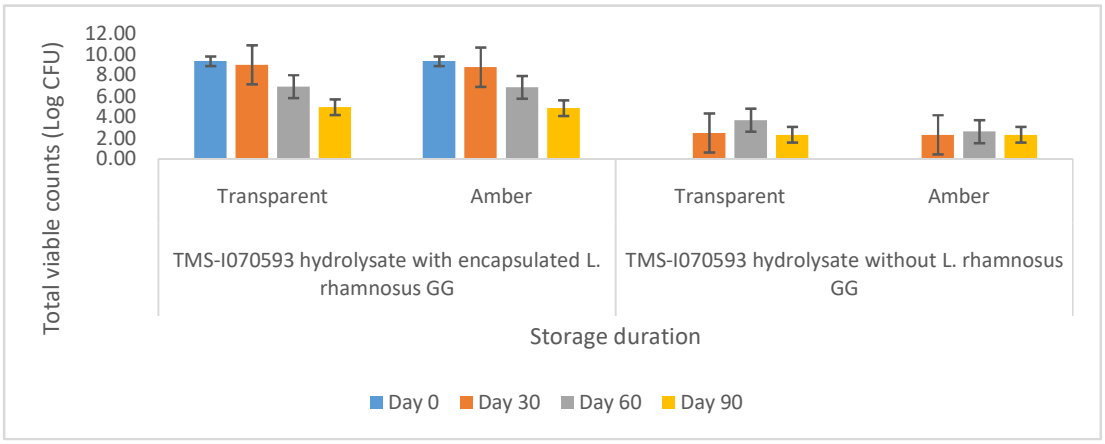
4.9.4 Viability of free and encapsulated *L. rhamnosus GG* in provitamin A cassava hydrolysate during storage at 4^{o}C

The viable cell counts of *L. rhamnosus GG* cells in the provitamin A cassava hydrolysates during storage are presented in Figures 4.15, 4.16 and 4.17.

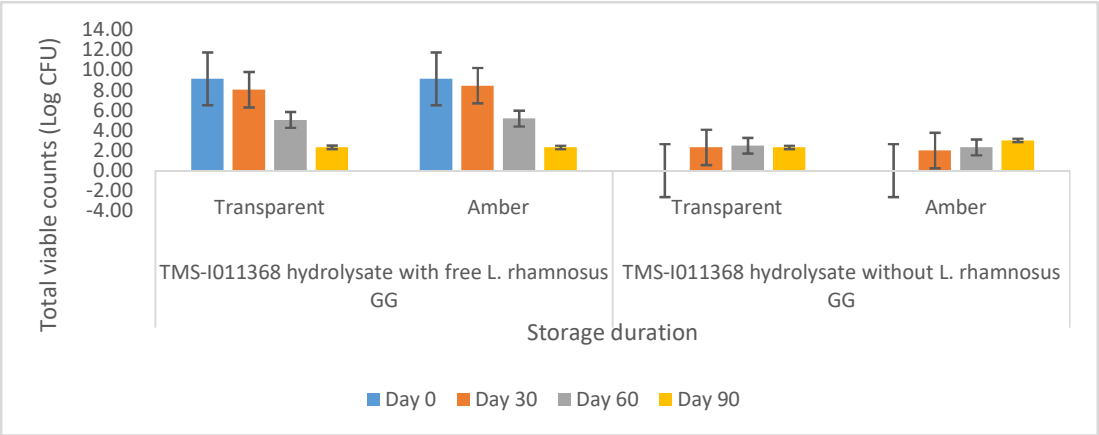
The viable cell count of free *L. rhamnosus GG* reduced from 9.12 - 9.23 log CFU/ml after fermentation to 8.04 – 8.86 log CFU/ml after the first 30 days, translating to 88.15 (TMS-I011368 hydrolysate with free *L. rhamnosus GG* stored in transparent bottle) – 95.99 % viability (TMS-I070593 hydrolysate with free *L. rhamnosus GG* stored in amber bottle). These values reduced to 7.94 – 8.02 log CFU/ml, which translated to 55.26 % (TMS-I011368 hydrolysate with free *L. rhamnosus GG* stored in transparent bottle) – 87.94 % viability (TMS-I11371 hydrolysate with free *L. rhamnosus GG* stored in amber bottle) after 60 days. *L. rhamnosus GG* underwent further reductions in cell viability in the range of 25.22 – 55.81% viability (TMS-I011371 hydrolysate with free *L. rhamnosus GG* stored in amber bottle) occurred by the end of 90 days.The cell viability of encapsulated *L. rhamnosus GG* reduced from 9.12 – 9.32 Log CFU/g after fermentation to 8.42 (TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG* storedin transparent bottle) – 8.98 Log CFU/g (TMS-I011371 hydrolysate withencapsulated *L. rhamnosus GG* stored in transparent bottle). This translated to 92.32 – 96.77% viability by the end of the first 30 days. The viability of *L. rhamnosus GG* further reduced to 6.83 – 7.94 Log CFU/g equivalent to 73.28% (TMS-I070593 hydrolysate with encapsulated *L. rhamnosus GG* stored in amber bottle) – 85.56% viability (TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG* stored in amber bottle) after 60 days. The viability at the end of 90 days of storage decreased to 52.04 (TMS-I070593 hydrolysate with encapsulated *L. rhamnosus GG* stored in amber bottle) – 75.22% (TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG* stored in amber bottle).



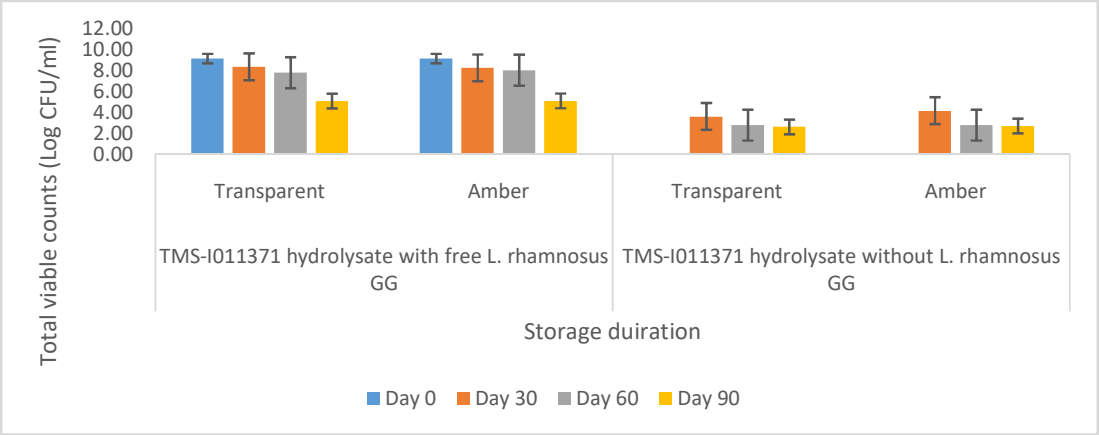
(a). Effect of packaging on the viability of free *L. rhamnosus GG* in TMS-I070593 hydrolysate during storage at 4°C.



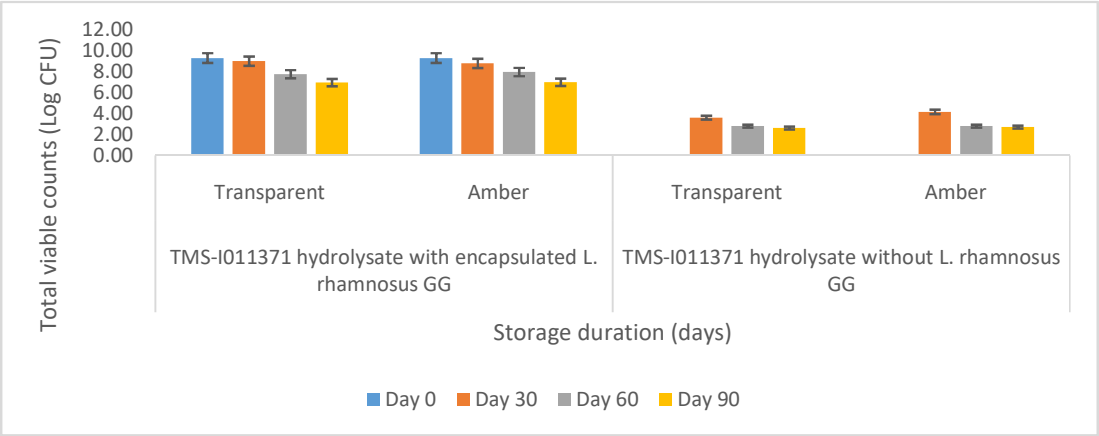
(b). Effect of packaging on the viability of encapsulated*L. rhamnosus GG* in TMS-I070593 hydrolysate during storage at 4°C.



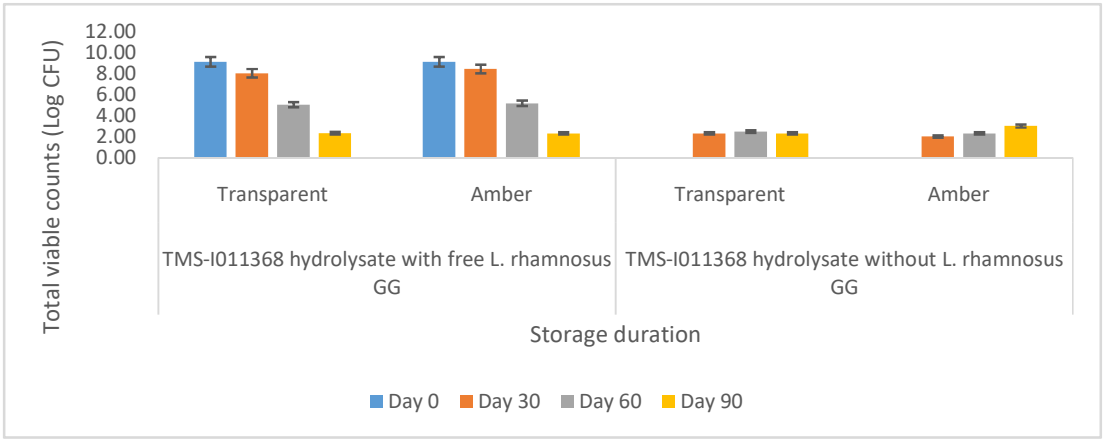
(a) Effect of packaging on the viability of free *L. rhamnosus GG* in TMS-I011368 hydrolysate during storage at 4°C



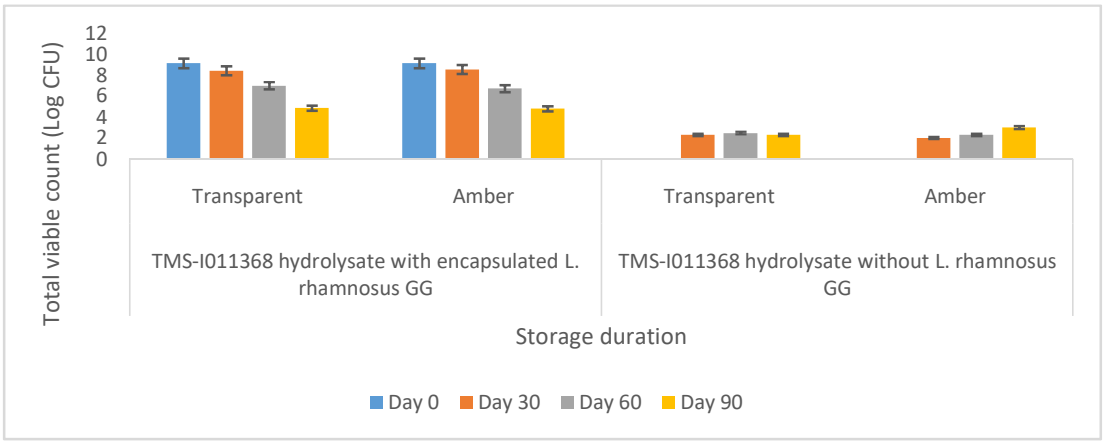
(a). Effect of packaging on the viability of free *L. rhamnosus GG* in TMS-I011371 hydrolysate during storage at 4°C.



(b). Effect of packaging on the viability of encapsulated *L. rhamnosus GG* in TMS-I011371 hydrolysate during storage at 4°C.



(a). Effect of packaging on the viability of free *L. rhamnosus GG* in TMS-I011368 hydrolysate during storage at 4°C



(b). Effect of packaging on the viability of encapsulated*L. rhamnosus GG* in TMS-I011368 hydrolysate during storage at 4°C

There was a rapid loss of free *L. rhamnosus GG* cells in the provitamin A cassavahydrolysates within the first 30 days of storage. This was evident in the notable manner in which the cell numbers of free *L. rhamnosus GG* reduced. However, the reduction in cell numbers of the encapsulated cells was a very gradual one. The observed cell number satisfies the WHO recommended minimum value of 10^6 CFU/ml. There was no observed significant differences in the cell reduction rates among all the samples containing encapsulated cells. This suggests that encapsulation was able to achieve sufficient protection of *L. rhamnosus GG* cells during refrigerated storage. At the end of the 90 days, there was a significant decrease in the viable cells compared to the counts after the first 30 days. The same pattern was observed in all the varieties of provitamin A cassava tested. This suggests that there was no observed effect of variety on the viability of *L. rhamnosus GG.* However, the transparency of the packaging material did not seem to significantly contribute to the viability of *L. rhamnosus GG* as the marginal differences observed in the viable cell counts did not follow any particular pattern in relation to the transparency/opacity of the packaging material.

Despite the observed cell losses, the viability of the *L. rhamnosus GG* cells in this study was maintained above 5 Log CFU/ml in the hydrolysates with free *L. rhamnosus GG*, and above 7 Log CFU/g in hydrolysates with encapsulated *L. rhamnosus GG* by the end of 60 days of storage.The observation in this study is consistent with other existing studies in which *L. rhamnosus GG* showed significant viability during refrigerated storage. Champagne and Gardner (2008) studied the viability of 9 probiotic strains including *L. rhamnosus* in a fruit drink for 80days and reported that *L. rhamnosus* showed significant viability ($> 10^6$ CFU) during storage despite indicating some cell losses. Saarela *et al.* (2009) also reported that despite high losses of freeze-dried *L. rhamnosus* in apple juice during storage, *L. rhamnosus GG* maintained significant viability. Although, the tendency of probiotic strains to maintain viability in an acid medium is strain-dependent, *L. rhamnosus* can survive an acidic medium (pH 4.0-4.5). However, sub-optimal pH medium such as pH ranges 3.4-3.6 might exert an inhibitory effect on its growth.

Other strains of *Lactobacillus* have also been reported to maintain viability during refrigerated storage. Klewicka and Czyżowska (2011) reported that the viability of*L. brevis* and *L. paracasei* in Beetroot juice during refrigerated storage was at 9.11 Log CFU/ml at 30 days, reduced further to 8.15 Log CFU/ml after 30 days while further storage led to a further decrease of 6.80 Log CFU/ml by the end of 180 days (Klewicka and Czyżowska, 2011). Several factors that have been reported to interfere with the viability of lactic acid strains of probiotics during storage are the level and permeation of oxygen in the product and through the packaging material, i.e. the porosity of the packaging material, the temperature of the storage environment, duration of storage and the acidity of the medium due to accumulation of organic acids such as lactic acid (Xu *et al.*, 2013).

4.9.5 Microbiological safety of provitamin A cassava hydrolysate during storage

Figure 4.18 shows the population of spoilage and pathogenic organisms in the provitamin A cassava hydrolysates during storage. No spoilage or pathogenic organism was detected in the provitamin A cassava hydrolysates after fermentation. All the provitamin A cassava hydrolysate with free or encapsulated *L. rhamnosus GG* did not show presence of any pathogenic organisms throughout the first 30 days. However, by day 60, all the hydrolysates had total aerobic organisms in the range of 0.4×10^1 - 1.6×10^1 CFU/ml, coliform counts in the range of 1×10^2 - 2×10^2 CFU/ml while fungal growths were present in the range of 1×10^2 - 2×10^2 CFU/ml. The storage study was therefore terminated by the end of 60 days owing to the presence of spoilage and pathogenic organisms. This showed that the samples of probiotic provitamin A cassava hydrolysate were stable up to a period of 30 days without any microbial deterioration. This is consistent with Panda *et al.* (2009). However, enumeration of viable *L. rhamnosus GG* was extended beyond 60 days so as to ascertain the extent to which probiotics were viable in the samples.

The above findings could be due in part to the bio-preservative ability of lactic acid bacteria through their bacteriocins and other metabolites produced in biological systems during fermentation. Biopreservation has been defined as the use of antagonistic non-pathogenic organisms such as *Lactobacilli* or their metabolic products to inhibit or destroy undesirable or pathogenic organisms, thus extending the shelf-life of food and making it safe for consumption (Nath *et al.*, 2014). Bacteriocins

are inhibitory towards gram-positive bacteria only. However, De Keersmaecker *et al.* (2006) argued that theantimicrobial activity of *L. rhamnosus GG* against strains of *Salmonella* is not due to bacteriocin rather to the accumulation of lactic acid. Makras *et al.* (2006) also reported

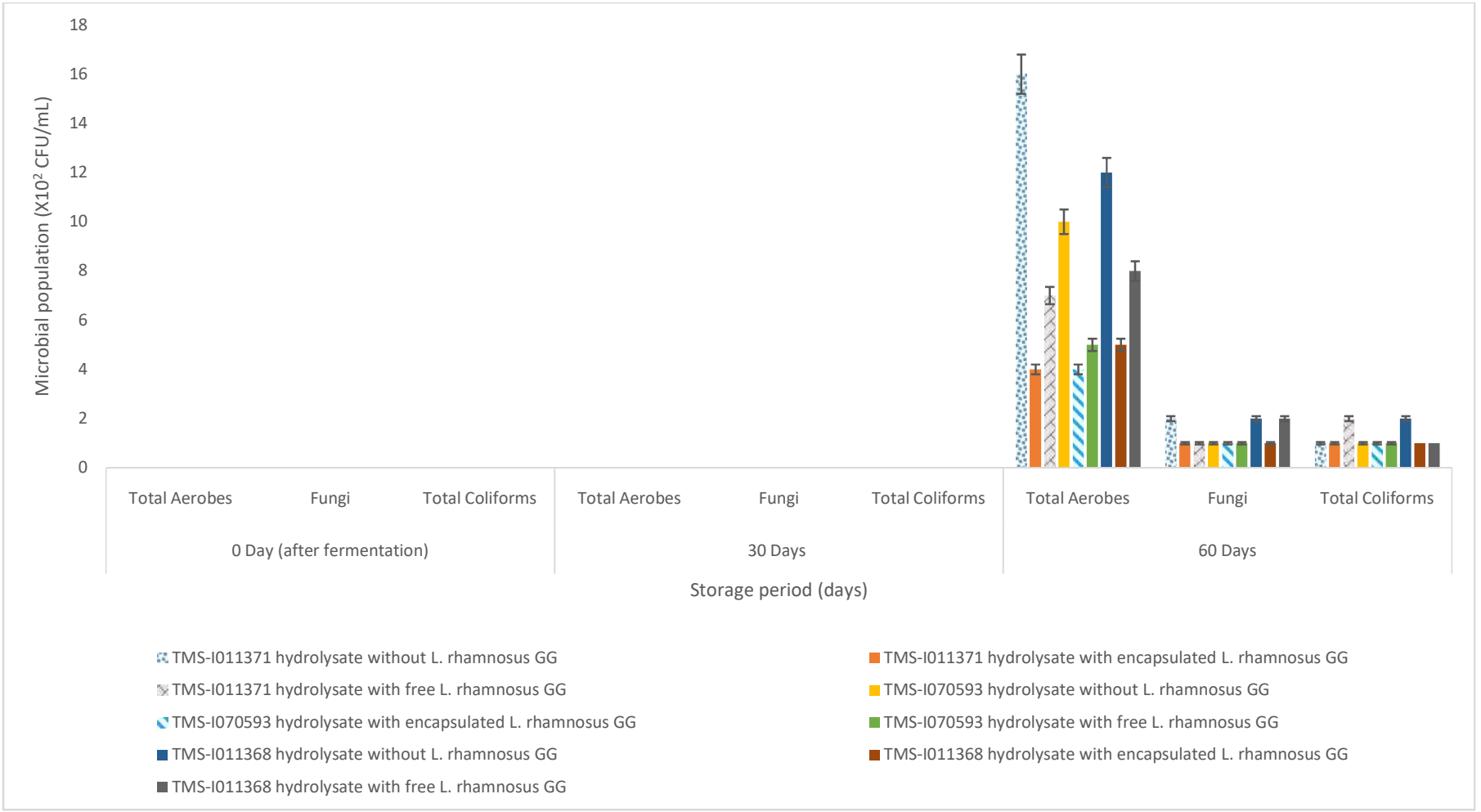


Figure 4.18: Evaluation of presence of spoilage and pathogenic organisms in provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG* during storage at 4^{0}C for 60 days.

that *L. rhamnosus GG* singly (among other probiotics tested) exerted antimicrobial activity against *Salmonella enterica Typhimurium,* a gram-negative pathogen, and that the antibacterial activity was solely due to the production of lactic acid. *Lactobacillus rhamnosus GG* was also reported to have antimicrobial activity which was particularly due to lactic acid, against *Shigella sonnei* (Zhang *et al*., 2011*).*De Keersmaecker *et al.* (2006) also identified that *L. rhamnosus GG* produced another antimicrobial agent apartfrom lactic acid. Acetic acid was attributed to having an inhibitory effect against other pathogenic bacteria in the pH range of 3-5.

Mycotoxins, which is usually initiated by mould growth, occur naturally as toxicants in foods especially where the raw materials have been contaminated (Adegoke and Letuma, 2013). These mycotoxins have been proved to be biologically de-contaminated by *L. rhamnosus GG. L. rhamnosus GG* and *L. rhamnosus LC705* were reported to remove aflatoxin B1 effectively (as much as 80% w/w toxin) from a culture media that was previously contaminated (Shetty and Jespersen, 2006).

4.10 Animal study

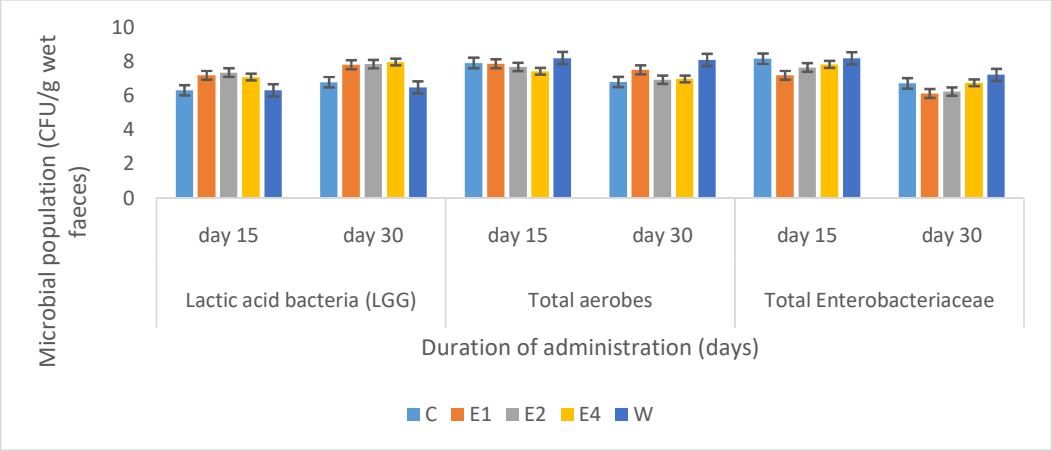
4.11 Effect of administration of provitamin A cassava hydrolysate on faecal microbial ecology and growth direction index of Wistar rats.

Figure 4.19 shows the counts of the total microflora cultivable in the faeces of Wistar rats on day 15 and day 30 after the administration of the provitamin A cassava hydrolysate without *L. rhamnosus GG* or with encapsulated $1x10^{10}$ CFU (PHE1), 2 $x10^{10}$ CFU (PHE2) or 4 x 10^{10} CFU *L. rhamnosus GG* (PHE4) or provitamin A cassava hydrolysate with free $1x10^{10}$ CFU/ml (PHF1), 2 $x10^{10}$ CFU/ml (PHF2) or 4 x 10^{10} CFU/ml *L. rhamnosus GG* (PHF4) As at day 15 (basal population), the population of lactic acid bacteria (LAB) enumerated in the faeces of the control rats was 6.30 Log CFU/g wet faeces while the population of total aerobes and the total enterobacteriaceaein control rats were 8.19 Log CFU and 8.18 Log CFU/g wet faeces respectively.

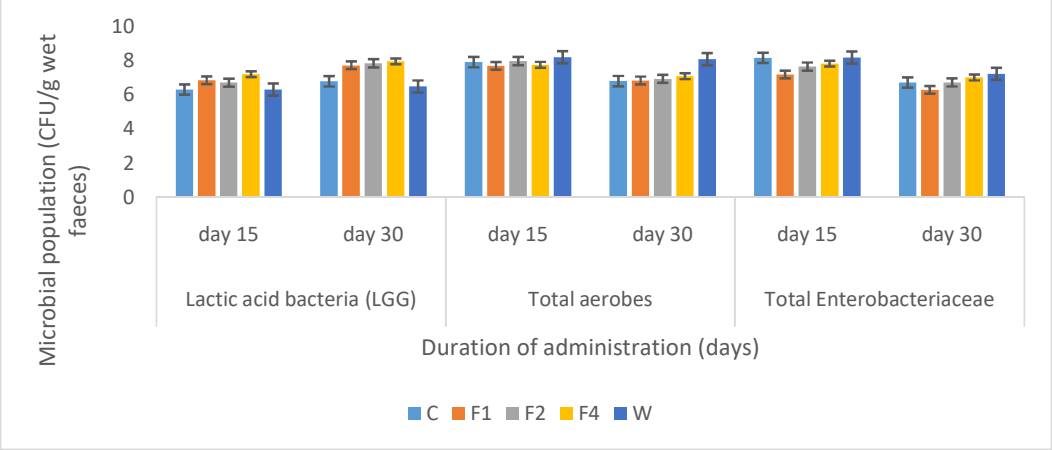
The population of LAB in rats administered PHE ranged between 7.08 and 7.34 Log CFU/g wet faeces on day 15, but increased to 7.81 (PHE1) – 7.96 (PHE4) Log CFU/g wet faeces by day 30. The population of LAB in rats administered PHF increased from 6.70 – 7.20 Log CFU/g wet faeces on day 15, to 7.71 (PHF1)) – 7.94 (PHF4) Log

CFU/g wet faeces on day 30.The population of total aerobes in control rats was 7.90 Log CFU/g

(a). Effect of provitamin A cassava hydrolysate with encapsulated *L. rhamnosus GG* on the faecal microbial ecology of Wistar rats



(b). Effect of provitamin A cassava hydrolysate with free *L. rhamnosus GG* on the faecal microbial ecology of Wistar rats

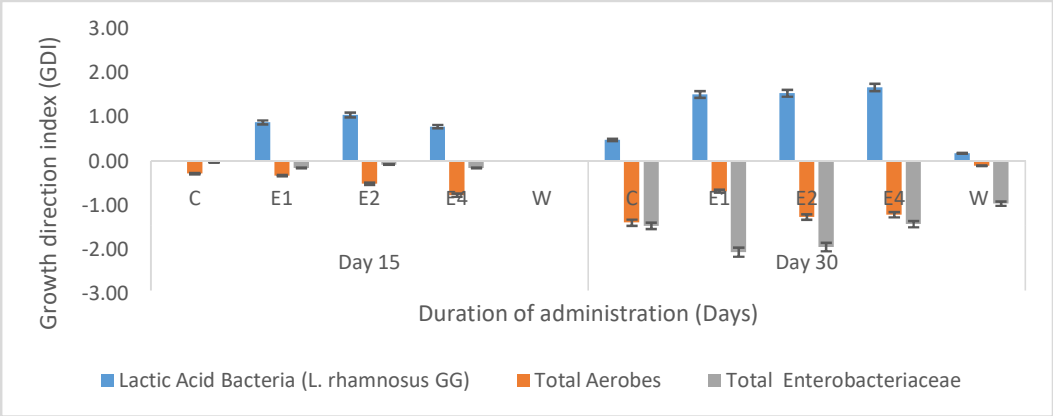


wet faeces. The rats administered PHE decreased in the population of total aerobes from 7.41 – 7.85 Log CFU/g wet faeces on day 15, to 6.91 – 7.51 by day 30. Rats administered PHF also decreased in the population of total aerobes from 7.68 – 7.96

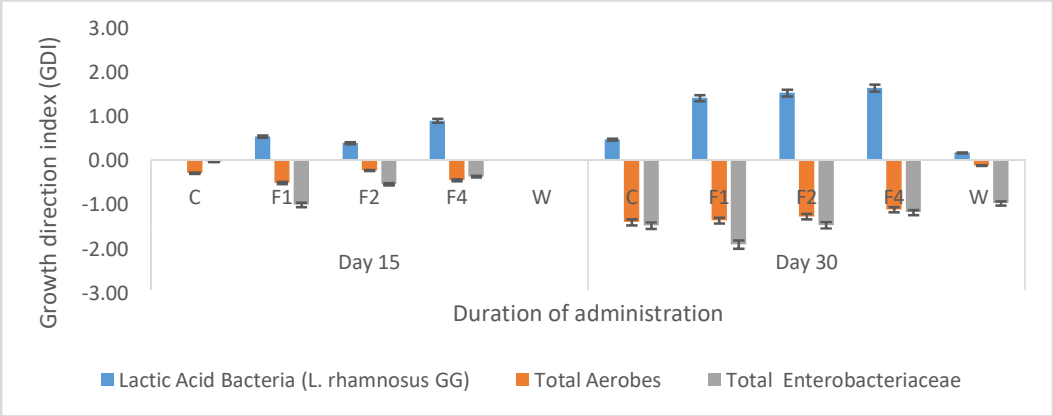
Log CFU/g wet faeces on day 15, to 6.83 – 7.08 Log CFU/g wet faeces by day 30. Total Enterobacteriaceaedecreased in rats administered PHE and PHF from 7.64 – 8.15 Log CFU/g wet faeces to 6.11 – 6.75 Log CFU/g wet faeces, andfrom 7.17 – 7.81 Log CFU/g wet faeces to 6.28 – 7.00 Log CFU/g wet faeces respectively between days 15 and 30.

The growth direction index of microbial population of Wistar rats administered provitamin A cassava hydrolysate is presented in Figure 4.20. The growth direction index (GDI) of faecal lactic acid bacteria (*L. rhamnosus GG*) expanded in rats administered PHE and PHF from 0.78 – 1.04 to 1.51 (PHE1) - 1.66 (PHE4), and from 0.40 – 0.90 to 1.41 (PHF1) – 1.64 (PHF4) respectively between days 15 and 30. GDI of total aerobes in rats administered PHE and PHF reduced from -0.77 – -0.33 to -1.27 (PHE2) – -0.68 (PHE1) and from -0.22 - -0.51 to -1.36 (PHFI) - -1.10 (PHF4) respectively between days 15 and 30. Total Enterobacteriaceaealso decreased in GDI in the rats administered PHE and PHF from -0.16 – -0.08 to -2.06 (PHE1) – -1.43 (PHE4), and -1.00 – -0.53 to -1.89 (PHF1) – -1.17 (PHF4).

There was a variation in the basal population of the gut microflora of the individual Wistar rats observed on day 15. However by day 30, there was a significant change in the population of the microflora compared to the basal population. Adak et al. (2013) noted that ratio of the bacteria population in rat intestines with respect to specific groups, may vary within species and between different individuals in the same species.The population of the lactic acid bacteria (*L. rhamnosus GG*) enumerated in the rats administered PHE or PHF increased significantly ($\alpha_{0.05}$) in a dose-dependent pattern, with the rats administered PHE4 or PHF4 having the highest population of lactic acid bacteria (*L. rhamnosus GG*) in the faeces by the 30th day. There was a significant ($\alpha_{0.05}$) reduction in the population of the total aerobic organisms, and total Enterobacteriaceaeenumerated in the faeces of all the rats administered PHE or PHF by day 30 in comparison with the basal population taken at day 15, and in comparison to the control rats. However the control group did not differ significantly from the group that received PH.



(a). Growth direction index (GDI) of faecal microflora of Wistar rats administered TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*



(a). Growth direction index (GDI) of faecal microflora of Wistar rats administered TMS-I011368 hydrolysate with free*L. rhamnosus GG*

The observed increase in the total *LAB* population was observed with a concurrent reduction in the total aerobic counts and total Enterobacteriaceaecounts in each group. There was no significant difference in the populations between days 15 and 30 in the groups that received PH. The effect of differences in inoculum concentration of the *L. rhamnosus GG* was observed to be more pronounced in the encapsulated cells. Themicrobial population of the rats administered TMS-I011368 hydrolysate without *L. rhamnosus GG* by day 30 did not differ significantly ($\alpha_{0.05}$) from the basal populations obtained from all the groups at day 15. The observed changes in microbial population was due to the ability of lactic acid bacteria (*L. rhamnosus GG*) to colonise the intestine, indicated by the significant increase in the population of lactic acid bacteria (*L. rhamnosus GG*). *L. rhamnosus GG*, being a facultative anaerobic bacteria tends to create an anaerobiosis in the environment, thereby reducing the activity of aerobic organisms (Adak *et al.*, 2013). Moreover, the competition for nutrients and energy source tends to shift in favour of the over-populated organisms (Maity *et al.*, 2012).

The effect of the probiotic *L. rhamnosus GG* was better explained by the Growth direction index. The growth direction index (GDI) of *L. rhamnosus GG* wasobserved to move in the positive direction for all the groups by day 30as compared to their basal population (day 15). This shows an expansion in their population while there was a contraction in the population of total aerobes and pathogens. This was indicated by the GDI of the total aerobes and total enterobacteriaceae as their GDI shifted more in the negative direction. It was explained by Samanta *et al.*(2014) that probiotics promote gut health by influencing enterocyte turnover, competing with pathogenic bacteria for nutrients and binding sites, and producing bacteriostatic compounds that limit the growth of pathogenic bacteria. Therefore, it was observed that as the population of lactic acid bacteria, (*L. rhamnosus GG*)increased, it resulted in a concurrent decrease of the total aerobes and total enterobacteriaceae. This observation further confirms the ability of *L. rhamnosus GG* to colonise the intestine as it has been established as a property characterising *L. rhamnosus GG* as a probiotics. The expansion of GDI of *L. rhamnosus GG* showed that the bacteria was able to out-compete the total aerobes and other pathogens in the intestine, and eventually colonise the intestine.

The observation in this study negates the findings of Maity *et al.*(2012) which reported a decrease in the population of total anaerobes (LAB) with a concurrent increase in the population of total aerobes and Enterobacteriaceae at hyperbaric conditions. Adak *et*

al.(2013) studied the changes in the gut microflora of humans at hypobaric conditions and reported a decrease in total aerobes with an increase in the population of total anaerobes (e.g. *Lactobacillus*), and increase in *E. coli* and otherEnterobacteriaceae. The altered air pressures in these reported studies were responsible for the contrast with the findings of this present study which did not involve any alteration of the normal atmospheric conditions.

Normal gut microbiota exerts many functions including resistance to infectionscaused by pathogenic organisms, vitamin B synthesis, and improvement in digestion (Azad *et al.*, 2018), improvement of the integrity of gut barrier, and reduction of oxidative stress and associated inflammation. Disease conditions e.g. cardiovascular disease, obesity, cancer, type 2 diabetes mellitus, and numerous immune disorders could alter the gut microbiota composition (Zhang *et al.*, 2018, Azad *et al.*, 2018). Imbalance of normal microflora has been shown to result inincreased rates of infections in the intestinal tract as well as systemic (McFarland, 2014). When a sub-population of the normal microbiota is absent, the ability to break down fibres and starches into absorbable short chain fatty acids may also be reduced or totally lost, resulting in incomplete digestion of carbohydrates which can trigger diarrhoea (Canani *et al.*, 2011, McFarland, 2014).

A higher relative abundance of Enterobacteriaceae can result in bloating and digestive discomfort, as a result of gas production through their metabolism (Tintore *et al.*, 2017). Lipopolysaccharide (LPS) molecules (also known as endotoxins) from Enterobacteriaceae are particularly pro-inflammatory, compared to LPS from other common gut Gram negative bacteria such as Bacteroides(Tintore *et al.*, 2017). In contrast, a high proportion of *Lactobacillus* and *Bifidobacterium* has a protective effect which might be associated with the ability of mucosal *Lactobacillus* to induce the expression of anti-inflammatory genes, thus normalising gut microflora balance (Chen *et al.*, 2019). In essence the anti-inflammatory activity of *Lactobacillus* in the gut counter-acts the pro-inflammatory activities of LPS produced from Enterobacteriaceae.

4.12 Toxicological evaluation of probiotic provitamin A cassava hydrolysate

4.12.1 Acute toxicity of probiotic provitamin A cassava hydrolysate

Following the single oral administration of probiotic provitamin A cassava hydrolysate at the doses presented in Table 4.12, there was no observed death or change in the

Table 4.12: Acute Toxicity of provitamin A cassava hydrolysate with or without *L. rhamnosus GG* in Wistar Rats: Toxicity and Mortality

Treatment groups	Dose (per kg body weight)	Number of Rats with signs of toxicity	Number of Deaths
Provitamin A cassava hydrolysate without *L. rhamnosus GG*	0.067mg	0	0
	0.134mg	0	0
	0.497mg	0	0
Provitamin A cassava hydrolysate with *L. rhamnosus GG*	1.0×10^{10} CFU/ml	0	0
	5.0×10^{10} CFU/ml	0	0
	1.0×10^{11} CFU/ml	0	0
	2.0×10^{11} CFU/ml	0	0
	4.0×10^{11} CFU/ml	0	0
	8.0×10^{11} CFU/ml	0	0

physical characteristics or appearance of the rats throughout the 24hours observation period. All the rats in each treatment group survived and showed normal behaviour including normal appetite. This suggests that the acute oral administration of TMS-I011368 hydrolysate without or with *L. rhamnosus GG* is safe.

4.12.2 Sub-chronic toxicity of provitamin A cassavahydrolysate

4.12.2.1 Effect of provitamin A cassava hydrolysate on the weight gain of Wistar Rats

The effect of provitamin A cassava hydrolysate on body weight gain (%) of rats is presented in Table 4.13.The rats administered water only (control) gained 11.65% body weight by the end of the 1st week and 40.00% by the end of the 4th week. The rats administered TMS-I011368 hydrolysate without *L. rhamnosus GG* (PH) gained 13.83% and 29.00% bodyweight by the end of the 1st and 4th week respectively. The body weight gain (%) of the rats administered TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG* (PHE) ranged from 18.24 to 30.34%, while the body weight gain (%) of rats administered with TMS-I011368 hydrolysate with free *L. rhamnosus GG* (PHF) ranged from 8.04 to 34.59% by the end of the 4th week.

The highest % body weight gain in this study was observed in the control rats, while the lowest % body weight gain in this study ($\alpha_{0.05}$) was observed in rats administered the highest concentration of free or encapsulated *L. rhamnosus GG* supplementation ($4x10^{10}$ CFU). It was generally observed that the weight gain of all the animals that received *L. rhamnosus GG* was well controlled when compared with the control rats. The % weight gain of rats administered PHE or PHF reduced with increasing doses of *L. rhamnosus GG* in a dose-dependent manner. This could be attributed to the anti-obesity effects of *L. rhamnosus GG.*

Christiakov *et al* (2015) reported that *L. rhamnosus GG* and other probiotics exert anti-obesity effects which was achieved through mechanisms such as reducing the frequency of obesity-associated commensalsFirmicutesandBacteroidetesin the intestines, reduction in epididymal fat depositions and modification by down-regulating the actions of lipid-synthesising enzymes in the liver. These in turn reduces the epididymal fat deposition and obesity tendency (Chistiakov *et al.*, 2015). This observation is consistent with the report of Ji *et al.* (2012) that established the anti-

obesity effect of *L. rhamnosus GG* and other probiotics in mice fed with a high-fat diet.

Table 4.13: Effect of provitamin A cassava hydrolysate with or without *L. rhamnosus GG* on percentage body weight gain of rats

Treatment groups	Body weight gain (%)			
	Week 1	Week 2	Week 3	Week 4
TMS-I011368 hydrolysate without *L. rhamnosus GG*	13.83	19.78	24.58	29.00
TMS-I011368 hydrolysate with $1x10^{10}$ CFU/g encapsulated *L. rhamnosus GG*	12.84	25.84	28.57	30.34
TMS-I011368 hydrolysate with $2x10^{10}$ CFU/g encapsulated *L. rhamnosus GG*	2.37	14.00	18.51	23.49
TMS-I011368 hydrolysate with $4x10^{10}$ CFU/g encapsulated *L. rhamnosus GG*	4.39	13.72	16.91	18.24
TMS-I011368 hydrolysate with $1x10^{10}$ CFU/mL free *L. rhamnosus GG*	10.50	18.91	29.97	34.59
TMS-I011368 hydrolysate with $2x10^{10}$ CFU/mL free *L. rhamnosus GG*	6.52	17.47	22.23	24.38
TMS-I011368 hydrolysate with $4x10^{10}$ CFU/mL free L. rhamnosus GG	7.81	10.68	9.18	8.04
Water only (Control)	11.65	26.14	36.69	40.00

The review by Cani and Delzenne (2009) concluded that changing the gut microbiota composition by the inclusion of probiotics (and/or prebiotics) may participate in the control of several parameters involved in the development of metabolic disorders associated with obesity.Kobyliak *et al.* (2017)evaluated probiotics for experimental obesity prevention with focus on strain dependence and viability of composition. The weight responses of rats placed on different combinations of high-fat diets were tested against different composition of probiotics (multi-strain probiotics combination and single strain probiotics). The study reported that when probiotic composed of live strains are used for supplementation, it resulted in significant weight reduction hence lowering the prevalence of obesity (Kobyliak *et al.*, 2017). There have been several studies showing evidences in support of the 'anti-obesity' effect of probiotics of the *Lactobacillus and Bifidobacterium* genera on diet-induced obesity in animal models (Lee *et al.*, 2006,Maity *et al.*, 2009, Miyoshi *et al.*, 2014). Therefore, weight management can be achieved through probiotic *L. rhamnosus GG* administration.

4.12.2.2 Effect of provitamin A cassava hydrolysate with or without*L. rhamnosus GG* on the haematological parameters of Wistar rats

The data showing the effect of provitamin A cassava hydrolysate on the haematological parameters of Wistar rats is presented in Table 4.14 and Table 4.15. The rats administered water had a packed cell volume (PCV) of 47.20±1.79% while rats administered PHhad a PCV of 42.80±0.84%. Rats administered PHE2had the highest PCV with a value of 47.40±2.30% while rats administered PHhad the lowest PCV of 42.80±0.84%. Red blood cell counts (RBC) of the rats administered water only was 10.85±1.23% while RBC in rats administered PH, PHE or PHF ranged between 7.88±1.10 and 11.63±1.61. The rats administered water only had mean corpuscular volume (MCV) and Mean cell haemoglobin (MCH) values of 43.00±4.74 *f*l and 13.40±0.89 pg respectively. The highest MCV and MCH values were 50.00±11.90 *f*l and 20.20±3.27 pg respectively in rats administered PHE2, while the lowest MCV and MCH were 39.00±8.60 *f*l and 13.40±3.44 pg respectively in rats administered PHF4. Mean corpuscular haemoglobin concentration (MCHC) was unchanged in all the rats in this study. Erythrocyte sedimentation rate (ESR) was 12.80±1.10 and 9.20±2.28 for water and PH respectively.

.

Table 4.14: Effect of provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on the haematological parameters of Wistar rats.

Treatment Groups	Packed cell volume (%)	Haemoglobin (g/dL)	Red blood cells ($x10^6\mu L$)	Mean corpuscular volume (*f*l)	Mean cell haemoglobin (pg)	Mean corpuscular haemoglobin concentration (g/dl)	Erythrocyte Sedimentation Rate
TMS-I011368 hydrolysate without *L. rhamnosus GG*	42.80±0.84^{b}	14.14±0.25^{a}	10.06±2.27^{ab}	44.00±11.27^{a}	14.40±3.65^{b}	33.04±0.06^{a}	9.20±2.28^{b}
TMS-I011368 hydrolysate with encapsulated $1x10^{10}$CFU/g *L. rhamnosus GG*	42.80±3.03^{b}	14.28±0.89^{b}	8.23±2.45^{ab}	44.60±13.01^{a}	18.00±6.67^{ab}	33.38±0.76^{a}	11.60±2.61^{ab}
TMS-I011368 hydrolysate with encapsulated $2x10^{10}$CFU/g *L. rhamnosus GG*	47.40±2.30^{a}	16.06±1.40^{ab}	7.88±1.10^{ab}	50.00±11.90^{a}	20.20±3.27^{a}	33.85±1.84^{a}	9.00±2.00^{b}
TMS-I011368 hydrolysate with encapsulated $4x10^{10}$CFU/g *L. rhamnosus GG*	46.20±1.48^{ab}	15.26±0.51^{ab}	10.84±2.71^{ab}	44.00±10.07^{a}	14.20±3.11^{b}	33.03±0.07^{a}	10.40±1.67^{ab}
TMS-I011368 hydrolysate with free $1x10^{10}$CFU/mL *L. rhamnosus GG*	43.80±5.85^{ab}	14.48±1.95^{ab}	10.39±1.97^{a}	43.00±10.32^{a}	14.00±3.24^{b}	33.06±0.05^{a}	11.40±2.61^{ab}
TMS-I011368 hydrolysate with free $2x10^{10}$CFU/mL *L. rhamnosus GG*	44.80±2.39^{ab}	14.82±0.80^{ab}	9.87±2.16^{ab}	47.40±13.96^{a}	15.20±4.60^{ab}	33.08±0.04^{a}	13.20±2.28^{a}
TMS-I011368 hydrolysate with free $4x10^{10}$CFU/mL *L. rhamnosus GG*	46.20±3.83^{ab}	15.68±2.01^{ab}	11.63±1.61^{ab}	39.00±8.60^{a}	13.40±3.44^{b}	33.85±1.79^{a}	12.00±2.45^{ab}
Water (Control)	47.20±1.79^{ab}	15.60±0.59^{ab}	10.85±1.23^{b}	43.00±4.74^{a}	13.40±0.89^{b}	33.05±0.05^{a}	12.80±1.10^{a}

*Means ± sd with the same superscripted alphabets in the same columns are not significantly different $_{(\alpha 0.05)}$

Table 4.15: Effect of probiotic provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on the differential white blood cell parameters of Wistar rats

Treatment groups	White Blood Cells (WBC) ($X10^3$cells/µl)	Lymphocyte ($X10^3$cells/µl)	Neutrophil ($X10^3$cells/µl)	Platelets ($X10^5$cells/µl)
TMS-I011368 hydrolysate without *L. rhamnosus GG*	9.60 ± 0.71^{ab}	2.87 ± 0.35^{ab}	6.73 ± 0.45^{ab}	1.20 ± 0.00^{b}
TMS-I011368 hydrolysate with encapsulated $1x10^{10}$CFU/g *L. rhamnosus GG*	9.68 ± 0.79^{ab}	2.24 ± 0.36^{c}	7.44 ± 0.82^{a}	1.28 ± 0.11^{ab}
TMS-I011368 hydrolysate with encapsulated $2x10^{10}$CFU/g *L. rhamnosus GG*	10.24 ± 0.83^{ab}	2.63 ± 0.58^{bc}	7.61 ± 0.65^{a}	1.32 ± 0.11^{ab}
TMS-I011368 hydrolysate with encapsulated $4x10^{10}$CFU/g *L. rhamnosus GG*	10.36 ± 0.70^{ab}	3.09 ± 0.38^{ab}	7.27 ± 0.45^{a}	1.36 ± 0.09^{ab}
TMS-I011368 hydrolysate with free $1x10^{10}$CFU/mL *L. rhamnosus GG*	10.48 ± 0.69^{a}	3.40 ± 0.11^{a}	7.08 ± 0.72^{ab}	1.30 ± 0.20^{ab}
TMS-I011368 hydrolysate with free $2x10^{10}$CFU/mL *L. rhamnosus GG*	9.80 ± 0.96^{ab}	3.20 ± 0.44^{a}	6.60 ± 0.59^{ab}	1.32 ± 0.11^{ab}
TMS-I011368 hydrolysate with free $4x10^{10}$CFU/mL *L. rhamnosus GG*	10.12 ± 1.42^{ab}	2.98 ± 0.26^{ab}	7.14 ± 1.17^{a}	1.32 ± 0.11^{ab}
Water (Control)	9.00 ± 1.28^{b}	3.09 ± 0.35^{ab}	5.91 ± 1.40^{b}	1.40 ± 0.00^{a}

*Means ± sd with the same superscripted alphabets in the same columns are not significantly different ($\alpha_{0.05}$)

ESR in the rats administered PHEranged between 9.00±2.00 and 11.60±2.61, while for rats administered PHF ranged between 11.40±2.61 and 13.20±2.28.Erythrocyte Sedimentation Rate (ESR) in control rats was 12.80±1.10. All treated rats had ESR values that were insignificantly different from control in the range of 10.40±1.67 and 13.20±2.28, except PHE2 (9.00±2.00) and PH (9.20±2.28) that were significantly ($\alpha_{0.05}$) lower than control. The white blood cell counts (WBC) were 9.00±1.28 and 9.60±0.71 $X10^3\mu L$ for the rats administered water (Control) and PH respectively. The rats administered PHE1 and PHE2had WBC counts of 9.68±0.79 $X10^3\mu L$ and 10.24±0.83 $X10^3\mu L$ respectively while PHF2 had WBC count of 9.80±0.96 $X10^3\mu L$. The differential white blood cell count showed that lymphocyte counts for rats administered PHF or PHE ranged between 2.98±0.26 to 3.40±0.11 $X10^3\mu L$ and 2.11±0.36 to 3.09±0.38 $X10^3\mu L$ respectively, while it was 3.09±0.35 $X10^3\mu L$ for water group.

The neutrophil counts for rats administered PHFand the rats administered PHEranged between 6.60±0.59 to 7.14±1.17 $X10^3\mu L$ and 7.27±0.45 to 7.61±0.65 $X10^3\mu L$ respectively, while that of rats which received only water was 5.91±1.40 $X10^3\mu L$. Platelet counts for the rats that received PHF or PHEranged between 1.30±0.20 to 1.32±0.11 $X10^5\mu L$ and 1.28±0.11 to 1.36±0.09 $X10^5\mu L$ respectively. The rats administered only water had platelet counts of 1.40±0.00 $X10^5\mu L$.

Evaluation of haematological parameters provide information on the effect of provitamin A cassava hydrolysate on the blood cells. The result showed a non-significant decline in the red blood cells and its indices which implies that the hydrolysate without or with the probiotic organism may be toxic to these blood cells if administered for a protracted period.The Erythrocyte indices i.e. mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (MCV, MCH and MCHC) were non-significantly increased by the provitamin A cassava hydrolysate at all doses of *L. rhamnosus GG* administered. The RBC, haemoglobin, and MCHC are parameters that give useful information about the size and haemoglobin content of the red blood cells, thus make it easy to understand the health condition or underlying cause of a disease (Sarma, 1990, Johnstone *et al.*, 2017).

These values compared well with the control at all the doses of *L. rhamnosus GG* tested- whether free or encapsulated. This is an indication that the provitamin A

cassava hydrolysate with or without probiotic may not have caused significant destruction of the existing red blood cells. It also indicates that the oxygen-carrying capacity of the blood has not been inhibited by the 30-day oral administration of the Provitamin A cassava hydrolysate. However, this study was for 30 days only, which may not be long enough to precipitate clinical anaemia that may occur with chronic administration of PH without or with *L. rhamnosus GG*. There was a significant ($\alpha_{0.05}$) leukocytosis, an increase in the white blood cell counts (WBC) of the rats dosed with PH without or with *L. rhamnosus GG* (free or encapsulated) when compared with the rats administered water only.A significant lymphopaenia, a decrease in the lymphocyte counts was also observed with a corresponding neutrophilia, increase in the neutrophil counts. Provitamin A hydrolysate (PH) administered alone induced minimal leukocytosis but when administered with *L. rhamnosus GG* may induce acute inflammatory responses with mobilisation of inflammatory cytokines (Garcia *et al.*, 2015). White blood cells (WBC) are known to carry out their defensive roles against agents that are capable of causing infections by producing, transporting and distributing antibodies in immune responses (Berinyuy *et al.*, 2015).

This current study suggests that the administration of PH with *L. rhamnosus GG* induced bacteremia with evidence of neutrophilia, especially if administered for a protracted period. These findings corroborate existing reports on the tendency of *L. rhamnosus GG* to induce bacteremia. Daily supplementation with *L. rhamnosus GG* may probably enhance the risk of developing *L. rhamnosus GG* sepsis owing to prolonged overload of microorganism (Dani *et al.*, 2016).Also, PH without *L. rhamnosus GG* induced a significant thrombocytopenia, which is reduced platelet count, but the depression was reversed with the probiotic. *L. rhamnosus GG* in itself is a natural commensal of the gastrointestinal tract, non-pathogenic, has been recognised as safe hence used as a probiotic, but may cause infections (Dani *et al.,* 2016). *L. rhamnosus GG* isolates have been implicated in infections. Although rare, cases of *Lactobacillus* infection such as bacteremia and septicemia have been reported in immuno-compromised patients and even immuno-competent individuals (Salminen *et al.*, 2002, Vahabnezhad *et al.*, 2013, Meini *et al.*, 2015, Koyama *et al.*, 2018). The ability of *L. rhamnosus GG* to attach to the intestine while effectively and temporarily colonising it could be responsible for the potential of this strain to cause infections (Salminen *et al.*, 2002).

Possibility of bacterial translocation as a result of damage to the oral or intestinal mucosa has been suggested as a probable reason for access of *L. rhamnosus GG* into the blood stream (Aroutcheva *et al.*, 2016). However, Dani *et al.* (2016) noted that there was no report of systemic infections due to administered *L. rhamnosus GG* in all the reports reviewed, thus supporting the safety of the probiotic supplementations. Results of this study also showed that administration of PH with *L. rhamnosus GG* will not adversely interfere with blood clotting. This is in agreement with Nissila *et al.* (2017) who reported that *L. rhamnosus GG* did not induce platelet aggregation activity in their study. Blood clots are formed by aggregation of platelet and clotting factors (Azizpour *et al.*, 2017)**.**

4.12.2.3 Effect of Provitamin A cassava hydrolysate on serum biochemical indices of Wistar rats

The effect of provitamin A cassava hydrolysate on plasma protein is presented in Table 4.16. The control rats had total protein concentration of 7.29±1.52 g/dL, with Albumin value of 4.41±1.12g/dL, globulin value of 2.88±0.62 and Albumin: Globulin ratio of 1.55±0.33. Total protein in rats administered PHE2 was the highest (7.56 ± 1.35 g/dL), with albumin and globulin concentrations of 4.74±0.83 and 2.82±0.56 g/dL respectively, and Albumin: Globulin ratio of 1.43±0.31, while rats administered PH had the lowest total protein (4.39±0.10 g/dL), with albumin (3.25±0.15 g/dL), globulin (1.14±0.07g/dL) and albumin: globulin ratio of 2.87±0.29 respectively. Table 4.17 presents the effect of provitamin A cassava hydrolysate on liver enzymesAST, ALT and ALP determined. Rats administered water only (Control) had AST values of 74.40±8.29 IU/L, with ALT values of 62.60±7.92 IU/L and ALP values of 49.20±4.87 IU/L. Rats administered PHE2 had the highest AST, ALT and ALP values of 79.80 ± 4.02, 72.00 ± 6.44 IU/L and 61.60 ± 1.52 IU/L respectively, while rats administered PHE1 had the lowest AST, ALT and ALP values of 48.60±3.13, 38.00±2.00 and 41.20±1.10 IU/L respectively.

Table 4.18 shows the effects of the probiotic provitamin A cassava hydrolysate on the blood lipid profile of the Wistar rats. The total cholesterol in rats administered water only was 65.40±1.14 mg/dL. The highest total cholesterol was recorded in rats administered PHE2 with values of 69.20 ± 0.84 while the lowest was observed in rats administered PHE1 (42.40±0.55 mg/dL). Triglycerides and plasma glucose concentration in control rats were 56.60±0.71mg/dL and 49.00±1.00 mg/dL

respectively. The highest was recorded in rats administered PHE2 with values of 55.60±1.14 and 63.40±1.34 mg/dL respectively, while the lowest triglycerides and

Table 4.16: Effect of Provitamin A cassava hydrolysatewith or without *L. rhamnosus GG* on Plasma protein

Treatment groups	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin: Globulin Ratio
TMS-I011368 hydrolysate without *L. rhamnosus GG*	4.39±0.10^{b}	3.25±0.15^{bc}	1.14±0.07^{d}	2.87±0.29^{a}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	4.49±1.04^{b}	2.72±0.64^{c}	1.78±0.40^{cd}	1.52±0.08^{c}
TMS-I011368 hydrolysate with encapsulated 2x10^{10}CFU/g *L. rhamnosus GG*	7.56±1.35^{a}	4.74±0.83^{a}	2.82±0.56^{ab}	1.69±0.16^{bc}
TMS-I011368 hydrolysate with encapsulated 4x10^{10}CFU/g *L. rhamnosus GG*	6.25±0.28^{a}	4.10±0.36^{ab}	2.15±0.09^{bc}	1.90±0.23^{b}
TMS-I011368 hydrolysate with free 1x10^{10}CFU/mL *L. rhamnosus GG*	7.48±1.51^{a}	4.65±0.88^{a}	2.84±0.66^{ab}	1.66±0.19^{bc}
TMS-I011368 hydrolysate with free 2x10^{10}CFU/mL *L. rhamnosus GG*	6.67±0.75^{a}	3.88±0.46^{ab}	2.80±0.55^{ab}	1.43±0.31^{c}
TMS-I011368 hydrolysate with free 4x10^{10}CFU/mL *L. rhamnosus GG*	7.15±1.70^{a}	4.22±1.03^{ab}	2.94±0.68^{a}	1.43±0.69^{c}
Water (Control)	7.29±1.52^{a}	4.41±1.12^{a}	2.88±0.62^{a}	1.55±0.33^{c}

*Means ± sd superscripted by the same alphabet in the same column are not significantly different ($\alpha_{0.05}$).

Table 4.17: Effect of provitamin A cassava hydrolysate with or without *L. rhamnosus GG* on liver enzymes of Wistar rats

Treatment groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
TMS-I011368 hydrolysate without *L. rhamnosus GG*	54.80±1.10^{c}	44.20±0.84^{c}	51.00±1.41^{bc}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	48.60±3.13^{c}	38.00±2.00^{c}	41.20±1.10^{cd}
TMS-I011368 hydrolysate with encapsulated 2x10^{10}CFU/g *L. rhamnosus GG*	79.80±4.02^{a}	72.00±6.44^{a}	61.60±1.52^{a}
TMS-I011368 hydrolysate with encapsulated 4x10^{10}CFU/g *L. rhamnosus GG*	72.40±1.67^{ab}	62.20±1.48^{b}	52.80±1.10^{ab}
TMS-I011368 hydrolysate with free 1x10^{10}CFU/mL *L. rhamnosus GG*	68.80±11.01^{b}	60.00±8.97^{b}	49.20±13.39^{bcd}
TMS-I011368 hydrolysate with free 2x10^{10}CFU/mL *L. rhamnosus GG*	66.60±7.92^{b}	56.40±7.73^{b}	45.60±7.80^{bcd}
TMS-I011368 hydrolysate with free 4x10^{10}CFU/mL *L. rhamnosus GG*	65.00±10.51^{b}	54.80±10.52^{b}	39.60±11.26^{d}
Water (Control)	74.40±8.29^{ab}	62.60±7.92^{b}	49.20±4.87^{bcd}

*Means ± sd with the same alphabet superscripts within the same column are not significantly different (α0.05).

Table 4.18: Effect of Provitamin A cassava hydrolysateswith or without *L. rhamnosus GG* on blood lipid profile of Wistar rats

Treatment groups	Cholesterol (g/dL)	Triglycerides (g/dL)	High density lipoprotein (HDL) (g/dL)	Glucose (g/dL)	Blood urea nitrogen (BUN) (g/dL)	Creatinine (g/dL)
TMS-I011368 hydrolysate without *L. rhamnosus GG*	45.40±0.55^{g}	42.60±1.67^{d}	24.40±4.89^{abc}	52.80±1.10^{c}	1.24±0.02^{c}	1.38±0.04^{c}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	42.40±0.55^{h}	44.40±1.34^{d}	19.37±1.03^{c}	43.00±1.00^{f}	2.25±0.16^{a}	2.41±0.21^{b}
TMS-I011368 hydrolysate with encapsulated 2x10^{10}CFU/g *L. rhamnosus GG*	69.20±0.84^{a}	55.60±1.14^{a}	28.34±2.05^{ab}	63.40±1.34^{g}	2.16±0.32^{ab}	3.13±0.41^{a}
TMS-I011368 hydrolysate with encapsulated 4x10^{10}CFU/g *L. rhamnosus GG*	62.40±0.55^{c}	46.60±1.34^{c}	23.12±0.99^{bc}	55.40±0.55^{b}	2.09±0.05^{ab}	2.24±0.17^{b}
TMS-I011368 hydrolysate with free 1x10^{10}CFU/mL *L. rhamnosus GG*	60.00±0.71^{d}	52.00±2.12^{b}	29.10±5.92^{ab}	53.20±1.30^{c}	1.91±0.44^{b}	1.93±0.46^{b}
TMS-I011368 hydrolysate with free 2x10^{10}CFU/mL *L. rhamnosus GG*	55.60±0.55^{e}	46.40±1.14^{c}	26.82±3.38^{ab}	47.20±0.84^{e}	1.22±0.08^{c}	1.35±0.09^{c}
TMS-I011368 hydrolysate with free 4x10^{10}CFU/mL *L. rhamnosus GG*	54.60±0.56^{f}	44.40±1.14^{d}	27.48±6.84^{ab}	40.40±1.14^{g}	1.15±0.10^{c}	2.17±0.72^{b}
Water (Control)	65.40±1.14^{b}	56.60±0.71^{a}	29.48±3.99^{a}	49.00±1.00^{d}	1.28±0.13^{c}	1.94±0.43^{b}

Means ± sd superscripted by the same alphabet in the same column are not significantly different ($\alpha_{0.05}$)

plasma glucose concentrations were observed in PHF4 with values of 44.40±1.14 and 40.40±1.14 mg/dL respectively. The highest HDL concentration was recorded in rats administered PHF1 (29.10±5.92 mg/dL), while the lowest was recorded in PHE1 (19.37±1.03 mg/dL) whereas the control rats had HDL values of 29.48±3.99 mg/dL.The blood urea nitrogen (BUN) and Creatinine concentrations in control rats were 1.28±0.13 and 1.94±0.43 mg/dL respectively. The highest and lowest BUN were 2.25±0.16 (PHE1) and 1.15±0.10 (PHF4) respectively, while the highest and lowest Creatinine concentrations were 3.13±0.41 mg/ dL (PHE2) and 1.35±0.09 mg/ dL.

The total plasma protein and albumin concentrations of the treated rats were not significantly altered ($\alpha_{0.05}$) when compared with the control at all of the doses of the free *L. rhamnosus GG* in the hydrolysates administered. The levels of total protein, albumin and globulin perform important roles in determining their synthesis by the liver(Mishra and Tandon, 2012). They can be used as an indicator of potential hepatic damage. Albumins and globulin are the main components of serum proteins. The major function of albumin is the maintenance of the colloidal osmotic pressure which relates to fluid balance in the body (Pacovsky *et al.*, 2018). Albumin also acts as antioxidants and it is also useful in the transport of compounds such as fatty acids, cholesterol, bile acids, drugs and metal ions (Kim *et al.*, 2017). Poor nutrition status is indicated by lower albumin levels while elevated globulins indicate chronic inflammation (He *et al.*, 2017). The albumin: globulin ratio (A/G ratio) is used as a marker of changes in serum proteins as a result of diseases such as liver disease which could manifest as a reduction in A/G ratio as a result of decrease in serum albumin or increase in serum globulins (Hong *et al.*, 2013). In this study, the albumin:globulin ratio of the rats did not significantly change from the control.

Alanine Transaminase (ALT) and Aspartate Transaminase (AST) are also used as biochemical indicators of liver damage. These liver aminotransferases catalyze the transfer of amino groups during the metabolism of amino acids(Marchesini and Targher, 2011). Alkaline phosphatase (ALP) is a hydrolase enzyme often used to assess the wholeness of the plasma membrane as it is a marker enzyme for the plasma membrane and the endoplasmic reticulum in different cell types (Kunutsor *et al.*, 2014). These enzymes are used to assess the integrity of the liver as changes in their activity could indicate a possible toxicity of the organ (Malakouti *et al.*, 2017). For all groups, serum ALP was not significantly altered $_{(\alpha 0.05)}$ when compared with the control

group. Since the integrity of the plasma membrane and endoplasmic reticulum are often assessed by the alkaline phosphatases, any alteration of ALP activity may suggest injury or damage to the plasma membrane (Akanji and Yakubu, 2000). Observations from this study therefore suggest that the plasma membranes of the organs was not compromised by the PH without or with *L. rhamnosus GG.*

The lipid profile showed that total cholesterol, triglycerides were significantly decreased in the experimental groups with *L. rhamnosus GG* inclusion, with the exception of PHE2. This observation is similar to the findings of Nguyen *et al.* (2007) that reported the ability of probiotics to reduce serum cholesterol. Hasiri *et al*. (2015) administered *L. rhamnosus GG* to adult dogs at doses 10^{10}CFU/ml and found that the serum cholesterol of the dogs were significantly reduced. This drop in serum cholesterol was attributed to the ability of probiotics to bring about changes in cholesterol metabolism such as via bile salt deconjugation which in turn leads to a decrease in the absorption of lipids from the gut (Hasiri *et al.*, 2015). Wang *et al.* (2016) tested 5 strains of *Lactobacillus* including *L. rhamnosus* along with 3 strains of *Bifidobacterium* on Hamsters using *Rosurastatin* (a drug used for cholesterol reduction in humans) as control, to compare the effects of the probiotics on plasma lipid profile. The study reported that all the five strains of *Lactobacillus* were effective in the reduction of cholesterol in animals and it was therefore concluded that probiotics are suitable for cholesterol reduction and also advantageous for its lack of drug-like side effects. In 2016, the effect of two strains of *Lactobacillus rhamnosus- L. rhamnosus SKG34 and L. rhamnosus FBB42* isolated from fermented milk and faeces of healthy infants were investigated on the blood lipids profile of rats. The results showed that the two strains of probiotics either applied singly or in combination significantly lowered total serum cholesterol in rats fed with high fat diets (Nocianitri *et al.*, 2017). Several mechanisms have been established through which probiotics exert lowering of lipid profile.

Some of such mechanisms include the ability of probiotics to facilitate decrease in blood lipid levels by either preventing the synthesis of hepatic cholesterol alone or in combination with redistributing the cholesterol in the plasma from plasma to the liver (Jenkins *et al.*, 1981, Pereira and Gibson, 2002, Homayouni *et al.*, 2012, Maldonado-Pereira *et al.*, 2018). Other mechanisms involve taking up and assimilation of cholesterol for the stabilisation of their cell membrane (Liong and Shah, 2005),

binding cholesterol to the cell walls of probiotics in the intestine (Lye *et al.*, 2010), conversion of cholesterol to coprostanol (Lye *et al.*, 2010), deconjugation of bile acids through bile salts hydrolase catalysis (Patel *et al.*, 2010). The latter mechanism has been established by several authors as a major mechanism by which *Lactobacillus* achieves cholesterol-lowering effect. *Lactobacillus* have been explained as the major producer of intestinal bile salt hydrolase which is an intestinal bacteria-produced enzyme that exerts negative impact on the fat digestion and utilisation of the host (Smith *et al.*, 2014, Geng and Lin, 2017). Another new possibility has been proposed that LAB achieves the cholesterol-lowering effects by means of modulating the compositions of intestinal microbiota (Hu *et al.*, 2013).

Furthermore, the results of this study showed that blood urea nitrogen (BUN) and creatinine did not significantly increase in the rats, apart from the rats administered PH, PHE2 and PHF2 which had marginal increase. The kidneys regulate the excretion of urea, creatinine and reabsorption of electrolytes into the blood (Iyer and Kailasapathy, 2005). When there is a reduced filtering capacity of the kidney to filter, waste products will accumulate and this will lead to an increase in serum urea and creatinine levels (Salazar, 2014, Chutani and Pande, 2017). The observation of insignificant and marginal differences in the urea and creatinine concentrations in the plasma of the rats suggested that PH without or with *L. rhamnosus GG* showed that the kidney functions of the rats tested were not compromised.

4.12.2.4 Relative Organ weights

The relative organs weight is shown in Table 4.19. The relative weights of the kidney, heart and liver for the rats administered water only (control) were 0.6±0.09%, 0.37±0.05%, and 3.27±0.45% respectively. The rats administered TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG* (PHE)and the rats administered TMS-I011368 hydrolysate with free *L. rhamnosus GG* (PHF) at all doseshad relative weights of kidneys ranging from 0.62±0.13 to 0.68±0.17% and 0.60±0.06 to 0.62±0.08% respectively, with relative weights of hearts ranged from 0.36±0.06 to 0.37±0.08% and 0.34±0.07 to 0.35±0.04% respectively. The relative weights of liver in rats administered PHE and PHF were in the range of 3.08±0.87 to 3.77±0.45% and 2.94±0.79 to 3.43±1.80% respectively.

Table 4.19: Effect of Provitamin A cassava hydrolysates with or without *L. rhamnosus GG* on Relative Organ Weights of Wistar rats

Treatment groups	Kidney	Heart	Liver	Spleen
TMS-I011368 hydrolysate without *L. rhamnosus GG*	0.57±0.16 ^{b}	0.34±0.08 ^{a}	2.89±1.08 ^{f}	0.30±0.27 ^{a}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	0.68±0.17^{ab}	0.37±0.05 ^{a}	3.47±1.15 ^{b}	0.33±0.22 ^{a}
TMS-I011368 hydrolysate with encapsulated 2x10^{10} CFU/g *L. rhamnosus GG*	0.62±0.14^{ab}	0.36±0.06 ^{a}	3.08±0.87 ^{d}	0.34±0.17 ^{a}
TMS-I011368 hydrolysate with encapsulated 4x10^{10} CFU/g *L. rhamnosus GG*	0.62±0.13^{ab}	0.37±0.08 ^{a}	3.77±0.45 ^{a}	0.31±0.03 ^{a}
TMS-I011368hydrolysate with free 1x10^{10}CFU/g *L. rhamnosus GG*	0.62±0.08^{ab}	0.35±0.05 ^{a}	3.43±1.80 ^{b}	0.34±0.12 ^{a}
TMS-I011368hydrolysate with free 2x10^{10} CFU/g *L. rhamnosus GG*	0.60±0.09 ^{b}	0.34±0.07 ^{a}	2.94±0.79^{ef}	0.32±0.16 ^{a}
TMS-I011368 hydrolysate with free 4x10^{10} CFU/g *L. rhamnosus GG*	0.60±0.06 ^{b}	0.35±0.04 ^{a}	2.98±0.70 ^{e}	0.30±0.16 ^{a}
Water (Control)	0.60±0.09^{ab}	0.37±0.05 ^{a}	3.27±0.45 ^{c}	0.30±0.06 ^{a}

Means ± sd superscripted by the same alphabet in the same column are not significantly different ($\alpha_{0.05}$).

There were no significant differences $_{(\alpha 0.05)}$ in the relative weights of kidneys, heart, liver of all the rats administered TMS-I011368 hydrolysate with or without *L. rhamnosus GG* in comparison with the group administered only water (control). This suggests that there was no toxicity imparted on the organs by the provitamin A cassava hydrolysate with or without *L. rhamnosus GG.* Changes in organ weights have been employed as a quick marker in the assessment of general toxicity.

4.12.2.5 Assessment of Markers of Oxidative Stress in Wistar rats administered with probiotic provitamin A cassava hydrolysate

Tables 4.20, 4.21 and 4.22 presents the kidney, heart and liver oxidative stress profiles of Wistar rats administered with TMS-I011368 hydrolysate with or without probiotics for 30 days.Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) in the kidney of control rats were 39.48±4.22 and 2.43±0.40 respectively, while those administered PH without or with *L. rhamnosus GG* significantly increased when compared to the control. All the treated rats had kidney H_2O_2 levels in the range of 41.19±1.87 to 69.31±3.31 units/mg protein, except for PHE4 which had significantly ($\alpha_{0.05}$) decreased H_2O_2 levels (33.98±3.22 units/mg protein). For kidney MDA, the lowest level was observed in the rats administered PHF2 (2.39±0.31 units/mg protein) and the highest was in rats administered PHE1 (3.36±0.43 units/mg protein).Heart H_2O_2 levels in the control rats was 31.98±3.78 unit/mg protein, while it reduced significantly ($\alpha_{0.05}$)in rats administered PH (27.56±3.04 units/mg protein) and it was elevated significantly ($\alpha_{0.05}$) in rats administered PHF2 to a value of 42.94±4.44 units/mg protein.

Heart MDA observed in PHE2 was the lowest (2.05±0.62 units/mg protein) while the highest was observed in PHE1 (4.52±0.18 units/mg protein). Liver H_2O_2 was 127.52±6.92 units/mg protein while it ranged from 76.06±2.35 units/mg protein (rats administered PH) to 167.98±3.57 units/mg protein (in rats administered PHF2). The lowest Liver MDA was observed in rats administered PHE4 (2.15±0.17 units/mg protein) and the highest was observed in rats administered PHE2 (9.60±0.29 units/mg protein).Superoxide dismutase (SOD) in the kidney of the control rats was 22.23±8.29 units/mg protein, while it was 37.64±3.43 and 13.94±1.38 in the heart and Liver respectively. The levels of kidney SOD increased significantly ($\alpha_{0.05}$) in the range of 22.26±2.61 to35.32±7.52 units/mg protein in all the rats that received PH without or

with *L. rhamnosus GG* (free or encapsulated) except PHE2 which was significantly reduced with a value of18.22±1.48 units/mg protein.

Table 4.20: Effect of Provitamin A cassava hydrolysateswith or without *L. rhamnosus GG* on kidney oxidative stress profile of Wistar rats

Treatment groups	Oxidants		Antioxidants				
	Hydrogen peroxide	Malondialdehyde	Superoxide Dismutase	Glutathione	Glutathione peroxidase	Glutathione S-transferase	Nitric oxide
TMS-I011368 hydrolysate without *L. rhamnosus GG*	42.94±2.03^{cd}	3.24±0.25^{a}	22.26±2.61^{cd}	78.68±2.77^{abc}	559.96±13.69^{d}	0.81±0.36^{bc}	0.75±0.14^{d}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	41.89±1.87^{d}	3.36±0.43^{a}	35.32±7.52^{a}	77.13±3.15^{bcd}	605.92±5.88^{c}	0.71±0.61^{bc}	1.40±0.32^{ab}
TMS-I011368 hydrolysate with encapsulated 2x10^{10}CFU/g *L. rhamnosus GG*	41.19±2.89^{d}	2.90±0.70^{ab}	18.22±1.48^{d}	79.73±4.50^{ab}	506.79±10.81^{f}	0.60±0.29^{bc}	1.62±0.18^{a}
TMS-I011368 hydrolysate with encapsulated 4x10^{10}CFU/g *L. rhamnosus GG*	33.98±3.22^{e}	3.30±0.32^{a}	31.34±3.68^{ab}	76.74±3.06^{bcd}	750.42±2.07^{a}	0.52±0.23^{bc}	1.39±0.20^{ab}
TMS-I011368 hydrolysate with free 1x10^{10}CFU/mL *L. rhamnosus GG*	45.81±2.80^{c}	2.86±0.55^{ab}	26.50±6.30^{bc}	75.74±2.20^{cd}	539.98±6.45^{e}	1.38±0.56^{a}	1.18±0.16^{bc}
TMS-I011368 hydrolysate with free 2x10^{10}CFU/mL *L. rhamnosus GG*	69.31±3.31^{a}	2.39±0.31^{b}	25.25±4.42^{bcd}	82.16±2.30^{a}	686.62±32.02^{b}	0.48±0.24^{bc}	1.56±0.17^{a}
TMS-I011368 hydrolysate with free 4x10^{10}CFU/mL *L. rhamnosus GG*	50.06±2.88^{b}	3.27±0.56^{a}	23.84±7.45^{bcd}	70.64±0.80^{e}	763.22±2.65^{a}	0.37±0.12^{bc}	1.11±0.46^{bc}
Water (control)	39.48±4.22^{d}	2.43±0.40^{b}	22.23±8.29^{cd}	74.57±3.29^{d}	601.56±17.29^{c}	0.99±0.55^{ab}	0.95±0.33^{cd}

*Means ± sd superscripted by the same alphabet in the same column are not significantly different ($\alpha_{0.05}$).

Table 4.21: Effect of Provitamin A cassava hydrolysateswith or without *L. rhamnosus GG* on Heart oxidative stress profile of Wistar rats

	Oxidants		Antioxidants				
Treatment groups	Hydrogen peroxide	Malondialdehyde	Superoxide Dismutase	Glutathione	Glutathione peroxidase	Glutathione S-transferase	Nitric oxide
TMS-I011368 hydrolysate without *L. rhamnosus GG*	27.56±3.04^{d}	3.26±0.33^{ab}	40.85±2.66^{ab}	73.55±1.87^{ab}	1159.18±36.32^{abc}	1.21±0.52^{a}	0.55±0.13^{bcd}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	32.73±3.68^{c}	4.52±0.18^{b}	38.51±2.94^{bc}	70.16±1.70^{b}	1078.35±53.77^{cd}	1.02±0.61^{a}	0.71±0.29^{bc}
TMS-I011368 hydrolysate with encapsulated 2x10^{10}CFU/g *L. rhamnosus GG*	35.98±1.31^{c}	2.05±0.62^{ab}	37.13±1.96^{bc}	72.13±2.62^{ab}	1041.66±53.63^{d}	0.69±0.34^{a}	0.81±0.40^{ab}
TMS-I011368 hydrolysate with encapsulated 4x10^{10}CFU/g *L. rhamnosus GG*	32.06±2.99^{c}	2.58±1.26^{ab}	39.68±2.84^{abc}	75.67±3.18^{ab}	1115.01±33.98^{abcd}	0.78±0.22^{a}	0.41±0.07^{d}
TMS-I011368 hydrolysate with free 1x10^{10}CFU/mL *L. rhamnosus GG*	37.23±4.39^{b}	3.96±0.66^{ab}	39.23±1.48^{abc}	72.47±4.82^{ab}	1193.69±151.59^{ab}	0.76±0.14^{a}	0.57±0.18^{bcd}
TMS-I011368 hydrolysate with free 2x10^{10}CFU/mL *L. rhamnosus GG*	42.94±4.44^{a}	3.22±0.70^{ab}	43.11±4.73^{a}	73.33±1.85^{ab}	1206.94±86.96^{a}	0.80±0.58^{a}	0.97±0.07^{a}
TMS-I011368 hydrolysate with free 4x10^{10}CFU/mL *L. rhamnosus GG*	41.35±2.92^{a}	3.68±0.31^{ab}	35.99±2.84^{c}	71.38±2.55^{b}	1099.83±41.32^{bcd}	1.38±0.99^{a}	0.53±0.22^{cd}
Water (control)	31.98±3.78^{c}	2.10±0.91^{a}	37.64±3.43^{bc}	77.91±9.65^{a}	1076.78±60.35^{cd}	1.41±0.46^{a}	0.39±0.07^{d}

*Means ± sd superscripted by the same alphabet in the same column are not significantly different ($\alpha_{0.05}$)

Table 4.22: Effect of Provitamin A cassava hydrolysateswith or without *L. rhamnosus GG* on Liver oxidative stress profile of Wistar rats

Treatment groups	Oxidants		Antioxidants				
	Hydrogen peroxide	Malondialdehyde	Superoxide Dismutase	Glutathione	Glutathione peroxidase	Glutathione S-transferase	Nitric oxide
TMS-I011368 hydrolysate without *L. rhamnosus GG*	76.06±2.35^{e}	4.15±0.57^{b}	14.31±2.84^{ab}	78.51±2.51^{b}	328.26±14.70^{bc}	1.46±1.13^{b}	2.40±0.21^{bcd}
TMS-I011368 hydrolysate with encapsulated 1x10^{10}CFU/g *L. rhamnosus GG*	118.52±7.78^{c}	4.38±0.29^{c}	12.66±1.66^{ab}	80.90±6.69^{b}	339.30±12.8-9^{ab}	1.02±0.80^{b}	2.32±0.14^{a}
TMS-I011368 hydrolysate with encapsulated 2x10^{10}CFU/g *L. rhamnosus GG*	86.02±3.56^{d}	9.60±0.29^{a}	15.31±2.80^{a}	83.05±2.12^{b}	333.10±84.67^{bc}	1.09±0.52^{b}	3.16±1.03^{ab}
TMS-I011368 hydrolysate with encapsulated 4x10^{10}CFU/g *L. rhamnosus GG*	112.67±3.08^{c}	2.15±0.17^{d}	11.18±2.63^{ab}	96.60±3.54^{a}	293.63±16.10^{c}	1.56±0.62^{b}	2.76±0.41^{ab}
TMS-I011368 hydrolysate with free 1x10^{10}CFU/mL *L. rhamnosus GG*	117.29±7.66^{c}	2.38±0.20^{d}	13.31±2.72^{ab}	81.86±2.64^{b}	340.92±11.39^{ab}	1.82±0.89^{b}	2.12±0.86^{bcd}
TMS-I011368 hydrolysate with free 2x10^{10}CFU/mL *L. rhamnosus GG*	167.98±3.57^{a}	2.24±0.13^{d}	12.53±2.63^{ab}	72.93±0.66^{c}	334.89±13.07^{bc}	1.43±0.76^{b}	2.59±0.40^{abc}
TMS-I011368 hydrolysate with free 4x10^{10}CFU/mL *L. rhamnosus GG*	162.23±4.26^{a}	3.60±0.38^{c}	13.83±1.89^{ab}	79.32±2.69^{b}	369.81±47.62^{ab}	1.58±0.56^{b}	1.70±0.36^{d}
Water (control)	127.52±6.92^{b}	4.44±0.19^{b}	13.94±1.38^{ab}	79.72±3.17^{b}	380.35±9.03^{a}	3.22±1.00^{a}	2.02±0.20^{cd}

*Means ± sd superscripted by the same alphabet in the same column are not significantly different ($\alpha_{0.05}$).

Heart SOD was significantly elevated in PHF2 (43.11±4.73), while it showed a non-significant reduction in PHE2 (37.13±1.96) and PHF4 (35.99±2.84 unit/mg protein). The liver SOD in the treated rats ranged from 11.18±2.63 (PHE4) to 15.31±2.80 (PHE2).Kidney GSH in the control rats was 74.57±3.29 units/mg protein in the control rats were 22.23±8.29 mg/unit protein. Kidney GSH increased significantly ($\alpha_{0.05}$) in all the rats that received PH without or with *L. rhamnosus GG* (free or encapsulated) with the lowest (75.74±2.20) in PHF1 and the highest (82.16±2.30) observed in PHF2. Heart GSH ranged from 70.16±1.70 (PHE1) to 75.67±3.18 (PHE4). Liver GSH ranged from 72.93±0.66 in PHF2 to 96.60±3.54 in PHE4.

Glutathione peroxidase (GPx) in the kidney of control rats was 601.56±17.29 while the control heart and liver GPx were 1076.78±60.35 and 380.35±9.03 respectively. Kidney GPX significantly reduced in the order 559.96±13.69 (PH)>539.98±6.45 (PHF1)>506.79±10.81 (PHE2) while it increased significantly ($\alpha_{0.05}$) in PHE1, PHE4, PHF2 and PHF4 with the highest values, 750.42±2.07 (PHE4) and 763.22±2.65 (PHF4) recorded in the highest doses of *L. rhamnosus GG* (encapsulated or free respectively). Heart GPX significantly increased in the range of 1078.35±53.77 (PHE1) and 1206.94 ±86.96 (PHF2) while GPX in the liver decreased in the range from 293.63±16.10 in PHE4 to 369.81±47.62 in PHF4.

Glutathione S-transferase (GST) in the kidney of control rats was 0.99±0.55 while the levels in the heart and liver of control rats were 1.41± 0.46 and 3.22±1.00 unit/mg protein respectively. Kidney GST significantly reduced in all rats dosed with PH without or with *L. rhamnosus GG* (free or encapsulated) in the range of 0.37±0.12 unit/mg protein (PHF4) to 0.81±0.36 unit/mg protein (PH) except PHF1, which showed a significant elevation of 1.38±0.56 unit/mg protein. Heart GST ranged from 0.69±0.34 unit/mg protein (PHE2) to 1.38±0.99 unit/mg protein (PHF4) while Liver GST significantly increased from 1.02±0.80 in PHE1 to 1.82±0.89 in PHF1.

Nitric oxide in the kidney, heart and liver of control rats were 0.95±0.33, 0.39±0.07 and 2.02±0.20 unit/mg proteinrespectively. Kidney Nitric oxide decreased significantly to 0.75±0.14 in rats administered with PH only while it increased significantly in the range of 1.11±0.46 (PHF4) to 1.62±0.18 (PHE2) in all other rats dosed with PHE and PHF at all doses. Heart Nitric oxide and the Nitric oxide in the liver significantly

increased in the range of 0.41±0.07 (PHE4) to 0.97±0.07 (PHF2) and 1.70±0.36 in PHF4 to 3.16±1.03 in PHE2 respectively.

H_2O_2 and MDA, markers of lipid peroxidation were marginally increased by the administration of PH without or with *L. rhamnosus GG*. This showed that oxidative stress may be induced by PH if administered for a prolonged period. However, the concurrent elevation of SOD, GPx and GST, which are the enzymatic defenses against lipid peroxidation, and GSH which is a non-enzymatic defense in all the PH without or with *L. rhamnosus GG* when compared with the control rats showed that the oxidative stress induced in the rats was probably reversed. Oxidative stress refers to a situation whereby there is an imbalance between oxidants and antioxidants, and especially when this imbalance shifts more towards oxidants. The results obtained was corroborated by several authors. Singh *et al.* (2016) reported increased activities of SOD and GPX with a concurrent reduced lipid peroxidation, in rats fed with high fat diet with fermented milk containing either *L. rhamnosus GG* or *L. rhamnosus SCDC 17.* Yadav *et al.* (2019) also reported an increase in levels of antioxidants SOD and GPX in the kidney and liver of rats fed with high cholesterol diet with fermented milk containing either *L. rhamnosus* MTCC 5957 or *L. rhamnosus* MTCC 5897. The same trend was reported by Sharma *et al.* (2014). The above further established the anti-oxidative properties of probiotic strains which have been reported to carry out these anti-oxidative functions through several mechanisms. Oxidants can be directly neutralised in the intestine by the expression of antioxidants thus having a direct modulatory effect on gut inflammation and the related oxidative stress (Martarelli *et al.*, 2011).

Superoxide dismutase (SOD) is an enzyme that eliminates superoxide radicals and thus protects the cellular constituents against oxidative damage (Mansuroˇglua *et al.*, 2015). It destroys free radical superoxide by converting it to peroxide which can then be destroyed by GPX reactions by catalyzing its removal, converting it to non-toxic products through the utilisation of GSH as a reductant (Rathi *et al.*, 2015). Through this mechanism, SOD prevents damage to the membrane biological system, thus protecting the tissue against oxidative damage (Mates, 2000). GST are GSH-dependent enzymes which is involved in catalyzing the conjugation of GSH to various compounds (Baltruskeviciene *et al.*, 2016). They are detoxification mechanisms which metabolise toxic compounds that may generate ROS, by making them less toxic, more water soluble and thus more quickly eliminated from a biological system (Suthar,

2017). Findings in this study is consistent with Deabes *et al.* (2012) which reported that mice administered *L. rhamnosus GG* alone exhibited an insignificant increase in GSH content in liver tissues. The study concluded that oral administration of *L. rhamnosus GG* to mice significantly mitigated toxicity by means of Glutathione (GSH) as well as a stable SOD activity thereby preventing oxidative stress.

Okafor *et al.* (2006) documented a contrary report which stated that the significant depletion of glutathione with concurrent elevation of ALT, AST and ALP in rats fed with high cyanide containing food. This has been explained as the effect of consuming foods containing toxic levels of cyanide. PH cyanide content was earlier reported in this current study to be in the range of 1.00±0.02 and 1.80±0.002 mg/kgfresh weight basis which is significantly lower than the maximum limits of 10mg/kg specified by WHOfor cyanide.This may explain the deviation from Okafor *et al.* (2006) observed with insignificant alterations of glutathione and the serum concentrations of liver enzymes; ALT, AST and ALP. This finding corroborates the fact that low cyanide levels will not in the overall lead to toxicity which could present as increase in ROS.Wang (2012) administered *L. rhamnosus GG* supernatant to mice at a dose of 10^9 CFU/ml per day and also observed that pre-treatment with *L. rhamnosus GG* normalised the intestinal dysfunction changes due to oxidative stress generated by alcohol exposure which leads to alcohol liver injury. Previous study established that culture supernatant pretreatment had a protective role against oxidative stress induced in rats by alcohol which could lead to alcohol liver disease (Forsyth *et al.*, 2009).

Nitric oxide, involved in vasodilation, is one of the protective mechanism in the gastro intestinal tract and *LGG* has been reportedly involved in the biosynthesis of nitric oxide (Korhonen *et al*., 2001).In this study, nitric oxide synthesis was observed to increase in the kidney, heart and liver tissues as well. This increase was also observed to be more pronounced in the TMS-I011368 hydrolysate with encapsulated 2 x 10^{10} CFU group. Functions of Nitric Oxide include vasodilation, regulation of the cardiovascular disorders such as hypertension (Zhao *et al.*, 2015), anti-aggregation of platelets (Bondonno *et al.*, 2015), host defense, etc. The Nitric Oxide synthases are the specific enzymes which are responsible for converting oxygen molecules to nitric oxide. Inert nitrate anion can also be converted by gastrointestinal facultative anaerobic bacteria such as *L. rhamnosus GG* during respiration, to nitrite which can be used to generate nitric oxide (Cevher *et al.*, 2015; Sobko *et al.*, 2005). Decreased levels

of GSH promotes the generation of ROS and oxidative stress thereby affecting the structural integrity of the cell.Elevation of plasma levels of liver enzymes indicate loss of liver integrity which may be as a result of cellular leakages of cell membrane in the liver (Mordi *et al.*, 2015).

Azcárate-Peril *et al.* (2011) highlighted several possible mechanisms by which probiotics may protect the host against oxidative stress by possessing intracellular enzymes that chelates metal ions and scavenges free radicals thus preventing the formation of ROS.

4.13 Glycemic index

4.13.1 Estimation of glycemic index of provitamin A cassava hydrolysate containing free and encapsulated *Lactobacillus rhamnosus GG* in rats

Tables 4.23-24 shows the fasting blood glucose levels, peak blood glucose levels, peak times, incremental area under the 2-hr curve and glycemic indices of glucose and provitamin A cassava hydrolysate without or with *L. rhamnosus GG* in normoglycemic rats and humans respectively. Figures 4.21-4.22 show the effect of provitamin A cassava hydrolysate without or with 2 x 10^{10} or 4 x 10^{10} CFU free or encapsulated *L. rhamnosus GG* cells on Blood Glucose Levels (BGL) of normoglycemic rats and humans respectively. Fasting blood glucose levels in all the rats ranged between 60.67±0.58 and 73.33±1.53 mg/dL. The peak blood glucose levels (BGL) for glucose was 101.15±3.13 mg/dL, with peak time of 15 min. the highest peak BGL for PHE was 98.33±13.64 mg/dL (PHE4) with peak time of 60 min. The highest peak BGL for PHF was 62.33±0.58 (PHF4), attained at a peak time of 30 min.The iAUC of glucose was 2156, with glycemic index (GI) of 100, while iAUC ranged between 1545 (PHE4) and 1598 (PHE2) in PHE, while it ranged between 1498 (PHF4) and 1635 (PHF2), in PHF. These resulted in glycemic index (GI) of 100 for glucose,maximum GI values of 74.11 (PHE2),75.83 (PHF2) and 83.72 (PH).

In humans, peak BGL of 102.30±8.74 and 104.70±3.05 mg/dL were achieved in PH and PHF4 at peak times of 15 and 30 min respectively, with IAUC of 1500 and 1413 respectively. These resulted in GI of 71.60 and 67.45 respectively.Peak BGL was significantly reduced with increasing doses of *L. rhamnosus GG* inclusion in the TMS-I011368 hydrolysate. This resulted in significantly lower AUCsand glycemic indices

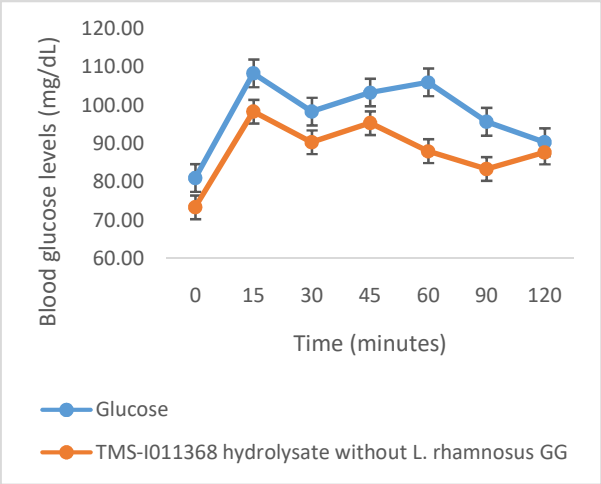
of PHE and PHF than that of PH. The glycemicindices of TMS-I011368hydrolysate were

Table 4.23: Blood glucose indices of normoglycemic Wistar rats administered TMS-I011368 hydrolysate with or without *L. rhamnosus GG*

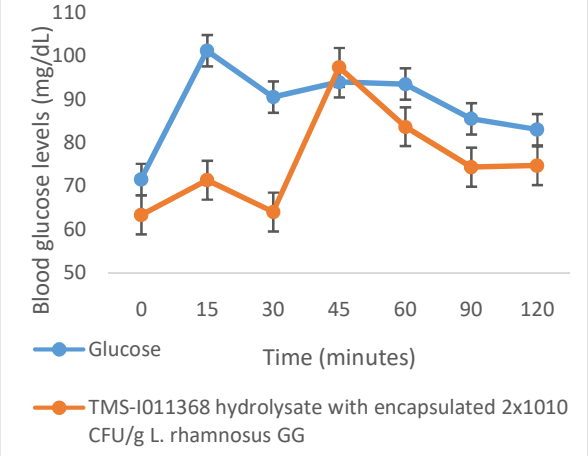
	Fasting BGL (Baseline)	Peak Blood Glucose Level (mg/dL)	Peak Time (Minutes)	Incremental Area Under Curve (IAUC)	Glycemic Index
Glucose	62.00±1.73	101.15±3.63	15	2156	100
TMS-I011368 hydrolysate without *L. rhamnosus GG*	73.33±1.53	98.33±3.05	15	1805	83.72
TMS-I011368 hydrolysate with encapsulated $2x10^{10}$CFU/g *L. rhamnosus GG*	63.33±2.31	97.33±1.53	45	1598	74.11
TMS-I011368 hydrolysate with encapsulated $4x10^{10}$ CFU/g *L. rhamnosus GG*	73.00±6.56	98.33±14.64	60	1545	71.66
TMS-I011368 hydrolysate with free $2x10^{10}$ CFU/mL *L. rhamnosus GG*	60.67±0.58	91.67±10.01	30	1635	75.83
TMS-I011368 hydrolysate with free $4x10^{10}$ CFU/mL *L. rhamnosus GG*	62.33±0.58	83.67±8.74	30	1498	69.48

Table 4.24: Blood glucose indices of normoglycemic humans administered TMS-I011368 hydrolysate with or without *L. rhamnosus GG*

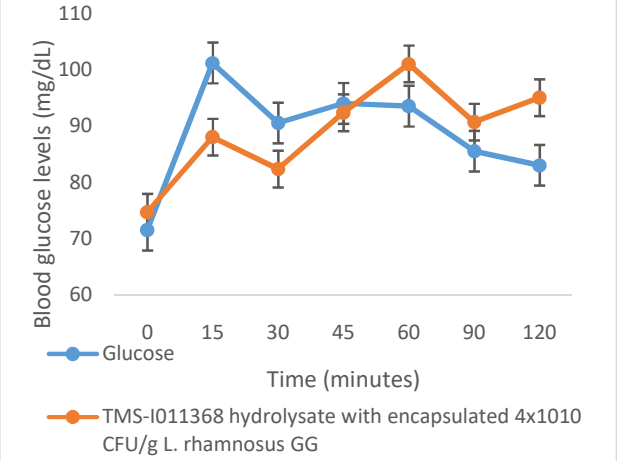
	Fasting BGL (Baseline)	Peak Blood Glucose Level (mg/dL)	Peak Time (Minutes)	Incremental Area Under Curve (IAUC)	Glycemic Index
Glucose	77.67±1.73	107.70±3.63	15	2095	100
TMS-I011368 hydrolysate without *L. rhamnosus GG*	82.00±1.53	104.70±3.05	15	1500	71.60
TMS-I011368 hydrolysate with free $4x10^{10}$ CFU/mL *L. rhamnosus GG*	81.33±0.58	102.30±8.74	30	1413	67.45



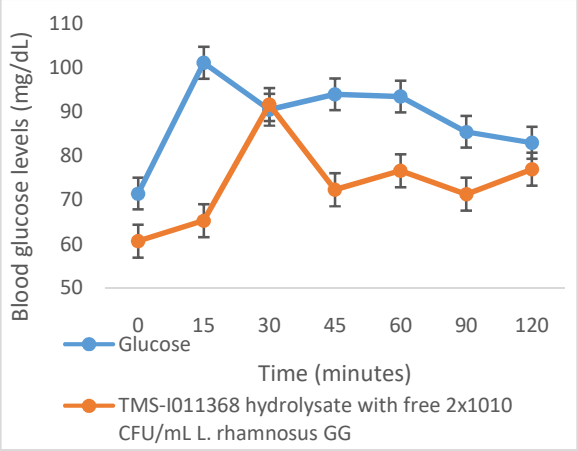
(a). Blood glucose response curves of TMS-I011368 hydrolysate without *L. rhamnosus GG*



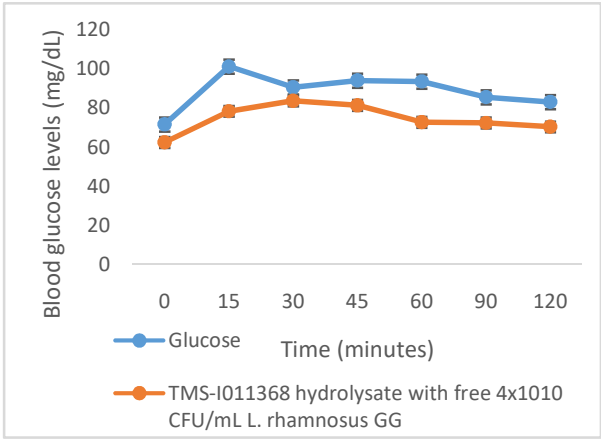
(b). Blood glucose response curves of TMS-I011368 hydrolysate with 2x 10^{10} CFU/g encapsulated*L. rhamnosus GG*



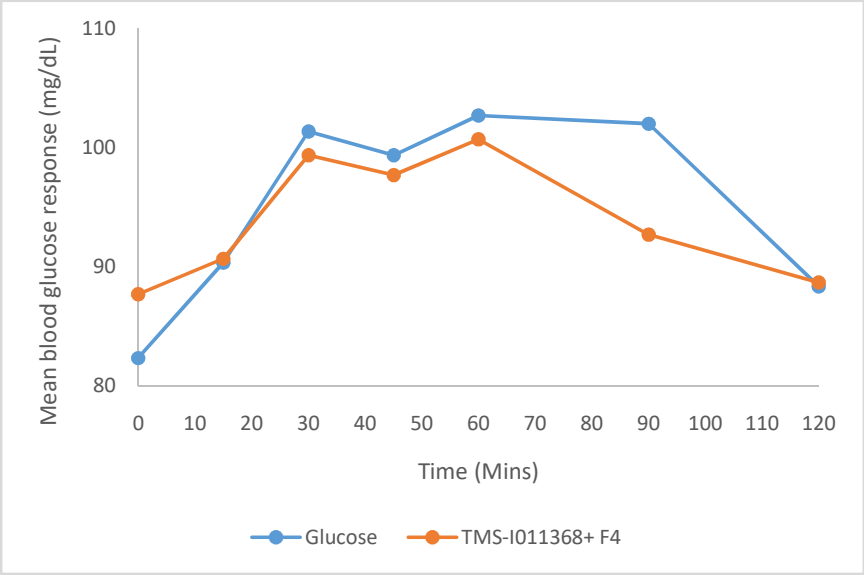
(c). Blood glucose response curves of TMS-I011368 hydrolysate with 4 x 10^{10} CFU/g encapsulated*L. rhamnosus GG*



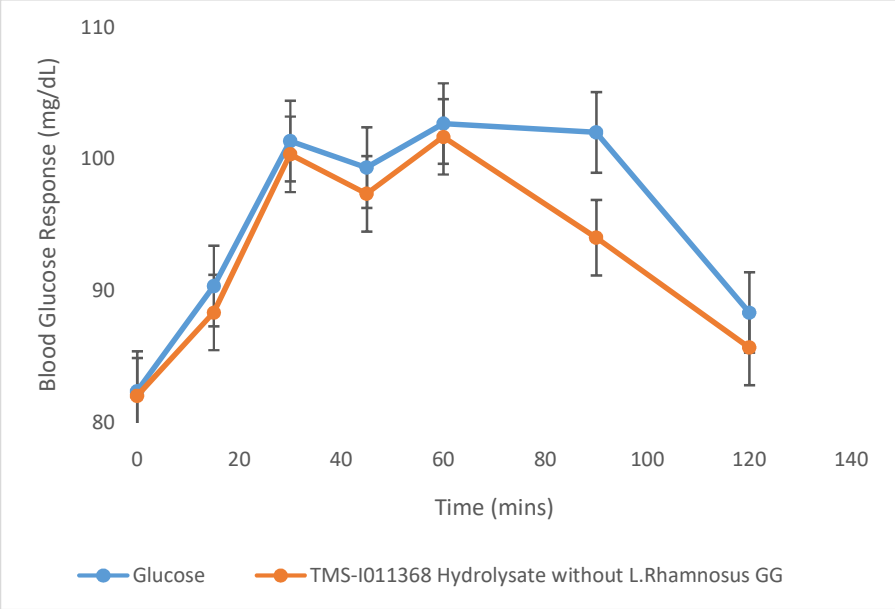
(d). Blood glucose response curves of TMS-I011368 hydrolysate with 2x 10^{10} CFU/mL free*L. rhamnosus GG*



(e). Blood glucose response curves of TMS-I011368 hydrolysate with 4 x 10^{10} CFU/mL free*L. rhamnosus GG*



(a). Blood glucose response curve of TMS-I011368 hydrolysate containing $4x10^{10}$ CFU/mL/kg body weight free *L. rhamnosus GG* in normoglycemic human subjects



(b). Blood glucose response curve of TMS-I011368 hydrolysate without *L. rhamnosus GG* in normoglycemic human subjects.

also observed to be reduced with increasing doses of *L. rhamnosus GG* inclusion, with the lowest value observed in PHF4.

The above observations indicated that the inclusion of *L. rhamnosus GG* in provitamin A cassava hydrolysate helped toachieve a good glycemic control in the Wistar rats. A good glycemic control is an essential factor in preventing or delaying diabetes, obesity and other cardiovascular diseases (Unadike *et al.*, 2010). Castillo *et al.* (2018) explained that ability to achieve a good glycemic control is the primary target in the treatment of diabetes. There was no significant differences in the glycemic index values obtained for the rats in comparison with that obtained for human subjects. Provitamin A hydrolysates are categorised as high glycemic index beverage. Ogbuji and David-Chukwu (2010) stated that cassava food products have high glycemic index ranging from 88 to 92. Ogbonna *et al.* (2018) also reported that *Eba* made from Provitamin A cassava had a glycemic index of 94.7 in normoglycemic individuals. However, *L. rhamnosus GG* inclusion was observed to improve the glucose tolerance in the rats, and thus lowered the glycemic index. This result was corroborated by Park *et al.* (2015) which stated that *L. rhamnosus GG* significantly improved glucose tolerance in mice. Glucose intolerance is one of the factors that characterise diabetes (Balakumar *et al.*, 2016).

The results of determination of plasma glucose as a biochemical parameter determined on Wistar rats in this study further confirms the lowering of blood glucose in the rats supplemented with *L. rhamnosus GG* when compared with the control rats. *L. rhamnosus GG* has been reported by several researchers as having anti-hyperglycemic and anti-diabetic properties. Koh *et al.* (2018) stated that consumption of probiotics could improve postprandial hyperglycemia and to a large extent and help in controlling diabetes mellitus.Although high GI diets have been implicated as posing a high risk of obesity and diabetes to consumers, they may be advantageous in the rapid release of glucose, and help with energy recovery after exercise (Little *et al.*, 2009). Therefore, this beverage could be beneficial to athletes. It can also help in the management of hypoglycemic individuals as well as healthy individuals that are seeking an alternative to the commercially sweetened carbonated beverages currently available. However, it

is not recommended to diabetic individuals or people with pre-diabetes. Diabetic individuals need to focus on the consumption of low GI foods.

4.14 Descriptive sensory analysis

Table 4.25 illustrates the sensory and descriptive attributes of Provitamin A cassava hydrolysate after 48 hours of fermentation with free and encapsulated *L. rhamnosus GG* as well as the fresh un-inoculated hydrolysate. TMS-I011368 hydrolysate with free *L. rhamnosus GG* scored 4.49±1.98 in yellow colour followed by TMS-I011371 hydrolysate and TMS-I070593 with free *L. rhamnosus GG* with a score of 4.12±2.25 and 4.02±2.01 respectively. All the hydrolysates with encapsulated *L. rhamnosus GG* ranged between 3.76±2.14 and 4.39±2.11. The un-inoculated hydrolysates ranked lowest in yellow colour, with scores ranging from 2.83±1.75 to 3.49±2.19. The provitamin A cassava hydrolysate without *L. rhamnosus GG* ranked highest in sweetness among all the samples with values ranging from 4.24±2.11 to 4.98±1.82; and lowest in sourness with values ranging from 3.88±2.25 to 4.37±2.12. The ranking of sweetness for hydrolysates with free *L. rhamnosus GG* and the hydrolysates with encapsulated *L. rhamnosus GG* ranged from 3.12±1.81 to 3.61±1.81 and 3.24±1.98 to 3.80±2.18 respectively.The hydrolysates inoculated with free *L. rhamnosus GG* ranked lowest in sweetness and highest in sourness. The texture/mouthfeel of hydrolysates with encapsulated *L. rhamnosus GG* ranged between 4.41±1.96 and 5.22±2.09 while the texture/mouthfeel for hydrolysates without *L. rhamnosus GG* ranked between 4.78±2.40 and 5.85±2.46.

The provitamin A cassava hydrolysate without *L. rhamnosus GG* ranked better overall acceptability with scores between 5.32±2.09 and 5.76±1.97 than the fermented hydrolysates. The hydrolysates with encapsulated *L. rhamnosus GG*ranked between 4.46±1.94 and 5.02±2.02while the hydrolysates with free *L. rhamnosus GG* ranked between 4.63±1.56 to 5.07±2.27, which translated to a range of 49.56 - 56.33% in overall acceptability, respectively, on a scale of 1-9. There was no significant difference $_{(\alpha 0.05)}$in the values of the yellow colour of the hydrolysates. However, the fermented hydrolysates were more preferred to the unfermented hydrolysates. This could be because provitamin A carotenoids are improved by fermentation. There was a

significant ($P<0.05$) influence of variety differences among the samples. The hydrolysate from TMS-I011368 variety scored highest in yellow colour while the hydrolysates from TMS-I070593 variety scored lowest. This could be attributed to differences in the retention of their provitamin A carotenoid contents.

Table 4.25: Sensory evaluation of provitamin A cassava hydrolysate containing free and encapsulated *L. rhamnosus GG*

Samples	Yellow colour	Sweetness	Sourness	Swallowability	Texture/Mouthfeel	Off-Flavour	After-taste	Cloudiness	Overall Acceptability
TMS-I011371 hydrolysate without *L. rhamnosus GG*	3.37±2.12^{bc}	4.98±1.82^{a}	4.37±2.12^{bc}	6.46±1.85^{a}	5.85±2.46^{a}	4.78±2.34^{ab}	5.10±2.53^{a}	5.37±2.07^{a}	5.76±1.97^{a}
TMS-I011368 hydrolysate without *L. rhamnosus GG*	3.49±2.19^{abc}	4.56±1.64^{ab}	4.29±2.15^{bc}	6.02±1.89^{ab}	5.00±2.25^{ab}	4.00±1.88^{b}	4.63±2.18^{a}	5.27±2.12^{a}	5.56±1.82^{ab}
TMS-I070593 hydrolysate without *L. rhamnosus GG*	2.83±1.75^{c}	4.24±2.11^{abc}	3.88±2.25^{bc}	5.61±2.31^{abc}	4.78±2.40^{ab}	4.44±2.31^{ab}	4.44±2.38^{a}	4.83±2.06^{a}	5.32±2.09^{abc}
TMS-I011371 hydrolysate with encapsulated *L. rhamnosus GG*	3.88±2.05^{ab}	3.24±1.93^{cd}	4.98±2.23^{ab}	4.15±2.10^{e}	4.41±1.96^{b}	5.10±2.28^{a}	4.41±2.37^{a}	4.95±1.86^{a}	4.46±1.94^{c}
TMS-I011368 hydrolysate with encapsulated *L. rhamnosus GG*	4.39±2.11^{ab}	3.56±1.48^{cd}	4.71±2.33^{abc}	5.17±2.10^{bc}	5.22±2.09^{ab}	5.27±2.07^{a}	4.07±2.27^{a}	5.22±2.31^{a}	4.76±1.83^{bc}
TMS-I070593 hydrolysate with encapsulated *L. rhamnosus GG*	3.76±2.14^{abc}	3.80±2.18^{bcd}	5.12±2.22^{ab}	4.90±2.17^{cd}	5.12±2.16^{ab}	5.37±2.29^{a}	4.90±2.56^{a}	5.37±1.97^{a}	5.02±2.02^{abc}
TMS-I011371 hydrolysate with free *L. rhamnosus GG*	4.12±2.25^{ab}	3.61±1.81^{cd}	5.27±2.07^{ab}	5.63±2.33^{abc}	4.56±2.34^{b}	4.80±2.24^{ab}	4.56±1.94^{a}	4.88±2.16^{a}	5.00±1.91^{abc}
TMS-I011368 hydrolysate with free *L. rhamnosus GG*	4.49±1.98^{a}	3.12±1.81^{cd}	5.49±2.10^{a}	5.49±2.06^{abc}	4.39±2.26^{b}	4.71±2.27^{ab}	4.24±2.26^{a}	4.98±2.01^{a}	4.63±1.56^{bc}
TMS-I070593 hydrolysate with free *L. rhamnosus GG*	4.02±2.01^{ab}	3.54±2.07^{cd}	5.27±2.51^{ab}	5.51±2.48^{abc}	4.63±2.15^{b}	5.39±2.11^{a}	4.88±2.38^{a}	5.39±1.81^{a}	5.07±2.27^{abc}

*Means ± standard deviation with the same alphabet superscripts in the same row are not significantly different at P>0.05.

The unfermented samples ranked higher ($\alpha_{0.05}$) in sweetness than all the fermented samples, showing an inverse relationship with the sourness attribute. The ranking of the "sourness" attribute follows a direct opposite trend observed in sweetness. There was a higher rating of sourness in the fermented samples than the unfermented. The fermented samples were significantly sourer in taste ($\alpha_{0.05}$) than the unfermented samples. This could be due to the slightly astringent taste that the presence of organic acids, mainly lactic acid could impart to the fermented samples, thereby reducing the sweetness and increasing the sourness. The reduction in sweetness of the fermented samples might also be as a result of the consumption of the sugars in the hydrolysate samples by the probiotic organism during fermentation for microbial growth and metabolism (MaiaCosta *et al*., 2013). An increase in the sugar content might be suggested for commercial production so as to compensate for the depleted sugar content of the hydrolysate and to appeal more to consumers taste. The unfermented samples were rated highest in their ease of swallow attribute while the least ranked were the provitamin A cassava hydrolysate containing encapsulated *L. rhamnosus GG.* This is because the latter contained probiotic beads which could give a feeling of particles in the mouth. A typical fruit juice does not contain these beads or particles. Hence, the presence of probiotic beads created difficulty in swallowing.

The influence of viscosity on the texture of the provitamin A cassava hydrolysates is reflected in the mouthfeel of the hydrolysates. All the samples with free *L. rhamnosus GG* cells were significantly different ($\alpha_{0.05}$) in mouthfeel from the hydrolysates with encapsulated cells. There was no significant difference in the mouthfeel of the hydrolysate samples with encapsulated *L. rhamnosus GG* cells when compared with the control i.e. the provitamin A cassava hydrolysate without *L. rhamnosus GG*. This could be due to the fact that the spherical beads tend to sink down and settle through the watery hydrolysate instead of being held by it (Krasaekoopt and Kitsawad, 2010) while the hydrolysate with free *L. rhamnosus GG* cells have the cells evenly dispersed within it, giving a slight increase in the viscosity of the hydrolysate. Krasaekoopt and Kitsawad (2010) explained that the viscosity of a product plays a major role in the texture/mouthfeel as probiotic beads tends to sink down in a watery-type product (such as fruit juice), while a more viscous product (such as yoghurt) can hold probiotic beads.

This observation corroborates the higher kinematic viscosity recorded in hydrolysates with free *L. rhamnosus GG* (Table 4.9).The flavour and after-taste of the fermented provitamin A hydrolysates did not show any significant difference when compared with the unfermented samples. This could be because the panelists were unfamiliar with the taste of provitamin A cassava hydrolysate as it is an innovative product. The attribute of cloudiness ranked higher in hydrolysates with free *L. rhamnosus GG* cells than the other hydrolysates. However, there was no significant statistical difference $(\alpha_{0.05})$ in the cloudiness of all the samples. Addition of *L. rhamnosus GG* beads did not significantly affect the cloudiness of the provitamin A cassava hydrolysates. This could be because there was no contrast in the white colour of the beads and the off-white-yellow colour of the hydrolysate samples. The free cells were dispersed within the samples untrapped, so this could contribute to the increase in cloudiness. Thisis consistent with the observation of Krasaekoopt and Kitsawad, (2010) who noted that addition of probiotic beads did not affect the cloudiness of orange juice because of the colour.

There was no significant differences in the overall acceptability of fermented hydrolysates when compared with the unfermented ones. All the provitamin A cassava hydrolysates samples ranked from 4.46±1.94 to 5.07±2.27 in overall acceptability on the scale of 1-9. This shows that despite that the unfermented samples ranked higher in the other attributes (sweetness, ease of swallow, texture/mouthfeel), the hydrolysates inoculated with *L. rhamnosus GG* were no less accepted.

CHAPTER FIVE

CONCLUSIONS, RECOMMENDATIONS AND CONTRIBUTIONS TO KNOWLEDGE

5.1 Conclusions

The findings in this study showed that it was possible to develop a ready-to-drink probiotic beverage from provitamin A cassava varieties by starch hydrolysis using α-amylase and glucoamylase. The provitamin A cassava hydrolysate developed was a good substrate for the growth of *Lactobacillus rhamnosus GG* which maintained viable cell population > 10^6 CFU/ml during storage.

The provitamin A cassava hydrolysate showed properties that supported the growth of *Lactobacillus rhamnosus GG* thus proved to be a good probiotic carrier. The hydrolysate produced was microbiologically stable up to a storage period of 30 days at 4ºC, as there was no presence of pathogenic organisms during the first 30 days. The provitamin A hydrolysate had significant beta carotene retention and thus a significant antioxidant activity.

Encapsulation of *Lactobacillus rhamnosus GG* gave a significant improvement to the viability of probiotic *L. rhamnosus GG* during simulated gastrointestinal conditions and also in the provitamin A cassava hydrolysates. The protection conferred on *L. rhamnosus GG* by encapsulation assisted in probiotic delivery to the colon as reflected in faecal microbial population of the rats. The expansion of growth direction index (GDI) of *L. rhamnosus GG* showed that lactic acid bacteria (*L. rhamnosus GG*) was able to out-compete total aerobes and other pathogenic organisms in the intestine, and eventually colonising the intestine.

In-vivo toxicity evaluation showed that the administration of provitamin A cassava hydrolysate without or with *L. rhamnosus GG* also did not induce any toxic/adverse effects on the haematological and biochemical parameters of Wistar rats.

The administration of provitamin A cassava hydrolysate without or with *L. rhamnosus GG* did not induce any significant increase in lipid peroxidation in the kidney, heart and liver tissues of the Wistar rats. It can be concluded that supplementation with *L. rhamnosus GG* reversed lipid peroxidation.

The findings in this current study showed that the administration of provitamin A hydrolysate with *L. rhamnosus GG* induced anti-obesity effects in the Wistar rats due to a well-controlled and gradual body weight loss observed in the rats. This was associated with significant lowering of serum cholesterol and improved glycemic control in Wistar rats. Thus, provitamin A hydrolysate with *L. rhamnosus GG* could be recommended for weight management.

The inclusion of *L. rhamnosus GG* in provitamin A hydrolysate exerted a significant dose-dependent lowering of the glycemic index (GI) of the hydrolysate from high GI towards the intermediate GI range. The consumption of provitamin A hydrolysate with *L. rhamnosus GG* would provide the energy needed transiently but it would not induce hyperglycemia. Provitamin A cassava hydrolysate is an innovative non-dairy probiotics products that can suit specialised market segments such as children, and chronically ill patients. It is also suitable for consumers with certain dietary restrictions like lactose intolerance, other milk allergies, vegetarianism, or low cholesterol. However, caution should be taken by pre-diabetic individuals or individuals with diabetes in consuming the provitamin A cassava hydrolysate.

The provitamin A hydrolysate fermented with *L. rhamnosus GG* recorded a significant overall acceptability despite that the provitamin A hydrolysate samples without *L. rhamnosus GG* (unfermented) ranked higher in the other attributes (sweetness, ease of swallow, texture/mouth-feel).

The probiotic provitamin A cassava hydrolysate produced therefore proved to be a good carrier of probiotic thus serving as a good non-dairy matrix for probiotic *L. rhamnosus GG*. It can serve as an antioxidant drink due to the antioxidant properties of beta-carotene and other phenolic compounds contained in the hydrolysates. This probiotic provitamin A cassava hydrolysate could therefore serve as a good alternative for consumers who are

lactose intolerant and vegetarians who would not be able to consume beverages from dairy sources.

5.2 Recommendations

In large scale application, it is recommended that fermentation of provitamin A cassava hydrolysate should be carried out with encapsulated *L. rhamnosus GG* beads produced by extrusion technique as this enhanced the viability of *L. rhamnosus GG* during processing, storage and gastrointestinal transit.

The maximum recommended storage duration of provitamin A cassava hydrolysate under refrigerated (4^{o}C) condition is 30 days.

Sensory attributes, especially taste of provitamin A cassava hydrolysate may be improved for commercial purposes, by the addition of non-caloric sweeteners such as stevia or sucralose. The population of obesity-associated commensals may be enumerated in further studies, to evaluate the effect of sweetener on gut microbiota.

It is recommended that future research attention should be focused on optimisation of the process variables to further reduce the glycemic index of the hydrolysate.

Further studies is recommended to evaluate the functionality of probiotic *L. rhamnosus GG* as affected by duration of storage.

Further research should also employ the use of metagenomics sequencing procedures in characterising the bacteria constituents of provitamin A cassava hydrolysate as well as faecal microbiota.

Provitamin A cassava hydrolysate should be promoted industrially as a non-dairy probiotic beverage, as this could contribute to its wider utilisation and expansion of its economic importance.

5.3 Contributions to knowledge

The findings of this research have shown that it is possible to produce a safe, lactic acid fermented beverage, with acceptable sensory attributes from provitamin A cassava varieties.

The successful application of provitamin A cassava hydrolysate as carrier for probiotic *Lactobacillus rhamnosus GG* has expanded the non-dairy food matrices for probiotics.

A suitable microencapsulation material (alginate) was applied to enhance the viability and survivability of probiotic *Lactobacillus rhamnosus GG* in provitamin A cassava hydrolysate.

This study was able to evaluate the stability and viability of the encapsulated probiotic *Lactobacillus rhamnosus GG* under specific gastrointestinal conditions and in the non-dairy food carrier (provitamin A cassava hydrolysate).

It was able to establish the microbiological and toxicological safety of provitamin A cassava hydrolysate with or without probiotic *Lactobacillus rhamnosus GG* at doses up to $4x10^{10}$ CFU for up to 30 days, using haematological, clinical biochemistry and assessment of oxidative markers procedures.

The glycemic index of provitamin A cassava hydrolysate with or without probiotic *Lactobacillus rhamnosus GG* was established.

REFERENCES

Abdel-Rahman, M. A., Y. Xiao, Y. Tashiro, Y. Wang, T. Zendo, K. Sakai and K. Sonomoto. 2015. Fed-batch fermentation for enhanced lactic acid production from glucose/xylose mixture without carbon catabolite repression. *Journal of Bioscience and Bioengineering* 119(2): 153-158.

Aberoumand, A. 2012. Screening of phytochemical compounds and toxic proteinaceous protease inhibitor in some lesser-known food based plants, and heir effects and potential applications in food. *International Journal of Food Science and Nutrition Engineering* 2(3): 16-20.

Adak, A., C. Maity, K. Ghosh, B. R. Pati and K. C. Mondal. 2013. Dynamics of predominant microbiota in the human gastrointestinal tract and change in luminal enzymes and immunoglobulin profile during high-altitude adaptation. *Folia Microbiologica- Springer* 58(6): 523-528.

Adebayo-Tayo, B. and A. Stephanie. 2016. Probiotic viability, physicochemical and sensory properties of probiotic pineapple juice. *Fermentation* 2(4): 1-20.

Adedayo, B. C., A. A. Adebayo, E. E. Nwanna and G. Oboh. 2018. Effect of cooking on glycemic index, antioxidant activities, α-amylase, and α-glucosidase inhibitory properties of two rice varieties. *Food Science and Nutrition* 6: 2301–2307.

Adegoke, G. O. and P. Letuma. 2013. Strategies for the prevention and reduction of mycotoxins in developing countries. In: *Mycotoxin and food safety in developing countries*. H. A. Makun (eds). 5**:** *123-136*.

Adegunwa, M. O., L. O. Sanni and B. Maziya-Dixon. 2010. Effects of fermentation length and varieties on the pasting properties of sour cassava starch. Proceedings of the 11th International Society for Tropical Root Crops, African Branch (ISTRC-AB) Symposium, Kinshasa, DR Congo.

Adenle, A. A., O. C. Aworh, R. Akromah and G. Parayil. 2012. Developing GM super cassava for improved health and food security: future challenges in Africa.*Agriculture and Food Security*1:11. *http://www.agricultureandfood security.com/content/1/1/11*

Aderibigbe, F. A. and L. A. Adejumo. 2015. Effect of different hydrolysis methods on starch degradation. *African Journal of Biotechnology* 14(3): 264-271.

Adewusi, S. R. A., T. V. Ojumu and O. S. Falade. 1999. The effect of processing on total organic acid content and mineral availability of simulated cassava-vegetable diets. *Plant foods for Human Nutition* 53: 367-380.

Adhikari, K., I. U. Gruw, A. Mustapha and L. N. Fernando. 2002. Changes in the profile of organic acids in plain set and stirred yogurts during manufacture and refrigerated storage. *Journal of Food Quality* 25: 435-451.

Affognon, H., C. Mutungi, P. Sanginga and C. Borgemeister. 2014. Unpacking postharvest losses in sub-saharan africa: A meta-analysis. *Journal of World Development* 66: 49–68.

Agamennone, V., C. A. M. Krul, G. Rijkers and R. Kort. 2018. A practical guide for probiotics applied to the case of antibiotic-associated diarrhea in the netherlands. *BMC Gastroenterology* 18(103): 1-12.

Aguilar-Toalá, J. E., R. Garcia-Varela, H. S. Garcia, V. Mata-Haro, A. F. González-Córdova, B. Vallejo-Cordoba and A. Hernández-Mendoza. 2018. Postbiotics: An evolving term within the functional foods field. *Trends in Food Science and Technology* 75: 105-114.

Ai, Z., X. Lv, S. Huang, G. Liu, X. Sun, H. Chen, J. Sun and Z. Feng. 2017. The effect of controlled and uncontrolled ph cultures on the growth of *lactobacillus delbrueckii* subsp. *Bulgaricus*. *LWT - Food Science and Technology* 77: 269-275.

Ajala, L., O. Lawrence-Otutu and A. Bamgbose. 2012. Effect of delayed processing on some physico-chemical properties of cassava starch. *American Journal Of Food And Nutrition* 2(2): 31-36.

Ajani, E. O., S. Sabiu, F. A. Bamisaye, S. Ibrahim and B. A. Salau. 2015. Evaluation of the acute and sub-acute toxicity effects of ethanolic leaves extract of lagenaria

brevifolia (bitter gourd) on hepatic and renal function of rats. *European Journal of Medicinal Plants* 5(2): 210-219.

Ajibola, F. O., M. O. Edema and O. B. Oyewole. 2012. Enzymatic production of ethanol from cassava starch using two strains of *saccharomyces cerevisiae*. *Nigerian Food Journal* 30(2): 114-121.

Akanji, M. A. and M. T. Yakubu. 2000. A-tocopherol protects against metabisulphite-induced tissue damage in rats. *Nigerian Journal of Biochemistry and Molecular Biology* 15: 179-183.

Akinwale, M. G., R. D. Aladesanwa, B. O. Akinyele, A. G. O. Dixon and A. C. Odiyi. 2010. Inheritance of ß-carotene in cassava (manihot esculenta crantza). *International Journal of Genetics and Molecular Biology* 2(10): 198-201.

Al-Harbi, M. S. 2016. Hepatoprotective effect and antioxidant capacity of naringenin on arsenic-induced liver injury in rats *International Journal of Pharmacy and Pharmaceutical Sciences* 8(4): 103-108.

Ali, A. A. and M. M. Mustafa. 2009. Use of starter cultures of lactic acid bacteria and yeasts in the preparation of kisra, a sudanese fermented food. *Pakistan Journal of Nutrition, 8: 1349-1353.*

Amaretti, A., M. d. Nunzio, A. Pompei, S. Raimondi, M. Rossi and A. Bordoni. 2013. Antioxidant properties of potentially probiotic bacteria: In vitro and in vivo activities. *Applied Microbiology and Biotechnology* 97: 809–817.

Ambesh, P., S. Stroud, E. Franzova, J. Gotesman, K. Sharma, L. Wolf and S. Kamholz. 2017. Recurrent lactobacillus bacteremia in a patient with leukemia. *Journal of Investigative Medicine High impact case reports* 2017(October-December Issue): 1-3.

Aminlari, M., A. Malekhusseini, F. Akrami and H. Ebrahimnejad. 2007. Cyanide-metabolising enzyme rhodanese in human tissues: Comparison with domestic animals. *Comparative Clinical Pathology* 16: 47-51.

Amund, O. D. 2016. Exploring the relationship between exposure to technological and gastrointestinal stress and probiotic functional properties of *Lactobacilli* and *Bifidobacteria*. *Canadian Journal of Microbiology* 62(715-725).

Anal, A. K. and H. Singh. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology* 18: 240-251.

Anandharaj, M., R. P. Rani and M. R. Swain. 2018. Production of high-quality probiotics by fermentation. In: *Microbial functional foods and nutraceuticals*. V. K. Gupta, H. Treichel, V. O. Shapaval, L. A. d. Oliveira and M. G. Tuohy (eds). 1st. John Wiley and Sons Ltd. 10**:** *235-266*.

AOAC. 2002. Aoac official methods of analysis. 15th ed. Association of official analytical chemists, arlington, va.

Arora, S., I. P. Kaur, K. Chopra and P. Rishi. 2014 Efficiency of double layered microencapsulated probiotic to modulate proinflammatory molecular markers for the management of alcoholic liver disease. *Mediators of Inflammation* 2014: 1-11.

Aroutcheva, A., J. Auclair, M. Frappier, M. Millette, K. Lolans, D. d. Montigny, S. Carrie`re, S. Sokalski4, W. E. Trick and R. A. Weinstein. 2016. Importance of molecular methods to determine whether a probiotic is the source of lactobacillus bacteremia. *Probiotics and Antimicrobial Proteins* 8: 31-40.

Asogwa, B. C., J. A. C. Ezihe and P. I. Ater. 2013. Socio-economic analysis of cassava marketing in Benue state, Nigeria. *International Journal of Innovation and Applied Studies* 2(4): 384-391.

Asonye, C. C. 2001. Fortification of common nigerian food-cassava meals. *The United Nations University Food and Nutrition Bulletin* 22(4).

Ayetigbo, O., S. Latif, A. Abass and J. Müller. 2018. Comparing characteristics of root, flour and starch of biofortified yellow-flesh and white-flesh cassava variants, and sustainability considerations: A review. *Sustainability* 10(3089): 1-32.

Ayichew, T., A. Belete, T. Alebachew, H. Tsehaye, H. Berhanu and A. Minwuyelet. 2017. Bacterial probiotics their importances and limitations: A review. *Journal of Nutritional Health and Science* 4(2):202.

Ayoola, A. A., A. O. Adeeyo, C. V. Efeovbokhan and D. A. Olasimbo. 2013. Optimum hydrolysis conditions of cassava starch for glucose production. *International Journal of Advanced Research in IT and Engineering* 2(1): 93-101.

Azad, M. A. K., M. Sarker, T. Li and J. Yin. 2018. Probiotic species in the modulation of gut microbiota: An overview. *BioMed Research International* 9478630: 1-8.

Azcárate-Peril, M. A., M. Sikes and J. M. Bruno-Bárcena. 2011. The intestinal microbiota, gastrointestinal environment and colorectal cancer: A putative role for probiotics in prevention of colorectal cancer? *American Journal of Physiology-Gastrointestinal and Liver Physiology* 301: G401–G424.

Azizpour, K., K. v. Kessel, R. Oudega and F. Rutten. 2017. The effect of probiotic lactic acid bacteria (lab) strains on the platelet activation: A flow cytometry-based study. *Journal of Probiotics and Health* 5(3): 1-5.

Bafana, B. (2011). Nigeria: Not everyone pleased with new vitamin a-fortified cassava. Inter Press Service (IPS) News Agency. Ibadan, IPS.

Bajpai, S. K. and S. Sharma. 2004. Investigation of swelling/degradation behaviour of alginate beads crosslinked with ca^{2+} and ba^{2+} ions. *Reactive Functional polymer* 59: 129-140.

Balakumar, M., D. Prabhu, C. Sathishkumar, P. Prabu, N. Rokana, R. Kumar, S. Raghavan, A. Soundarajan, S. Grover, V. K. Batish, V. Mohan and M. Balasubramanyam. 2016. Improvement in glucose tolerance and insulin sensitivity by probiotic strains of indian gut origin in high-fat diet-fed c57bl/6j mice. *European Journal of Nutrition* 57: 279-295.

Baltruskeviciene, E., B. Kazbariene, R. Badaras, L. Bagdonaitė, Aurelija Krikštaponienė, L. Zdanavičius, E. Aleknavicius and J. Didziapetrienė. 2016. Glutathione and glutathione s-transferase levels in patients with liver metastases of colorectal cancer and other hepatic disorders. *Turkish Journal of Gastroenterology* 27: 336-341.

Bartley, G. E. and P. A. Scolnik. 1995. Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. *The Plant Cell, Vol. 7, 1027-1038, July 1995.*

Bayless, T. M., E. Brown and D. M. Paige. 2017. Non-persistence and lactose intolerance. *Current Gastroenterology Report* 19(5): 23.

Bellik, Y., L. Boukraâ, H. A. Alzahrani, B. A. Bakhotmah, F. Abdellah, S. M. Hammoudi and M. Iguer-Ouada. 2013. Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: An update. *Molecules* 18: 322-353.

Berinyuy, E. B., B. Lawal, A. A. Olalekan, I. A. Olalekan, A. A. Yusuf and S. Sakpe. 2015. Haematologigal status and organ body-weight parameters in wistar rats during chronic administration of *cassia occidentalis. International Blood Research and Reviews* 4(3): 1-7.

Betiku, E., O. O. Akindolani and A. R. Ismaila. 2013. Enzymatic hydrolysis optimisation of sweetpotato *(ipomoea batatas*) peel using a statistical approach. *Brazillian Journal of Chemical Engineering* 30(3): 467-476.

Bondonno, C. P., K. D. Croft, N. Ward, M. J. Considine and J. M. Hodgson. 2015. Dietary flavonoids and nitrate: Effects on nitric oxide and vascular function. *Nutrition Reviews* 73(4): 216-235.

Bonsnes, R. and H. H. Tausslay. 1945. Introduction to colorimetric determination of creatinine by Jaffe reaction. *Biochemistry* 158: 581-591.

Boon, C. S., D. J. McClements, J. Weiss and E. A. Decker. 2010. Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition* 50: 515-532.

Borgogna, M., B. Bellich, L. Zorzin, R. Lapasin and A. Cesrro. 2010. Food microencapsulation of bioactive compounds: Rheological and thermal characterisation of non-conventional gelling system. *Food Chemistry* 122(2): 416-423.

Brashears, M. M., A. Amezquita, D. Jaroni and L. T. Steve. 2005. Lactic acid bacteria and their uses in animal feeding to improve food safety. *Advanced Food and Nutrition Research* 50: 11-31.

Bruce, R. D. 1985. An up-and-down procedure for acute toxicity testing. *Toxicological Sciences* 5(1): 151-157.

Bucolo, G. and H. David. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry* 19(5): 476-482.

Bujna, E., N. A. r. Farkas, A. M. Tran, M. S. Dam and Q. D. Nguyen. 2018. Lactic acid fermentation of apricot juice by mono- and mixed cultures of probiotic lactobacillus and bifidobacterium strains. *Food Science and Biotechnology* 27(2): 547-554.

Burgain, J., C. Gaiani, M. Linder and J. Scher. 2011. Encapsulation of probiotic living cells: from laboratory scale to industrial applications *Journal of Food Engineering* 104(4): 467-483.

Buruleanu, L. C., C. L. Nicolescu, G. Gorghiu, M. G. Bratu, D. Avram and I. Manea. 2009. Lactic acid fermentation of carrot and red beet juices by probiotic bacteria. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca 66, 252-258.

Canani, R. B., M. D. Costanzo, L. Leone, M. Pedata, R. Meli and A. Calignano. 2011. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World Journal of gastroenterology* 17: 1519–1528.

Cani, P. D., & Delzenne, N. M. (2009). The role of the gut microbiota in energy metabolism and metabolic disease. Current Pharmaceutical Design, 15(13), 1546-1558.

Capela, P., T. K. C. Hay and N. P. Shah. 2007. Effect of homogenisation on bead size and survival of encapsulated probiotic bacteria. *Food Research International* 40: 1261-1269.

Cardoso, A. P., E. Mirione, M. Ernesto, Fernando Massaza, J. Cliff, M. R. Haque and J. H. Bradbury. 2005. Processing of cassava roots to remove cyanogens. *Journal of Food Composition and Analysis* 18: 451-460.

Carvalho, L. M. J., A. R. G. Oliveira, R. L. O. Godoy, S. Pacheco, M. R. Nutti, J. L. V. de-Carvalho, E. J. Pereira and W. G. Fukuda. 2012. Retention of total carotenoid and β-carotene in yellow sweet cassava (*manihot esculenta crantz*) after domestic cooking. *Food and Nutrition Research* 56: 15788

Castillo, E. D., A. Nieto-Ruíz, M. Escudero-Marín and C. Campoy. 2018. Influence of glycaemic control on cognitive function in diabetic children and adolescents. In: *Diabetes food plan*. E. D. Castillo, A. Nieto-Ruíz, M. Escudero-Marín and C. Campoy (eds). Intechopen. 8**:** *121-143*.

Cevher, S. C., B. Balabanli and B. Aslim. 2015. Effects of probiotic supplementation on systemic and intestinal oxidant–antioxidant events in splenectomised rats. *Surgery today* 45(9): 1166-1172.

Chaluvadi, S., A. T. Hotchkiss and K. L. Yam. 2016. Gut microbiota: Impact of probiotics, prebiotics, synbiotics, pharmabiotics, a postbiotics on human health. In: *Probiotics, prebiotics and synbiotics. Bioactive foods in health promotion*. R. R. Watson and V. R. Preedy (eds). London. Elsevier**:** *515–523*.

Champagne, C. P. and N. J. Gardner. 2008. Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses. *Food Research International* 41(5): 539-543.

Chang, C.-H., H.-Y. Lin, C.-Y. Chang and Y.-C. Liu. 2005. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. *Journal of Food Engineering 77, (2006): 478–485.*

Charalampopoulos, D., S. S. Pandiella and C. Webb. 2002. Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. *Journal of Applied Microbiology* 92: 851–859.

Chatterjee, M. and M. Janarthan. 2012. Biological activity of carotenoid: Its implication in cancer risk and prevention. *Current pharmacology and Biotechnology* 13(1): 180-190.

Chaudhary, M. and R. Gupta. 2012. Cyanide detoxifying enzyme: Rhodanese. *Current Biotechnology* 1: 327-335.

Chauhan, P., R. Yadav, V. Kaushal and P. Beniwal. 2016. Evaluation of serum biochemical profile of breast cancer patients. *International Journal of Medical Research and Health Sciences* 5(7): 1-7.

Chávarri, M., I. Marañón, R. Ares, F. C. Ibáñez, F. Marzo and V. María del Carmen. 2010. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *international journal of food Microbiology* 142: 185-189.

Chede, P. S. 2013. Phytochemical analysis of citrus sinensis peel. *International Journal of Pharmacy and Biological Sciences* 4(1): 339-343.

Chen, C., Y. Lu, H. Yu, Z. Chen and H. Tian. 2019. Influence of 4 lactic acid bacteria on the flavor profile of fermented apple juice. *Food Bioscience* 27: 30-36.

Chen, L., H. Li, J. Li, Y. Chen and Y. Yang. 2019. *Lactobacillus rhamnosus GG* treatment improves intestinal permeability and modulates microbiota dysbiosis in an experimental model of sepsis. *International Journal of Molecular Medicine* 43: 1139-1148.

Chen, Y., S. Huang, Z. Tang, X. Chen and Z. Zhang. 2011. Structural changes of cassava starch granules hydrolyzed by a mixture of α-amylase and glucoamylase. *Carbohydrate Polymers* 85: 272–275.

Chinedu, E., D. Arome and F. S. Ameh. 2013. A new method for determining acute toxicity in animal models. *Toxicological International* 20(3): 224-226.

Chiocchetti, G. M., C. Jadán-Piedra, V. Monedero, M. Zúñiga, D. Vélez and V. Devesa. 2018. Use of lactic acid bacteria and yeasts to reduce exposure to chemical food contaminants and toxicity. *Critical Reviews in Food Science and Nutrition. Assessed online at doi: 10.1080/10408398.2017.1421521 on 28/02/2019.*

Chistiakov, D. A., Y. V. Bobryshev, E. Kozarov, I. A. Sobenin and A. N. Orekhov. 2015. Role of gut microbiota in the modulation of atherosclerosis-associated immune response. *Frontiers in Microbiology* 6 (671): 1-7. *doi: 10.3389/fmicb.2015.00671*

Chutani, A. and S. Pande. 2017. Correlation of serum creatinine and urea with glycemic index and duration of diabetes in type 1 and type 2 diabetes mellitus: A comparative study. *National Journal of Physiology, Pharmacy and Pharmacology* 7(9): 914-919.

Colehour, A. M., J. F. Meadow, Melissa A. Liebert, T. J. Cepon-Robins, T. E. Gildner, S. S. Urlacher, B. J. M. Bohannan, J. J. Snodgrass and L. S. Sugiyama. 2014. Local domestication of lactic acid bacteria via cassava beer fermentation. *PeerJ 2:e479;doi:10.7717/peerj.479.ecollection2014.*

Cook, M. T., G. Tzortzis, D. Charalampopoulos and V. V. Khutoryanskiy. 2012. Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release* 162(1): 56-57.

Corcoran, B. M., R. P. Ross, G. F. Fitzgerald and C. Stanton. 2004. Comparative survival of probiotic *lactobacilli* spray-dried in the presence of prebiotic substances. *Journal of Applied Microbiology* 96: 1024-1039.

Corcoran, B. M., C. Stanton, G. F. Fitzgerald and R. P. Ross. 2005. Survival of probiotic *Lactobacilli* in acidic environments is enhanced in the presence of metabolisable sugars. *Applied and eEnvironmental Microbiology* 71(6): 3060-3067.

Cruz, A. G., W. F. Castro, J. A. F. Faria, P. C. B. Lollo, J. Amaya-Farfán, M. Q. Freitas, D. Rodrigues, C. A. F. Oliveira and H. T. Godoy. 2012. Probiotic yogurts manufactured with increased glucose oxidase levels: Postacidification, proteolytic patterns, survival of probiotic microorganisms, production of organic acid and aroma compounds. *Journal of Dairy Science,95 :2261–2269.*

Dacie, J. V. and S. M. Lewis. 2008. Basic haematological techniques. In: *Textbook of practical haematology* (eds). 10th Edition. Churchill, Livingstone**:** *50-56*.

Daneshi, M., M. R. Ehsani, S. H. Razavi and M. Labbafi. 2013. Effect of refrigerated storage on the probiotic survival and sensory properties of milk/carrot jice mix drink. *Electronic Journal of Biotechnology* 16(5): 1-12.

Dani, C., C. C. Caterina, C. I. Luri, F. Arena, A. Antonelli and G. M. Rossolini. 2016. *Lactobacillus* sepsis and probiotic therapy in newborns: Two new cases and literature review. *American Journal of Perinatology* 6: e25–e29.

De Keersmaecker, S. C. J., T. L. A. Verhoeven, J. Desair, K. Marchal, J. Vanderleydem and I. Nagy. 2006. Strong antimicrobial activity of *lactobacillus rhamnosus gg* against *salmonellatyphimurium* is due to the accumulation of lactic acid. *FEMS Microbiology letters* 259: 89-96.

de Melo Pereira, G. V., A. M. Finco, L. A. J. Letti, S. G. Karp, M. G. B. Pagnoncelli, J. de Oliveira, V. T. Soccol, S. K. Brar and C. R. Soccol. 2019. Microbial metabolic pathways in the production of valued-added products. In: *Microbial sensing in fermentation*. S. K. Brar, R. K. Das and S. J. Sarma (eds). First Edition. John Wiley and Sons Ltd. 7**:** *137-167*.

Deabes, M. M., H. R. Darwish, K. B. Abdel-Aziz, I. M. Farag, S. A. Nada and N. S. Tawfek. 2012. Protective effects of *lactobacillus rhamnosus GG* on aflatoxin-induced toxicities in male albino mice. *Environmental and Analytical Toxicology* 2(3): 1-9.

Deepa, G., V. Singh and K. A. Naidu. 2010. A comparative study on starch digestibility, glycemic index and resistant starch of pigmented (‘njavara’ and ‘jyothi’) and a non-

pigmented (‘ir 64’) rice varieties. *Journal of Food Science and Technology* 47(6): 644-649.

Dellafiora, L., C. Dall’Asta and G. Galaverna. 2018. Toxicodynamics of mycotoxins in the framework of food risk assessment- an in silico perspective. *Toxins* 10(52): 1-23.

Dhital, S., V. M. Butardo Jr, S. A. Jobling and M. J. Gidley. 2015. Rice starch granule amylolysis- differentiating effects of particle size, morphology, thermal properties and crystalline polymorph. *Carbohydrate Polymers* 115: 305-316.

Ding, W. K. and N. P. Shah. 2008. Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *International Food Research Journal* 15(2): 219-232.

Dokic, L., J. Jakovljevic and P. Dokic. 2004. Relation between viscous characteristics and dextrose equivalent of maltodextrins. *Starch* 56: 520–525.

Donhowe, E. G., F. P. Flores, W. L. Kerr, L. Wicker and F. Kong. 2014. Characterisation and in vitro bioavailability of β-carotene: Effects of microencapsulation method and food matrix. *LWT- Food Science and Technology* 57: 42-48.

Dordevic, T. M., S. S. SilerMarinkovic and S. I. DimitrijevicBrankovic. 2010. Effect of fermentation on the antioxidant properties of some cereals and pseudo-cereals. *Food Chemistry* 119: 957-963.

Doughari, J. H. (2012). Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents, phytochemicals - a global perspective of their role in nutrition and health. *https://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/phytochemicals-extraction-methods-basic-structures-and-mode-ofoaction-as-potential-chemotherapeutic-* on 25/06/2017.

Eaton, D. L. and S. G. Gilbert. 2008. Principles of toxicology. In: *Casarett and doull’s toxicology: The basic science of poisons*. Curtis D Klaassen Ph.D (eds). seventh edition. United States of America. The McGraw-Hill Companies, Inc. 2**:** 1-1309.

Ebuehi, O. A. T. and D. C. Dibie. 2015. Hyperglycemic effect on brain cholinergic functions, oxidative stress and protein expression of brain derived neurotropic factor (bdnf) on cognitive functions in streptozotocin induced-diabetic rats. *Research in Neuroscience* 4(1):1-9.

Echebiri, R. N. and M. E. I. Edaba. 2008. Production and utilisation of cassava in nigeria: Prospects for food security and infant nutrition. *Journal of production Agriculture and Technology PAT 2008; 4 (1)*: 38-52

Egwim, E., A. Musa, Y. Abubakar and B. Mainuna (2013). Nigerian indigenous fermented foods: Processes and prospects. Mycotoxin and Food Safety in Developing Countries InTech**:** 153-180.

Eke, J., S. C. Achinewhu, L. Sanni, I. S. Barimalaa, B. Maziya-Dixon and A. Dixon. 2009. Pasting, color, and granular properties of starches from local and improved cassava varieties in high rainfall region of Nigeria. *International Journal of Food Properties* 12: 439-449.

El-Salam, M. H. A. and S. El-Shibiny. 2015. Preparation and properties of milk proteins-based encapsulated probiotics: A review. *Dairy Science and Technology* 95: 393–412.

El-Sharkawy, M. A. 2004. Cassava biology and physiology. *Plant Molecular Biology* 56(4): 481-501.

Eleazu, C. O. 2016. The concept of low glycemic index and glycemic load foods as panacea for type 2 diabetes mellitus; prospects, challenges and solutions. *African Health Sciences* 16(2): 468-479.

Ezeokonkwo, R. C., I. O. Ezeh, J. I. Onunkwo, I. W. Onyenwe, C. N. Iheagwam and W. E. Agu. 2012. Comparative serum biochemical changes in mongrel dogs following single and mixed infections of trypanosoma congolense and trypanosoma brucei brucei. *Elsevier Veterinary Parasitology* 190: 56- 61.

Falade, K. O. and J. O. Akingbala. 2010. Utilisation of cassava for food. *Food Reviews International* 27: 51–83.

FAO (2017). Food outlook: Biannual report on global food markets, food and agriculture organisation of the united nations (fao). FAO, Food and Agriculture Organisation of the United Nations (FAO)**:** 34-39.

Fawcett, J. K. and J. E. Scott. 1960. A rapid and precise method for the determination of urea. *Journal of Clinical Pathology* 13: 156-159.

Fijałkowski, K., D. Peitler, R. Rakoczy and A. Zywicka. 2016. Survival of probiotic lactic acid bacteria immobilised in different forms of bacterial cellulose in simulated

gastric juices and bile salt solution. *LWT - Food Science and Technology* 68: 322e328.

Filannino, P., Y. Bai, R. D. Cagno, M. Gobbetti and M. G. Ganzle. 2015. Metabolism of phenolic compounds by lactobacillus spp. During fermentation of cherry juice and broccoli puree. *Food Microbiology* 46: 272-279.

Florou-Paneri, P., E. Christaki and E. Bonos (2013). Lactic acid bacteria as source of functional ingredients. In: Lactic acid bacteria – R & D for food, health and livestock purposes, InTech.**:** 589-614.

Forsyth, C. B., A. Farhadi, S. M. Jakate, Y. Tang, M. Shaikh and A. Keshavarzian. 2009. *Lactobacillus GG* treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcohol steatohepatitis. *Alcohol* 43(2): 163-172.

Francis, G., Z. Kerem, H. P. S. Makkar and K. Becker. 2002. The biological action of saponins in animal systems: A review. *British Journal of Nutrition 2002, 88: 587-605.*

Francis, R. D., Y. Rodgers, C. Salmon, G. A. Junior, P. S. Bahado-Singh, A. M. Smith, A. O. Wheatley and H. N. Asemota. 2010. Glycemic index in the development of functional beverage. *European Journal of Experimental Biology* 8(2): 1-4.

Franko, B., P. Fournier, T. Jouve, P. Malvezzi, I. Pelloux, J. P. Brion and P. Pavese. 2017. Lactobacillus bacteremia: Pathogen or prognostic marker? *Médecine et maladies infectieuses* 47(1): 18-25.

Fugelsang, K. C. and C. G. Edwards (2007). Wine microbiology: Practical applications and procedures.http://www.springer.com/978-0-387-33341-0**:**29-44.

Gaanappriya, M., P. Guhankumar, V. Kiruththica, N. Santhiya and S. Anita. 2013. Probiotication of fruit juices *lactobacillus acidophilus*. *International Journal of Advanced Biotechnology and Research* 4: 72-77.

Galgano, F., N. Condelli, M. C. Caruso, M. A. Colangelo and F. Favati. 2015. Probiotics and prebiotics in fruits and vegetables: Technological and sensory aspects. In: *Beneficial microbes in fermented and functional foods*. V. R. Rai and J. A. Bai (eds). London. CRC Press**:** *189-206*.

Ganzle, M. G. 2015. Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science* 2: 106-117.

Garcia, C. E. V., M. Petrova, I. J. J. Claes, I. D. Boeck, T. L. A. Verhoeven, E. Dilissen, I. V. Ossowski, A. Palva, D. M. Bullens, J. Vanderleyden and S. Lebeer. 2015. Piliation of *Lactobacillus rhamnosus gg* promotes adhesion, phagoicytosis, and cytokine modulation in macrophages. *Applied and Environmental Microbiology* 81(6): 2050-2062.

Geng, W. and J. Lin. 2017. Bile salt hydrolase: An intestinal mocrobiome target for enhanced animal health. *Animal Health Research Reviews* 17(2):148-158.

Goldhaber, S. B. 2003. Trace element risk assessment: Essentiality vs. Toxicity. *Regulatory Toxicology and Pharmacology* 38(2): 232–242.

Goldstein, E. J. C., K. L. Tyrrell and D. M. Citron. 2015. Lactobacillus species: Taxonomic complexity and controversial susceptibilities. *Clinical Infectious Diseases* 60(2): 98–107.

Goodwin, J. W. and R. W. Hughes. 2000. Rheology for chemists, an introduction. Royal society of chemist. Cambridge, UK.

Gorbach, S., S. Doron and F. Magro (2017). *Lactobacillus rhamnosusGG*. The Microbiota in Gastrointestinal Pathophysiology, Elsevier inc.**:** 79-86.

Gosslau, A. 2016. Assessment of food toxicology. *Food Science and Human Wellness* 5: 103–115.

Gouriet, F., M. Million, M. Henri, P. E. Fournier and D. Raoult. 2012. *Lactobacillus rhamnosus* bacteremia: An emerging clinical entity *European Journal of Clinical Microbiology and Infectious Diseases* 31: 2469-2480.

Grune, T., G. Lietz, A. Palou, A. C. Ross, W. Stahl, G. Tang and H. K. Bielsalski. 2010. B-carotene is an important vitamin a source for humans. *Journal of Nutrition* 140(12): 2268S–2285S.

Guibad, G., N. Tixier and M. Baudu. 2005. Hysteresis area, a rheological parameter used as a tool to assess the ability of filamentous sludges to settle. *Journal of Process Biochemistry,* 40:2671-2676.

Gul, K., A. K. Singh and R. Jabeen. 2015. Nutraceuticals and functional foods: The foods for future world. *Critical Reviews in Food Science and Nutrition* 56(16): 2617-2627.

Gul, K., A. Tak, A. K. Singh, P. Singh, B. Yousuf and A. A. Wani. 2015. Chemistry, encapsulation, and health benefits of β-carotene - A review. *Cogent Food and Agriculture*http://dx.doi.org/10.1080/23311932.2015.1018696.

Guzmán-Maldonadoa, H. and O. Paredes-Lópezb. 1995. Amylolytic enzymes and products derived from starch: A review *Critical Reviews in Food Science and Nutrition* 35(5): 373-403.

Halim, S. Z., N. R. Abdullah, A. Afzan, B. A. A. Rashid, I. Jantan and Z. Ismail. 2011. Acute toxicity study of carica papaya leaf extract in sprague dawley rats. *Journal of Medicinal Plants Research* 5: 1867-1872.

Hasiri, M. A., H. R. Gheisari and A. A. Khademolhosseini. 2015. *Lactobacillus rhamnosus* as a probiotic for the health of adult dogs. *International Journal of Probiotics and Prebiotics* 10(2/3): 69-76.

He, J., H. Pan, W. Liang, D. Xiao, X. Chen, M. Guo and J. He. 2017. Prognostic effect of albumin-to-globulin ratio in patients with solid tumors: A systematic review and meta-analysis. *Journal of Cancer* 8 (19): 4002-4010.

Heidebach, T., P. Forst and U. Kulozik. 2009. Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. *Food Hydrocolloids* 23: 1670-1677.

Heidebach, T., P. Forst and U. Kulozik. 2012. Microencapsulation of probiotic cells for food applications. *Critical Reviews in Food Science and Nutrition* 52: 291–311.

Helland, M. H., T. Wickluund and J. A. Narvhus. 2004. Growth and metabolism of selected strain of probioticbacteria in maize porridge with added malt barley. *international Journal of Food Microbiology* 91: 305-313.

Homayouni, A., L. Payahoo and A. Azizi. 2012. Effect of probiotics on lipid profile: A review. *American Journal of Food Technology* 7: 251-265.

Hong, K.-W., H.-S. Jin, D. Song, H.-k. Kwak, S. S. Kim and Y. Kim. 2013. Genome-wide association study of serum albumin:Globulin ratio in korean populations. *Journal of Human Genetics* 58: 174–177.

Hu, X., T. Wang, W. Li, F. Jin and L. Wang. 2013. Effects of ns lactobacillus strains on lipid metabolism of rats fed a high-cholesterol diet. *Lipids in Health and Disease* 12(67): 1-12.

Hur, S. J., S. Y. Lee, Y. Kim, I. Choi and G. Kim. 2014. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry* 160: 346-356.

Ighodaro, O. M. and O. A. Akinloye. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine* 54: 287–293.

Itle, R. A. and E. A. Kabelka. 2009. Correlation between L*a*b* color space values and carotenoid content in pumpkins and squash (cucurbita spp.). *Hortscience* 44(3): 633-637.

Iyer, C. and K. Kailasapathy. 2005. Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt conditions and in yogurt. *Journal of Food Science* 70(1): 18-23.

Jeng, T. L., P. T. Ho, Y. J. Shih, C. C. Lai and J. M. Sung. 2012. Chemicals contents in non-alcoholic and alcoholic beverages produced from purple-fleshed and orange-fleshed sweet potato varieties. *Journal of Food Science and Technology Resource* 18(5): 639 – 644.

Jenkins, D. J. A., D. M. Thomas, M. S. Wolever, R. H. Taylor, H. Barker, H. Fielden, J. M. Baldwin, A. C. Bowling, H. C. Newman, A. L. Jenkins and D. V. Goff. 1981. Glycemic index of foods: A physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition* 34: 362-366.

Jenkins, D. J., T. M. Wolever and A. L. Jenkins. 1988. Starchy foods and glycemic index. *Diabetes Care* 11(2): 149-159.

Ji, Y. S., H. N. Kim, H. J. Park, J. E. Lee, S. Y. Yeo and J. S. Yang. 2012. Modulation of the murine microbiome with a concomitant anti-obesity effect by *Lactobacillus rhamnosus GG and lactobacillus sakei nr28. Beneficial Microbes* 3: 13-22.

Johnstone, C. P., A. Lill and R. D. Reina. 2017. Use of erythrocyte indicators of health and condition in vertebrate ecophysiology: A review and appraisal. *Biological Reviews* 92(1): 150-168.

Kachouri, F., H. Ksontini, M. Kraiem, K. Setti, M. Mechmeche and M. Hamdi. 2015. Involvement of antioxidant activity of lactobacillus plantarum on functional

properties of olive phenolic compounds. *Journal of Food Science and Technology* 52(12): 7924-7933.

Kanlaya, Y. and K. Jirasak. 2004. A study of optimal conditions for reducing sugars production from cassava peels by diluted acid and enzyme,. *Journal of Natural Science* 38:29-35.

Karbasi, M., M. S. Yarmand and M. Mousavi. 2015. Fermentation potential of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* in date syrup to develop a functional fermented beverage: A comparative study. *Journal of Food Processing and Preservation* 39: 863-870.

Karl, J. P., A. M. Hatch, S. M. Arcidiacono, S. C. Pearce, I. G. Pantoja-Feliciano, L. A. Doherty and J. W. Soares. 2013. Effects of psychological, environmental and physical stressors on the gut microbiota. *Frontiers in Microbiology* 9: 1-32.

Karovicova, J. and Z. Kohajdova. 2003. Lactic acid fermented vegetable juices. *journal of Horticultural Science (Prague).* 30(4): 152–158.

Karovicova, J. and Z. Kohajdova. 2005. Lactic acid fermented vegetable juices- palatable and wholesome foods. *Chemical Paper.* 59 (2): 143-148.

Kennedy, J. F., C. J. Knill and D. W. Taylor. 1995. Maltodextrins. In: *Handbook of starch hydrolysis products and their derivatives*. M. W. Kearsley and S. Z. Dziedzic (eds). Boston, MA. Springer.

Khosrokhavar, R. and A. M. Mortazavian. 2010. Effects probiotic-containing microcapsules on viscosity, phase separation and sensory attributes of drink based on fermented milk. *Milchwissenschaft Milk Science International* 65(2): 177-179.

Kim, D.-H., W.-T. Lim, M.-K. Lee and M.-S. Kim. 2012. Effect of temperature on continuous fermentative lactic acid (la) production and bacterial community, and development of LA-producing UASB reactor. *Bioresource Technology* 119: 355–361.

Kim, S., S. A. McClave, R. G. Martindale, K. R. Miller and R. T. Hurt. 2017. Hypoalbuminemia and clinical outcomes: What is the mechanism behind the relationship? *The American Surgeon* 83(11): 1220-1227.

Kim, Y. S., H. A. Lee, J. Y. Lim, Y. Kim, C. H. Jung, S. H. Yoo and Y. Kim. 2014. B-carotene inhibits neuroblastoma cell invasion and metastasis in vitro and in vivo by decreasing level of hypoxia-inducible factor-1α. *The Journal of Nutritional Biochemistry* 25: 655–664.

Klemashevich, C., C. Wu, D. Howsmon, R. C. Alaniz, K. Lee and A. Jayaraman. 2014. Rational identification of diet-derived postbiotics for improving intestinal microbiota function. *Current Opinion in Biotechnology* 26: 85-90.

Klewicka, E. and A. Czyżowska. 2011. Biological stability of lactofermented beetroot juice during refrigerated storage. *Polish Journal of Food and Nutrition Sciences* 61(4): 251-256.

Kobawila, S. C., D. Louembe, S. Keleke, J. Hounhouigan and C. Gamba. 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce *bikedi* and *ntobambodi*, two food products from Congo. *African Journal of Biotechnology.* 4*(7):* 689-696.

Kobyliak, N., T. Falalyeyeva, T. Beregova and M. Spivak. 2017. Probiotics for experimental obesity prevention: Focus on strain dependence and viability of composition. *Endokrynologia Polska* 68(6): 659–667.

Koh, W. Y., U. Utra, R. Ahmad, I. A. Rather and Y.-H. Park. 2018. Evaluation of probiotic potential and anti-hyperglycemic properties of a novel lactobacillus strain isolated from water kefir grains. *Food Science and Biotechnology* 27(5): 1369–1376.

Kohajdová, Z., J. Karovičová and M. Greifová. 2006. Lactic acid fermentation of some vegetable juices. *Journal of Food and Nutrition Research 45(3):* 115-119.

Korhonen, R., R. Korpela, M. Saxelin, M. Maki, H. Kankaanranta and E. Moilanen. 2001. Induction of nitric oxide synthesis by probiotic *Lactobacillus rhamnosus GG* in J774 macrophages and human T84 intestinal epithelial cells. *Inflammation* 25(4): 223-232.

Kotsonis, F. N. and G. A. Burdock. 2008. Food toxicology. In: *Casarett and doull's toxicology: The basic science of poisons*. Curtis D Klaassen Ph.D (eds). Seventh edition. USA. The McGraw-Hill Companies, Inc. 30**:** *1-1309*.

Kouamé, A. C., K. N. Kouassi, Y. D. N'dri and N. g. G. Amani. 2015. Glycaemic index and load values tested in normoglycemic adults for five staple foodstuffs: Pounded yam, pounded cassava-plantain, placali, attieke and maize meal stiff porridge. *Nutrients* 7: 1267-1281.

Koyama, S., H. Fujita, T. Shimosato, A. Kamijo, Y. Ishiyama, E. Yamamoto, Y. Ishii, Y. Hattori, M. Hagihara, E. Yamazaki, N. Tomita and H. Nakajima. 2018. Septicemia from *Lactobacillus rhamnosus GG,* from a probiotic enriched yogurt, in a patient with autologous stem cell transplantation. *Probiotics and Antimicrobial Proteins accessed online at https://doi.org/10.1007/s12602-018-9399-6.* on 23/04/2016

Krasaekoopt, W., B. Bhandari and H. Deeth. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal* 13: 3-13.

Krasaekoopt, W. and K. Kitsawad. 2010. Sensory characteristics and consumer acceptance of fruit juice containing probioitcs beads in thailand. *AU Journal of Technology* 14(1): 33-38.

Ktari, N., I. Bkhairia, R. Nasri, R. B. A. Kolsi, R. B. S.-B. Salem, I. B. Amara, N. Zeghal, B. B. Salah, R. B. Salah and M. Nasri. 2017. Zebra blenny protein hydrolysates as a source of bioactive peptides with prevention effect against oxidative dysfunctions and DNA damage in heart tissues of rats fed a cholesterol-rich diet. *Food Research International* 100(1): 423-432.

Kumar, M., V. Gupta, P. Kumari, C. R. K. Reddy and B. Jha. 2010. Assessment of nutrient composition and antioxidant potential of caulerpaceae seaweeds. *Journal of Food Composition and Analysis, 24:270–278.*

Kunutsor, S. K., T. A. Apekey, D. Seddoh and J. Walley. 2014. Liver enzymes and risk of all-cause mortality in general populations: A systematic review and meta-analysis. *International Journal of Epidemiology* 43: 187–201.

Ladeira, T., H. Souza and R. Pena. 2013. Characterisation of the roots and starches of three cassava cultivars. *International Journal of Agricultural Science Research* 2: 12–20.

Lahtinen, S., A. C. Ouwehand, S. Salminen and A. von Wright. 2011. Lactic acid bacteria: An introduction. In: *Lactic acid bacteria microbial and functional aspects*. S.

Lahtinen, A. C. Ouwehand, S. Salminen and A. von Wright (eds). 4th. Boca Raton, FL. CRC Press. 1**:** *1-16*.

Larmond, E. 1977. Laboratory method of sensory evaluation of food. Canada Department of Agriculture, Ottawa.

Lawal, B., O. K. Shittu, F. I. Oibiokpa, H. Mohammed, S. I. Umar and G. M. Haruna. 2016. Antimicrobial evaluation, acute and sub-acute toxicity studies of allium sativum. *Elsevier Journal of Acute Disease* 5(4): 296–301.

Lee, H.-Y., J.-H. Park, S.-H. Seok, M.-W. Back, D.-J. Kim, K.-E. Lee, K.-S. Paek, Y. Lee and J.- H. Park. 2006. Human originated bacteria, *lactobacillus rhamnosus pl60,* produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochimica et Biophysica Acta* 1761: 736-744.

Lee, K. Y. and D. J. Mooney. 2012. Alginate: Properties and biomedical applications. *Progress in Polymer Science* 37(1): 106-126.

Leo´n, K., D. Mery, F. Pedreschi and J. Leo´n. 2006. Color measurement in l*a*b* units from RGB digital images. *Food Research International* 39: 1084-1091.

Li, C., D. Fang, Z. Li, Z. Gu, Q. Yang, L. Cheng and Y. Hong. 2016. An improved two-step saccharification of high-concentration cornstarch slurries by granular starch hydrolyzing enzyme. *Industrial Crops and Products* 94: 259–265.

Li, N., W. M. Russell, M. Douglas-Escobar, N. Hauser, M. Lopez and J. Neu. 2009. Live and heat-killed *Lactobacillus rhamnosus GG*: Effects on proinflammatory and anti-inflammatory cytokines/chemokines in gastrostomy-fed infant rats. *Pediatric Research* 66(2): 203-207.

Li, X. Y., X. G. Chen, D. S. Cha, H. J. Park and C. S. Liu. 2009. Microencapsulation of a probiotic bacteria with alginate–gelatin and its properties. *Journal of Microencapsulation* 26: 315-324.

Li, Z., J. Teng, Y. Lyu, X. Hu, Y. Zhao and M. Wang. 2019. Enhanced antioxidant activity for apple juice fermented with *Lactobacillus plantarum ATCC14971*. *Molecules* 24(1): 51.

Liang, R., Q. Huang, J. Ma, C. F. Shoemaker and F. Zhong. 2013. Effect of relative humidity on the store stability of spray-dried beta carotene nanoemulsions. *Food Hydrocolloids* 33: 225-233.

Liong, M. T. and N. P. Shah. 2005. Acid and bile tolerance and cholesterol removal ability of *Lactobacilli* strains. *Journal of Dairy Science* 88(1): 55-66.

Little, J. P., P. D. Chilibeck, D. Ciona, A. Vandenberg and G. A. Zello. 2009. The effects of low–and high–glycemic index foods on high-intensity intermittent exercise. *International Journal of Sports Physiology and Performance* 4: 367-380.

Liu, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *Journal of Nutrition. 134:* 3479S–3485S.

Lye, H. S., G. R. Rahmat-Ali and M. T. Liong. 2010. Mechanisms of cholesterol removal by *Lactobacilli* under conditions that mimic the human gastrointestinal tract. *International Dairy Journal* 20(3): 169-175.

Lye, H. S., G. Rusul and M. T. Liong. 2010. Removal of cholesterol by *Lactobacilli* via incorporation and conversion to coprostanol. *Journal of Dairy Science* 93(4): 1383-1392.

Magerramov, M. A., A. I. Abdulagatov, I. M. Abdulagatov and N. D. Azizov. 2007. Viscosity of tangerine and lemon juices as a function of temperature and concentration. *International Journal of Food Science and Technology* 42: 804–818.

Mahmood, N. M. S. and K. R. Hamad. 2017. The study of body weight, haematological and serum biochemical parameters, liver and kidney texture in rats fed corn oil. *ZANCO Journal of Pure and Applied Sciences* 29(s4): s76-s86.

MaiaCosta, M. G., T. V. Fonteles, A. L. TibériodeJesus and S. Rodrigues. 2013. Sonicated pineapple juice as substrate for *L. casei* cultivation for probiotic beverage development: Process optimisation and product stability. *Food Chemistry* 139: 261-266.

Maity, C., A. Adak, T. K. Pathak, B. R. Pati and K. C. Mondal. 2012. Study of the cultivable microflora of the large intestine of the rat under varied environmental hyperbaric pressures. *Journal of Microbiology, Immunology and Infection* 45: 281-286.

Maity, C., S. Rana, B. R. Pati and K. C. Mondal. 2009. Effect of graded hyperbaric atmospheric pressure on the quantity and composition of faecal flora. *Microbal Ecology Health and Diseases* 21: 104-108.

Makras, L., V. Triantafyllou, D. Fayol-Messaoudi, T. Adriany, G. Zoumpopoulou, E. Tsakalidou, AlainServin and L. De Vuyst. 2006. Kinetic analysis of the antibacterial activity of probiotic *Lactobacilli* towards *Salmonellaentericaserovar typhimurium* reveals a role for lactic acid and other inhibitory compounds. *Research in Microbiology* 157: 241–247.

Malakouti, M., A. Kataria, S. K. Ali and S. Schenker. 2017. Elevated liver enzymes in asymptomatic patients – what should i do? *Journal of Clinical and Translational Hepatology* 5: 394–403.

Maldonado-Pereira, L., M. Schweiss, C. Barnaba and I. G. Medina-Meza. 2018. The role of cholesterol oxidation products in food toxicity. *Food and Chemical Toxicology* 118: 908-939.

Malik, T. F. and K. K. Panuganti. 2019. Lactose intolerance. *StatPearls Publisher. Accessed online at https://www.nbci.nlm.nih.gov/books/NBK532285_pubdet_*.on 01/07/2019

Manea, I., L. Buruleanu, D. Avram and M. Bratu (2009). Researches concerning the obtaining of lacto-fermented cabbage juice – functional food. Colloque Franco-Roumain de Chimie Appliquée – COFrRoCA 2008. Bacau, Romania.

Mansuro˘glua, B., S. Dermanb, A. Yabac and K. Kızılbey. 2015. Protective effect of chemically modified sod on lipid peroxidationand antioxidant status in diabetic rats. *International Journal of Biological Macromolecules* 72: 79-87.

Mantzourani, I., S. Kazakos, A. Terpou, A. Alexopoulos, E. Bezirtzoglou, A. Bekatorou and S. Plessas. 2019. Potential of the probiotic *Lactobacillus plantarumATCC 14917* strain to produce functional fermented pomegranate juice. *Foods8(4)*: 1-13.

Marchesini, G. and G. Targher. 2011. Commentary: Liver enzymes and the risk of adverse cardiovascular outcomes—the lower, the better? *International Journal of Epidemiology40*: 1539–1541.

Marineli, R. d. S., S. A. Lenquiste, É. A. Moraes and M. R. Maróstica Jr. 2015. Antioxidant potential of dietary chia seed and oil (*salvia hispanica l.*) in diet-induced obese rats. *Food Research International* 76(3): 666-674.

Marrocco, I., F. Altieri and I. Peluso. 2017. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxidative Medicine and Cellular Longevity accessed at https://doi.org/10.1155/2017/6501046 on 23/02/2019.*

Marshall, E. and D. Mejia. 2012. Traditional fermented food and beverages for improved livelihoods. *FAO Diversification booklet number 21.*: 1-79.

Martarelli, D., M. C. Verdenelli, S. Scuri, M. Cocchioni, S. Silvi, C. Cecchini and P. Pompei. 2011. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. *Current Microbiology* 62: 1689–1696.

Martinez, F. A. C., E. M. Balciunas, J. M. Salgado, J. M. D. Gonzalez, A. Converti and R. P. D. s. Oliveira. 2013. Lactic acid properties, applications and production: A review. *Trends in Food Science and Technology* 30*(1)*: 70-83.

Marwati, T., N. Cahyaningrum, S. Widodo, U. T. Astiati, A. Budiyanto, Wahyudiono, A. B. Arif and N. Richana. 2018. The effect of alpha amylase enzyme on quality of sweet sorghum juice for chrystal sugar. *IOP Conference Series: Earth and Environmental Science* 102*(012090)*: 1-9.

Mates, J. M., C. Perez-Gomez and M. Blanca. 2000. Chemical and biological activity of free radical 'scavengers' in allergic diseases. *Clinica Chimica Acta* 296: 1-15.

Mazumder, S. and D. Bera. 2013. Production of malto-dextrin from broken rice. *International Journal of Research in Engineering and Technology 2 (10):* 477-482.

Mbaebie, B. O., H. O. Edeoga and A. J. Afolayan. 2012. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia jacq*. *Asian Pacific Journal of Tropical Biomedicine 118-124.*

Mbajiuka, C. S., Y. S. Omeh and M. I. Ezeja. 2010. Fermentation of sorghum using yeast (*Saccharomyces cerevisiae*) as a starterculture for burukutu production. *Continental Journal of Biological Sciences 3: 63 - 74, 2010.*

McFarland, L. V. 2014. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: A systematic review. *BMJ Open* 4: e005047.

Meini, S., R. Laureano, L. Fani, C. Tascini, A. Galano, A. Antonelli and G. M. Rossolini. 2015. Breakthrough *Lactobacillus rhamnosusGG* bacteremia associated with probiotic use in an adult patient with severe active ulcerative colitis: Case report and review of the literature. *Infection 43 (6)*: 777-781.

Melekh, B., I. Ilkiv, A. Lozynskyi and A. Sklyarov. 2017. Antioxidant enzyme activity and lipid peroxidation in rat liver exposed to celecoxib and lansoprazole under epinephrine-induced stress. *Journal of Applied Pharmaceutical Science7(10)*: 094-099.

Miller, G. L. 1959. Use of dinitrosaiicyiic acid reagent for determination of reducing sugar. *Analytical Chemistry31(3)*: 426-428.

Min, M., C. R. Bunt, S. L. Mason and M. A. Hussain. 2018. Non-dairy probiotic food products: An emerging group of functional foods. *Critical Reviews in Food Science and Nutrition doi: 10.1080/10408398.2018.1462760.*

Mishra, A. and k. P. Sharma. 2014. Characterisation of probiotic microorganism from fermented dairy products. *GERF bulletin of Biosciences, 5(1): 10-14.*

Mishra, N. and V. L. Tandon. 2012. Haematological effects of aqueous extract of ornamental plants in male swiss rats. *Veterinary World* 5(1): 19-23.

Miyoshi, M., A. Ogawa and S. Higurashi. 2014. Anti-obesity effect of *Lactobacillus gasseri SBT2055* accompanied by inhibition of pro-inflammatory gene expression in the visceral adipose tissue in diet induced obese mice. *European Journal of Nutrition* 53(2): 599-606.

Mokarram, R. R., S. A. Mortazavi, M. B. Habibi Najafi and F. Shahidi. 2009. The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Research International* 42: 1040-1045.

Molin, G. 2010. Chapter 13: *Lactobacillus plantarum*: The role in foods and in human health. *In: Handbook of Fermented Functional Foods. Second and revised edition. E. R. Farnworth (ed). CRC Press, Boca Raton, London, New York. 353-393.*

Molin, G. 2014. *Lactobacillus plantarum 299V. Accessed online from http://probi.se/en/files/2009/LP299v-101.pdf on 04/09/2014.*

Montagnac, J. A., C. R. Davis and S. A. Tanumihardjo. 2009. Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comprehensive reviews in Food Science and Food Safety* 8(3): 181-194.

Mordi, J. C., U. E. Uzuegbu and A. Okunima. 2015. Alteration in antioxidant enzyme activities by nickel-induced hepatotoxicity in wistar rats: Ameliorating effect of the aqueous extract of myristica fragrans (nutmeg). *Biokemistri* 27(4): 145-152.

Mousavi, Z. E., S. M. Mousavi, S. H. Razavi, Z. EmamDjomeh and H. Kiani. 2010. Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of Biology and Biotechnology. doi: 10.1007/s11274-010-0436-1.*

Mousavi, Z. E., S. M. Mousavi, S. H. Razavi, M. Hadinejad, Z. Emam-Djomeh and M. Mirzapour. 2013. Effect of fermentation of pomegranate juice by lactobacillus plantarum and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. *Food Biotechnology* 27(1): 1-13.

Nahaisi, M. H., S. Ravisankar and G. D. Noratto. 2014. Probiotics as a strategy to improve overall human health in developing countries. *Journal of Probiotics and Health,* 2: 118.

Namitha, K. K. and P. S. Negi. 2010. Chemistry and biotechnology of carotenoids. *Critical Reviews in Food Science and Nutrition* 50: 728–760.

Narayanan, N., P. K. Roychoudhury and A. Srivastava. 2004. L (+) lactic acid fermentation and its product polymerisation. 2004. *Electronic Journal of Biotechnology*.7(2): 167-179.

Nath, S., S. Chowdhury, P. K. Dora and S. Sarkar. 2014. Role of biopreservation in improving food safety and storage *International Journal of Engineering Research and Applications* 4 (1): 26-32.

Nazzaro, F., F. Fratianni, A. Sada and P. Orlando. 2008. Symbiotic potential of carrot juice supplemented with *lactobacillus spp.* And inulin or fructooligosaccharides. *Journal of the Science of Food and Agriculture* 88 (13): 2271-2276.

Nestel, P., H. E. Bouis, J. V. Meenakshi and W. Pfeiffer. 2006. Biofortification of staple food crops. *Journal of Nutrition* 136: 1064–1067.

Nguyen, T. D. T., J. H. Kang and M. S. Lee. 2007. Characterisation of *Lactobacillus plantarum PH04,* a potential probiotic bacterium with cholesterol loweriing effects. *international Journal of food Microbiology* 113 (3): 358-361.

Nigam, P. and D. Singh. 1995. Enzyme involved and microbial systems in starch processing. *Enzyme and Microbial Technology* 17: 770-778.

Nissila, E., F. P. Douillard, J. Ritari, L. Paulin, H. M. Jarvinen, P. Rasinkangas, K. Haapasalo, S. Meri, H. Jarva and W. M. deVos. 2017. Genotypic and phenotypic diversity of *Lactobacillus rhamnosus* clinical isolates, their comparison with strain *GG* and their recognition by complement system. *PLoS ONE* 12(5): 1-18.

Njoku, V. O. and C. Obi. 2010. Assessment of some fermentation processes of cassava tubers. *International Archive of Applied Sciences and Technology* 1(1): 20 - 25.

Nocianitri, K. A., N. S. Antara, I. M. Sugitha, I. D. M. Sukrama, Y. Ramona and I. N. Sujaya. 2017. The effect of two lactobacillus rhamnosus strains on the blood lipid profile of rats fed with high fat containing diet *International Food Research Journal* 24(2): 795-802.

Nualkaekul, S., M. T. Cook, V. V. Khutoryanskiy and D. Charalampopoulos. 2013. Influence of encapsulation and coating materials on the survival of *Lactobacillus plantarum* and *Bifidobacterium longum in fruit juices*. *Food Research International* 53(1): 304-311.

O'Reilly, J., S. H. S. Wong and Y. Chen. 2010. Glycaemic index, glycaemic load and exercise performance. *Sports Medicine* 40(1): 27-39.

Oboh, G. and C. A. Elusiyan. 2007. Changes in the nutrient and anti-nutrient content of micro-f ungi fermented cassava flour produced from low- and medium-cyanide variety of cassava tubers. *African Journal of Biotechnology* 6(18): 2150-2157.

Ofor, M. O. 2011. Traditional methods of preservation and storage of farm produce in Africa. *New York Science Journal, 2011;4(3): 58-62. (ISSN: 1554-0200. Available at: http://www.sciencepub.net/newyork. Accessed online on 11/11/2014.*

Ogbonna, O. C., E. O. Fadeiye, R. T. Ikem, K. O. Oladipo, D. O. Soyoye, T. M. Olulana, O. Kalejaiye, P. Ilona and E. Boy. 2018. Blood glucose response on consumption of cassava varieties (garri) in healthy nigeria subjects. *Journal of Nutrition and Human Health* 2(1): 22-27.

Ogbuji, C. A. and N. P. David-Chukwu. 2010. Glycemic indices of different cassava food products. *European Journal of Basic and Applied Sciences* 3(3): 1-7.

Ogunmefun, O. T., T. R. Fasola, A. B. Saba and A. J. Akinyemi. 2015. Inhibitory effect of *Phragmanthera incana (schum.)* harvested from cocoa (*Theobroma cacao*) and kolanut (*Cola nitida*) trees on fe2+ induced lipid oxidative stress in some rat tissues- in vitro. *International Journal of Biomedical Sciences* 11(1): 16-22.

Okafor, P. N., V. O. Anyanwu and H. O. Onyema. 2006. Effects of cassava cyanide on the antioxidant (glutathione) status and some clinically important enzymes of rats. *Journal of Pharmacology and Toxicology* 1(1): 40-46.

Okereke, H. C., K. V. Ajunwa and J. O. Owanta. 2016. Physico-chemical properties of fresh and probioticated fruit juices with *Lactobacillus casei* produced in Nigeria. *Asian Journal of Natural and Applied Sciences* 5(2): 1-7.

Olaleye, S. B., O. A. Adaramoye, P. P. Erigbali and O. S. Adeniyi. 2007. Lead exposure increases oxidative stress in the gastric mucosa of hcl/ethanol-exposed rats. *World Journal of Gastroenterology* 13(38): 5121-5126.

Oloo, B. O., S. A, M. S, M. J. B and O. B. Rose. 2014. Effects of lactic acid fermentation on the retention of beta-carotene content in orange fleshed sweet potatoes. *International Journal of Food Studies* 3: 13-33.

Oloyo, R. A. 2001. Fundamentals of research methodology for social and applied sciences., ROA Educational Press, Nigeria.

Omodamiro, R. M. and C. Aniedu. 2012. Use of newly bred β-carotene cassava in production of value-added products: Implication for food security in Nigeria. *Global Journal of Science Frontier Research (D)* XII (X): 11-16.

Onwuka, G. I. and N. J. Ogbogu. 2007. Effect of fermentation on the quality and physicochemical properties of cassava based fufu products made from two cassava varieties *NR8212* and *nwangbisi*. *Jounal of Food Technology* 5(3): 261-264.

Orrù, S., Imperlini, E., Nigro, E., Alfieri, A., Cevenini, A., Polito, R., . . . Mancini, A. (2018). Review: Role of Functional Beverages on Sport Performance and Recovery. Nutrients, 10(1470), 1-21.

Ostlie, H. M., J. Treimo and J. A. Narhus. 2005. Effect of temperature on growth and metabolism of probiotic bacteria in milk. *International Dairy Journal* 15: 22-31.

Pacovsky, J., R. Hyspler, P. Husek, P. Navratil and M. Brodak. 2018. Colloid osmotic pressure participates on the post-transplant lymphocele pathogenesis. *Transplantaion Proceedings* 50(10): 3422-3425.

Panda, S. H., M. Parmanick and R. C. Ray. 2007. Lactic acid fermentation of sweet potato (*Ipomoea batatas l*.) into pickles. *Journal of Food Processing and Preservation* 31: 83–101.

Panda, S. H. and R. C. Ray. 2007. Lactic acid fermentation of beta-carotene rich sweet potato *(Ipomea batatas l*.) into lacto-juice. *Plant foods for Human Nutrition Journal* 62(2): 65-70.

Pandey, K. B. and S. I. Rizvi. 2010. Markers of oxidative stress in erythrocytes and plasma during aging in humans. *Oxidative Medicine and Cellular Longevity* 3(1): 2-12.

Paques, J. P., L. M. C. Sagis, C. J. M. v. Rijn and E. v. d. Linden. 2014. Nanospheres of alginate prepared through w/o emulsification and internal gelation with nanoparticles of $CaCO_3$. *Food Hydrocolloids* 40: 182-188.

Parasuraman, S. 2011. Toxicological screening. *Journal of Pharmacology and Pharmacotherapeutics* 2(2): 74-79.

Park, K.-Y., B. Kim and C.-K. Hyun. 2015. *Lactobacillus rhamnosus GG* improves glucose tolerance through alleviating ER stress and suppressing macrophage activation in db/db mice. *Journal of Clinical biochemistry and nutrition* 56(3): 240-246.

Patel, A. K., R. R. Singhania, A. Pandey and S. B. Chincholkar. 2010. Probiotic bile salt hydrolase: Current development and perspectives. *Applied Biochemistry and Biotechnology* 162(1): 166-180.

Patel, M. A., M. H. H. AbouGhaly, J. V. Schryer-Praga and K. Chadwick. 2017. The effect of ionotropic gelation residence time on alginatecross-linking and properties. *Carbohydrate Polymers* 155: 362–371.

Pathare, P. B., U. L. Opara and F. A.-J. Al-Said. 2013. Colour measurement and analysis in fresh and processed foods: A review. *Food and Bioprocess Technology* 6: 36-60.

Pearson, D. 1976. Chemical analysis of foods. Churchill, Livingstone, London.

Pedjie, N. (2012). Analysis of color additives in beverages with the PerkinElmer flexar FX-15 system equipped with a PDA detector. PerkinElmer, Inc. I. PerkinElmer.

Penelope, N., E. B. Howarth, J. V. Meenakshi and P. Wolfgang. 2014. Biofortification of staple food crops: A symposium of food fortification in developing countries. *Journal of Nutrition* 136 (4): 1064-1067

Pereira, D. I. A. and G. R. Gibson. 2002. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical Reviews in Biochemistry and Molecular Biology* 37(4): 259-281.

Podolak, I., A. Galanty and D. Sobolewska. 2010. Saponins as cytotoxic agents: A review. *Phytochemistry Reviews.* 9(3): 425–474.

Premkumar, T., G. Padmaja, S. N. Moorthy, S. K. Nanda, M. George and C. Balagopalan. 2014. New cassava products of future potentials in india. *Workshop proceedings by Central Tuber Research Institute, Shreekariyam, Thiruvananthapuram, 695017, Kerala, India.* 564-577. Accessed at *http://ciat-library.ciat.cgiar.org/articulos_ciat/asia/proceedings_workshop_00/564.pdf.* on 12/11/2014.

Puguan, J. M. C., X. Yu and H. Kim. 2014. Characterisation of structure, physico-chemical properties and diffusion behavior of ca-alginate gel beads prepared by different gelation methods. *Journal of Colloid and Interface Science* 432: 109–116.

Rajagopal, M. V. 2006. Production of beer from cassava. *Journal of food science* 42 (2): 532-533.

Ranadheera, C. S., J. K. Vidanarachchi, R. S. Rocha, A. G. Cruz and S. Ajlouni. 2017. Probiotic delivery through fermentation: Dairy vs. Non-dairy beverages. *Fermentation* 3(67): 1-17.

Rao, M. A. 2014. Chapter 2. Flow and functional models for rheological properties of fluid foods. In: *Rheology of fluid, semisolid, and solid foods, principles and applications. Food engineering series,* (eds). New York. 27-36.

Rathi, M. A., P. Meenakshi and V. K. Gopalakrishnan. 2015. Hepatoprotective activity of ethanolic extract of alysicarpus vaginalis against nitrobenzene-induced hepatic damage in rats. *South Indian Journal of Biological Sciences* 1(2): 60-65.

Rattanachaikunsopon, P. and P. Phumkhachorn. 2010. Lactic acid bacteria: Their antimicrobial compounds and their uses in food production. *Scholars Research Library: Annals of Biological Research,* 1(4):218-228. *(http://scholarsresearchlibrary.com/archive.html).*

Ray, R. C. and P. S. Sivakumar. 2009. Traditional and novel fermented foods and beverages from tropical root and tuber crops: Review. *International Journal of Food Science & Technology* 44:1073–1087.

Rayment, P., P. Wright, C. Hoad, E. Ciampi, D. Haydock, P. Gowland and M. F. Butler. 2009. Investigation of alginate beads for gastro-intestinal functionality, part 1: In vitro characterisation. *Food Hydrocolloids* 23: 816-822.

Reid, G. 2016. Probiotics: Definition, scope and mechanisms of action. *Best Practice and Research Clinical Gastroenterology* 30(1): 17-25.

Ritchie, M. L. and T. N. Romanuk. 2012. A meta-analysis of probiotic efficacy for gastrointestinal diseases. Plos one 7(4): E34938. doi:10.1371/journal.Pone.0034938.

Rivera-Espinoza, Y. and Y. Gallardo-Navarro. 2010. Non-dairy probiotic products. *Food Microbiology* 27: 1-11.

Roberfroid, M., G. R. Gibson, L. Hoyles, A. L. McCartney, R. Rastall and I. Rowland. 2010. Prebiotic effects: Metabolic and health benefits. *British Journal of Nutrition* 104(2): S1–S63.

Robin, F., C. Paillard, H. Marchandin, F. Demeocq, R. Bonnet and C. Hennequin. 2010. *Lactobacillus rhamnosus* meningitis following recurrent episodes of bacteremia in a child undergoing allogeneic hematopoietic stem cell transplantation. *Journal of Clinical Microbiology* 48(11): 4317–4319.

Rodriguez, E. B., M. E. Flavier, D. B. Rodriguez-Amaya and J. Amaya-Farfán. 2006. Phytochemicals and functional foods. Current situation and prospect for developing countries. *Segurança Alimentar e Nutricional, Campinas, 13(1): 1-22, 2006.*

Rokka, S. and P. Rantamäki. 2010. Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology* 231: 1-12.

Ross, A. C., J. E. Manson, S. A. Abrams, J. F. Aloia, P. M. Brannon, S. K. Clinton, R. A. Durazo-Arvizu, J. C. Gallagher, R. L. Gallo, G. Jones, C. S. Kovacs, S. T. Mayne,

C. J. Rosen and S. A. Shapses. 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: What clinicians need to know. *Journal of Clinical Endocrinology and Metabolism* 96(1): 53–58.

Saarela, M. H., H. L. Alakomi, A. Puhakka and J. Matto. 2009. Effect of fermentation ph on the storage stability of *lactobacillus rhamnosus* preparations and suitability of *in vitro* analyses of cell physiological functions to predict it. *Journal of Applied Microbiology* 106: 1204-1212.

Saganuwan, S. A. 2017. Toxicity studies of drugs and chemicals in animals: An overview. *Bulgarian Journal of Veterinary Medicine* 20(4): 291-318.

Salazar, J. H. 2014. Overview of urea and creatinine. *Laboratory Medicine* 45(1): e19-e20.

Salminen, M. K., S. Tynkkynen, H. Rautelin, M. Saxelin, M. Vaara, P. Ruutu, S. Sarna, V. Valtonen and A. J. rvinen. 2002. Lactobacillus bacteremia during a rapid increase in probiotic use of lactobacillus rhamnosus gg in finland. *Clinical Infectious Diseases* 35: 1155–1160.

Saltzman, A., E. Birol, H. E. Bouis, E. Boy and F. F. De Moura. 2013. Biofortification: progress toward a more nourishing future. *Global Food Security* 2: 9–17.

Samanta, A., A. Mandal, A. Patra, S. Mandal, S. Roy, S. Pradhan, K. Das and D. K. Nandi. 2014. Impact of probiotic supplementation on intestinal microflora of rat under environmental hypobaric pressure. *International Journal of Current Microbiology and Applied Sciencce* 3(8): 282-291.

Samarghandian, S., M. Azimi-Nezhad and F. Samini. 2015. Preventive effect of safranal against oxidative damage in aged male rat brain. *Experimental Animals* 64(1): 65-71.

Sanders, M. E. 2011. Impact of probiotics on colonising microbiota of the gut. *Journal of Clinical Gastroenterology* 45(3): S115–S119.

Santos, E. W., D. C. d. Oliveira, A. Hastreiter, G. B. d. Silva, J. S. d. O. Beltran, M. Tsujita, A. R. Crisma, S. Meiry, P. Neves, R. A. Fock and P. Borelli. 2016. Hematological and biochemical reference values for c57bl/6, swiss webster and balb/c mice.

Sarika, A. R., A. P. Lipton and M. S. Aishwarya. 2010. Bacteriocin production by a new isolate of *Lactobacillus rhamnosus GP1* under different culture conditions. *Advance Journal of Food Science and Technology* 2(5): 291-297.

Sarma, P. R. 1990. Red cell indices. In: *Clinical methods: The history, physical, and laboratory examinations.* H. K. Walker, W. D. Hall and J. W. Hurst (eds). 3rd. Boston. Butterworth. 152.

Scazzina, F., D. D. Rio, N. Pellegrini and F. Brighenti. 2009. Sourdough bread: Starch digestibility and postprandial glycemic response. *Journal of Cereal Science* 49: 419–421.

Schlabitz, C., A. Gennari, A. L. D. M. Araújo, J. A. Bald, C. F. V. D. Souza and L. Hoehne. 2015. Shelf life of a synbiotic fermented dairy beverage using ricotta cheese whey. *American Journal of Food Technology* 10(6): 254-264.

Shahriari, Z., B. Heidari, M. cheraghi and A. G. Shahriari. 2013. Biofortification of staple food crops: Engineering the metabolic pathways. *International Research Journal of Applied and Basic Sciences* 5(3): 287-290.

Sharma, R., R. Kapila, G. Dass and S. Kapila. 2014. Improvement in th1/th2 immune homeostasis, antioxidative status and resistance to pathogenic e. Coli on consumption of probiotic *Lactobacillus rhamnosus* fermented milk in aging mice. *Age* 36(9686): 1-17.

Sherid, M., S. Samo, S. Sulaiman, H. Husein, H. Sifuentes and S. Sridhar. 2016. Liver abscess and bacteremia caused by lactobacillus: Role of probiotics? Case report and review of the literature. *BMC Gastroenterology* 16(138): 1-6.

Shetty, P. H. and L. Jespersen. 2006. Saccharomyces cerevisiae and lactic acid bacteria as potential mycotoxin decontaminating agents *Trends in Food Science and Technology* 17: 48-55.

Sies, M. F., N. Asmuin, Fakhrulrazi, N. A. Norsalim, H. Zakaria, R. Pairan and A. Sadikin. 2017. Determine physical properties of an organic citric acid (processed lime juice) dissolve with water using experimental apparatus. *International Journal of Engineering and Technology* 8(6): 2530-2537.

Simsek, S. and S. N. El. 2015. In vitro starch digestibility, estimated glycemic index and antioxidant potential of taro (colocasia esculenta l. Schott) corm. *Food Chemistry* 168: 257–261.

Singh, S., R. K. Sharma, S. Malhotra, R. Pothuraju and U. K. Shandilya. 2016. *Lactobacillus rhamnosus NCDC17* ameliorates type-2 diabetes by improving gut function, oxidative stress and inflammation in high-fat-diet fed and streptozotocintreated rats. *Beneficial Microbes* 8(2): 243-255.

Sivudu, S. N., K. Umamahesh and O. V. S. Reddy. 2014. A comparative study on probiotication of mixed watermelon and tomato juice by using probiotic strains of *Lactobacilli*. *International Journal of Current Microbiology and Applied Sciencce* 3(11): 977-984.

Smith, K., X. Zeng and J. Lin. 2014. Discovery of bile salt hdrolase inhibitors using an efficient through-put screening system. *PLoS ONE* 9(1):e85344. doi:85310.81371/journal.pone.0085344.

Sobko, T., C. I. Reinders, E. Å. Jansson, E. Norin, T. Midtvedt and J. O. Lundberg. 2005. Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite. *Nitric Oxide* 13: 272-278.

Sobrun, Y., A. Bhaw-Luximon, D. Jhurry and D. Puchooa. 2012. Isolation of lactic acid bacteria from sugar cane juice and production of lactic acid from selected improved strains. *Advances in Bioscience and Biotechnology* 3: 398-407.

Soetan, K. O., C. O. Olaiya and E. O. Oyewole. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science* 4(5): 200-222.

Sohail, A., M. S. Turner, E. K. Pabawati, A. G. A. Coombes and B. Bhandari. 2012. Evaluation of *Lactobacillus rhamnosus GG* and *Lactobacillus acidophilus NCFM* encapsulated using a novel impinging aerosol method in fruit juice products. *International Journal of Food Microbiology* 157(2): 162–166.

Sohail, M., R. U. Khan, S. R. Afridi, M. Imad and B. Mehrin. 2013. Preparation and quality evaluation of sweet potato ready to drink beverage. *Asian Research Publishing Network (ARPN) Journal of Agricultural and Biological Science* 8(4): 279-282.

Soleimani, N. A., R. K. Kermanshahi, B. Yakhchali and T. N. Sattari. 2010. Antagonistic activity of probiotic lactobacilli against staphylococcus aureus isolated from bovine mastitis. *African Journal of Microbiology Research* 4(20): 2169-2173.

Song, D., S. Ibrahim and S. Hayek. 2012. Chapter 1: Recent application of probiotics in food and agricultural science. In: *Probiotics*. D. Song, S. Ibrahim and S. Hayek (eds).*1-35. Accessed online at http://dx.doi.org/10.5772/50121 on 01/07/2019*.

Soto, J. L. M., L. M. García, J. V. González, A. B. Nicanor and L. G. Cruz. 2012. Influence of starch source in the required hydrolysis time for the production of maltodextrins with different dextrose equivalent. *African Journal of Biotechnology* 11 (69): 13428-13435.

Souza, E. C., P. O. d. S. d. Azevedo, J. M. Domínguez, A. Converti and R. P. d. S. Oliveira. 2017. Influence of temperature and ph on the production of biosurfactant, bacteriocin and lactic acid by *Lactococcus lactis cect-4434*. *CyTA-Jounal of Food* 15(4): 525-530.

Steinkraus, K. H. 2002. Fermentations in world food processing. *Comprehensive reviews in Food Science and Food Safety* 1: 23-32.

Stinco, C. M., R. Fernández-Vázquez, M. L. Escudero-Gilete, F. J. Heredia, A. J. Meléndez-Martínez and I. M. Vicario. 2012. Effect of orange juice's processing on the color, particle size, and bioaccessibility of carotenoids. *Journal of Agricultural and Food Chemistry* 60: 1447-1455.

Suda, I., T. Oki, M. Masuda, M. Kobayashi, Y. Nishiba and S. Furuta. 2003. Physiological functionality of purple-fleshed sweet potatoes containing anthocyanins and their utilisation in foods. *Japan Agricultural Research Quarterly (JARQ):* 37 (3): 167 – 173.

Sun, J., R. Zhao, J. Zeng, G. Li and X. Li. 2010. Characterisation of destrins with different dextrose equivalents. *Molecules* 15: 5162-5173.

Suthar, P. C. 2017. Glutathione s-transferases: A brief on classification and gstm1-t1 activity. *International Journal of Pharmaceutical Science and Research* 8(3): 1023-1027.

Sy, C., O. Dangles, P. Borel and C. Caris-Veyrat. 2013. Iron-induced oxidation of (all-e)-β-carotene under model gastric conditions: Kinetics, products, and mechanism. *Free Radical Biology and Medicine* 63: 195–206.

Tabilo-Munizaga, G. and G. V. Barbosa-Ca´novas. 2005. Rheology for the food industry. *Journal of Food Engineering* 67: 147–156.

Talsma, E. F., I. D. Brouwer, H. Verhoef, G. N. Mbera, A. M. Mwangi, A. Y. Demir, B. Maziya-Dixon, E. Boy, M. B. Zimmermann and A. Melse-Boonstra. 2016. Biofortified yellow cassava and vitamin a status of kenyan children: A randomised controlled trial. *American Journal of Clinical Nutrition* 103: 258-267.

Talsma, E. F., A. Melse-Boonstra, B. P. H. d. Kok, G. N. K. Mbera, A. M. Mwangi and I. D. Brouwer. 2013. Biofortified cassava with pro-vitamin A is sensory and culturally acceptable for consumption by primary school children in kenya. *Journal of PLoS ONE* 8(8): e73433.

Tamminen, M., S. Salminen and A. C. Ouwehand. 2013. Fermentation of carrot juice by probiotics: Viability and preservation of adhesion. *International Journal of Biotechnology for Wellness Industries* 2: 10-15.

Tanasupawat, S. 2009. Thai lactic acid bacteria: Diversity and applications. *SWU Scientific Journal* 25 (1): 1

Taverniti, V. and S. Guglielmetti. 2011. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: Proposal of paraprobiotic concept). *Genes and Nutrition* 6: 261-274.

Tian, H., Y. Shen, H. Yu, Y. He and C. Chen. 2017. Effects of 4 probiotic strains in co-culture with traditional starters on the flavor profile of yogurt. *journal of food science* 82(7): 1693–1701.

Tintore, M., G. Colome, J. Santas and J. Espadaler. 2017. Gut microbiota dysbiosis and role of probiotics in infant colic. *Archives of Clinical Microbiology* 8(4):56.

Trinder, P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology* 22(2): 158-161.

Tsen, J.-H., Y.-P. Lin and V. A.-E. King. 2004. Banana puree fermentation by *Lactobacillus acidophilus* immobilised in Ca-alginate. *The Journal of General and Applied Microbiology* 49(6): 357-361.

Tumuhimbise, G. A., A. Namutebi, F. Turyashemererwa and J. Muyonga. 2013. Provitamin a crops: Acceptability, bioavailability, efficacy and effectiveness. *Food and Nutrition Sciences* 4:430-435.

Uchendu, F. N. 2013. The role of biofortification in the reduction of micronutrient food insecurity in developing countries. *African Journal of Biotechnology* 12(37): 5559-5566,.

Ugbogu, A. E., E. I. Akubugwo, E. J. J. Iweala, F. O. Uhegbu, G. C. Chinyere and N. A. Obasi. 2013. Role of phytochemicals in chemoprevention of cancer: A review. *International Journal of Pharmaceutical and Chemical Sciences* 2(2)

Unadike, B. C., A. Eregie and A. E. Ohwovoriole. 2010. Glycaemic controlamongst persons with diabetes mellitus in benin city. *Nigerian Medical Journal* 51: 164-166.

Uthumporna, U., I. S. M. Zaidulb and A. A. Karima. 2010. Hydrolysis of granular starch at sub-gelatinisation temperature using a mixture of amylolytic enzymes. *Journal of Food and bioproducts processing,* 88:47–54.

Vahabnezhad, E., A. B. Mochon, L. J. Wozniak and D. A. Ziring. 2013. *Lactobacillus* bacteremia associated with probiotic use in a pediatric patient with ulcerative colitis. *Journal of Clinical Gastroenterology* 47: 437–439.

Valik, L., A. Medvedova and D. Liptakova. 2008. Characterisation of the growth of *Lactobacillus rhamnosus GG* in milk at suboptimal temperatures. *Journal of food and Nutrition Reesearch* 47(2): 60-67.

Van der Maarel, M. J. E. C., B. van der Veen, J. C. M. Uitdehaag, H. Leemhuis and L. Dijkhuizen. 2002. Properties and applications of starch-converting enzymes of the α-amylase family. *Journal of Biotechnology* 94: 137-155.

Vella, M. N., L. M. Stratton, J. Sheeshka and A. M. Duncan. 2013. Exploration of functional food consumption in older adults in relation to food matrices, bioactive ingredients, and health. *Journal of Nutrition in Gerontology and Geriatrics* 32(2): 122-144.

Wang, H.-f., X.-h. Zhong, W.-y. Shi and B. Guo. 2011. Study of malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in chickens infected with avian infectious bronchitis virus. *African Journal of Biotechnology* 10(45): 9213-9217.

Wang, Y., Y. Liu, A. Sidhu, Z. Ma, C. McClain and W. Feng. 2012. *Lactobacillus rhamnosus GG* culture supernatant ameliorates acute alcohol-induced intestinal permeability and liver injury.*American Journal of Physiology - Gastrointestinal and Liver Physiology* 303(1): G32-G41.

Wang, W.-H., Y.-W. Chiu, S.-H. Kao, W.-J. Ting, C.-Y. Hsu, H.-H. Cheng, H.-H. Lee, K. Hsieh, J.-Y. Liu and J.-M. Hwang. 2016. Eight probiotics are effective in lowering serum lipids. *Adaptive Medicine* 8: 1-5.

Wang, Y., Y. Tashiro and K. Sonomoto. 2014. Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits. *Journal of Bioscience and Bioengineering*http://dx.doi.org/10.1016/j.jbiosc.2014.06.003.

Wang, Y., Y. Tashiro and K. Sonomoto. 2015. Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits. *Journal of Bioscience and Bioengineering* 119(1): 10-18.

Wang, Y., Y. Wu, Y. Wang, H. Xu, X. Mei, D. Yu, Y. Wang and W. Li. 2017. Antioxidant properties of probiotic bacteria. *Nutrients* 9(521): 1-15.

Wangpor, J., P. Prayoonyong, C. Sakdaronnarong, A. Sungpet and W. Jonglertjunya. 2017. Bioethanol production from cassava starch by enzymatic hydrolysis, fermentation and ex-situ nanofilteration. *Energy Procedia* 138: 883-888.

Westby, A. 2002. Utilisation, storage and small-scale processing. In: *Cassava: Biology, production and utilisation.* R. J. Hillocks, J. M. Thresh and A. C. Bellotti (eds). Natural Resources Institute, University of Greenwich, Chattam Maritime, Kent ME4 4TB, UK. CAB International 14**:** *281-300*.

Weydert, C. J. and J. J. Cullen. 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nature Protocols* 5(1): 51-66.

White, P. J. and M. R. Broadley. 2009. Biofortification of crops with seven mineral elements often lacking in human diets- iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* 182(1): 49-84.

WHO (2009). Global prevalence of vitamin a deficiency in populations at risk 1995–2005: WHO global database on vitamin a deficiency. WHO Library Cataloguing-in-Publication Data. World Health Organisation**:** 68.

WHO. 2014. Biofortification of staple crops. e-Library of Evidence for Nutrition Actions (eLENA): A-Z list of Interventions.

Williams, N. T. 2010. Probiotics. *American Journal of Health-System Pharmacy 67:6, 449-458.*

Williams, P. 2012. Food toxicity and safety. In: *Essentials of human nutrition*. J. Mann and A. Truswell (eds). Oxford. Oxford University Press.**:** *449-466*.

Willowski, I., G. Rechkemmer and B. L. Pool-Zobel. 2001. Protective role of probiotics and prebiotics in colon cancer. *American Journal of Clinical Nutrition, 73: 451-455.*

Wireko-Manu, F. D., W. O. Ellis and I. Oduro. 2010. Production of a non-alcoholic beverage from sweet potato (ipomoea batatas l.). *African Journal of Food Science* 4(4): 180 -183.

Wisløff, H., S. Uhlig, E. Scheie, J. Loader, A. Wilkins and A. F. øyen. 2008. Toxicity testing of saponin-containing yucca schidigera roetzl. Juice in relation to hepato- and nephrotoxicity of narthecium ossifragum (l.) huds. *Toxicon* 51: 140-150.

Wong, J. Y. and F. Y. Chye. 2008. Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis,* 22:269–277.

Xu, Y., C. Grizzard, E. N. Sismour, H. L. Bhardwaj and Z. Li. 2013. Resistant starch content, molecular structure and physicochemical properties of starches in virginiagrown corn, potato and mungbean yixi. *Journal of Cereals and Oil seeds* 4(1): 10-18.

Yadav, R., R. Vij, S. Kapila, S. H. Khan, N. Kumar, S. Meena and R. Kapila. 2019. Milk fermented with probiotic strains *Lactobacillus rhamnosus* MTCC 5957 and *Lactobacillus rhamnosus* MTCC 5897 ameliorates the diet-induced hypercholesterolemia in rats. *Annals of Microbiology.* Accessed online at *https://doi.org/10.1007/s13213-018-1433-0*on *10/02/2019*

Yao, Y., W. Sang, M. Zhou and G. Ren. 2010. Phenolic composition and antioxidant activities of 11 celery cultivars. *Journal of Food Science* 75(1): C9-C13.

Yazhini, P., P. Visha, P. Selvaraj, P. Vasanthakumar, V. Thavasiappan, M. G. Preeda and J. Ramavathi. 2017. Survival efficiency of encapsulated probiotic bacteria - *Lactobacillus lactis* and *Bifidobacterium bifidum* in different ph. *Indian Veterinary Journal* 94(12): 35-37.

Yoon, K. Y., E. E. Woodams and Y. D. Hang. 2004. Probiotication of tomato juice by lactic acid bacteria. *Journal of Microbiology* 42 (4): 315-318.

Yoon, K. Y., E. E. Woodams and Y. D. Hang. 2006. Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology* 97: 1427-1430.

Yuasa, M., Y. Aoyama, R. Shimada, H. Sawamura, S. Ebara, M. Negoro, T. Fukui and T. Watanabe. 2016. Effects of biotin deficiency on biotinylated proteins and biotin-related genes in the rat brain. *Journal of Nutritional Science and Vitaminology* 62: 81-87.

Yuwa-Amornpitak, T. 2010. Ethanol production from cassava starch by selected fungi from an-koji and *Saccaromycetes cereviseaeJournal of Biotechnology* 9 (1): 84-88.

Zalan, Z., J. Hudacek, J. Stetina, J. Chumchalova and A. Halasz. 2010. Production of organic acids by lactobacillus strains in three different media. *European Food Research and Technology*230: 395–404.

Zamora, L. L., J. A. G. Calderón, E. T. Vázquez and E. B. Reynoso. 2010. Optimisation of ethanol production process from cassava starch by surface response. *Journal of Mexican Chemical Society* 54(4): 198-203.

Zhang, W., Z. Y-H, Y. G-Y, X. Liu, B. Xia, X. Hu, J.-H. Su and J.-F. Wang. 2018. *Lactobacillus rhamnosus GG* affects microbiota and suppresses autophagy in the intestines of pigs challenged with salmonella infantis. *Frontiers in Microbiology* 8: 2705.

Zhang, Y., L. Zhang, M. Du, H. Yi, C. Guo, Y. Tuo, Xue Hana , J. Li, L. Zhang and L. Yang. 2011. Antimicrobial activity against shigella sonnei and probiotic properties of wild *Lactobacilli* from fermented food. *Microbiological Research* 167: 27-31.

Zhao, Y., P. M. Vanhoutte and S. W. S. Leung. 2015. Vascular nitric oxide: Beyond enos. *Journal of Pharmacological Sciences* 129(2): 83-94.

Zhao, Z., X. Xie, Z. Wang, Y. Tao, X. Niu, X. Huang, L. Liu and Z. Li. 2016. Immobilisation of *Lactobacillus rhamnosus* in mesoporous silica-based material: An efficiency continuous cell-recycle fermentation system for lactic acid production. *Elsevier Journal of Bioscience and Bioengineering*121(6): 645-651. http://dx.doi.org/10.1016/j.jbiosc.2015.11.010: 1-7.

Zhou, Y., E. Martins, A. Groboillot, C. P. Champagne and R. J. Neufeld. 1998. Spectrophotometric quantification of lactic bacteria in alginate and control of cell release with chitosan coating. *Journal of Applied Microbiology,* 84: 342-348.

Zhu, L.-J., Q.-Q. Liu, J. D. Wilson, M.-H. Gu and Y.-C. Shi. 2011. Digestibility and physicochemical properties of rice (oryza sativa l.) flours and starches differing in amylose content. *Carbohydrate Polymers* 86: 1751– 1759.

Zhu, S. C., D. Y. Ying, L. Sanguansri, J. W. Tang and M. A. Augustin. 2013. Both stereo-isomers of glucose enhance the survival rate of microencapsulated *Lactobacillus rhamnosus GG* during storage in the dry state. *Journal of Food Engineering* 116: 809–813.

Zidenga, T., D. Siritunga and R. T. Sayre. 2017. Cyanogen metabolism in cassava roots: Impact on protein synthesis and root development. *Frontiers in Plant Science* 8(220): 1-12.

Zohra, S. F., B. Meriem, S. Samira and A. M. M. S. 2012. Phytochemical screening and identification of some compounds from mallow. *Journal of Natural Products and Plant Resources.* 2 (4):512-516.

APPENDICES

APPENDIX 1: PICTURES OF PROVITAMIN A CASSAVA ROOTS, STARCH AND HYDROLYSATE

Plate 1: Provitamin A cassava roots

Plate 2: Cross-section of the provitamin A cassava varieties used

Plate 3: Provitamin A cassava starch

Plate 4: Gelatinised provitamin A cassava starch

Plate 5: Liquefied provitamin A cassava starch

Plate 6: Provitamin A cassava hydrolysate

APPENDIX 11: **Pictures of food products from provitamin A cassava**

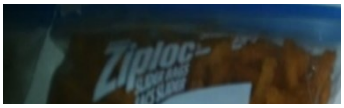


Plate 7: Pictures of food products from provitamin A cassava (Source: Author's compilation from Cassava breeding unit, IITA)

APPENDIX III: POST HOC TEST FOR COLOUR DETERMINATION

L

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05		
		1	2	3
LCJF1371	3	37.80000		
LCJC1371	3	38.36667	38.36667	
LCJE1371	3	38.54000	38.54000	38.54000
LCJE593	3	38.66667	38.66667	38.66667
LCJC1368	3	39.26333	39.26333	39.26333
LCJE1368	3	39.34333	39.34333	39.34333
LCJF1368	3		39.89333	39.89333
LCJC593	3		39.97667	39.97667
LCJF593	3			40.11000
Sig.		.064	.056	.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
LCJF1368	3	-1.74333					
LCJE1368	3		-1.56667				
LCJF593	3			-1.28667			
LCJC1371	3			-1.27667			
LCJC1368	3			-1.25333			
LCJC593	3				-1.08667		
LCJE593	3				-.97667	-.97667	
LCJF1371	3					-.89667	-.89667
LCJE1371	3						-.80667
Sig.		1.000	1.000	.607	.084	.200	.152

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05				
		1	2	3	4	5
LCJF1371	3	1.45000				
LCJE1371	3	1.92333	1.92333			
LCJE593	3	2.00000	2.00000			
LCJF593	3		2.57333	2.57333		
LCJC593	3		2.57667	2.57667		
LCJC1371	3			3.15000	3.15000	
LCJC1368	3				3.69000	
LCJE1368	3					5.20000
LCJF1368	3					5.78333
Sig.		.140	.091	.122	.127	.101

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX IV: ANOVA AND POST HOC TEST FOR SENSORY EVALUATION

ANOVA for sensory evaluation

		Sum of Squares	df	Mean Square	F	Sig.
Yellowcolour	Between Groups	90.591	8	11.324	2.642	.008
	Within Groups	1542.878	360	4.286		
	Total	1633.469	368			
Sweetness	Between Groups	125.729	8	15.716	4.416	.000
	Within Groups	1281.073	360	3.559		
	Total	1406.802	368			
Sourness	Between Groups	96.249	8	12.031	2.435	.014
	Within Groups	1778.585	360	4.941		
	Total	1874.835	368			
Swallowability	Between Groups	143.415	8	17.927	3.870	.000
	Within Groups	1667.463	360	4.632		
	Total	1810.878	368			
Mouthfeel	Between Groups	72.341	8	9.043	1.812	.074
	Within Groups	1796.878	360	4.991		
	Total	1869.220	368			
Offlavour	Between Groups	70.038	8	8.755	1.802	.075
	Within Groups	1748.976	360	4.858		
	Total	1819.014	368			
Aftertaste	Between Groups	36.119	8	4.515	.836	.571
	Within Groups	1943.610	360	5.399		
	Total	1979.729	368			
Turbidity	Between Groups	17.024	8	2.128	.508	.850
	Within Groups	1506.927	360	4.186		
	Total	1523.951	368			
QAcceptability	Between Groups	58.878	8	7.360	1.949	.052
	Within Groups	1359.561	360	3.777		
	Total	1418.439	368			

Post hoc test for sensory evaluation

Sweetness

Yellowcolour

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05		
		1	2	3
SZW 593C	41	2.82927		
HJY 1371C	41	3.36585	3.36585	
XFR 1368C	41	3.48780	3.48780	3.48780
QWV 593E	41	3.75610	3.75610	3.75610
JTV 1371E	41		3.87805	3.87805
HBW 593F	41		4.02439	4.02439
XTD 1371F	41		4.12195	4.12195
DXR 1368E	41		4.39024	4.39024
JHG 1368F	41			4.48780
Sig.		.064	.053	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 41.000.

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05			
		1	2	3	4
JHG 1368F	41	3.12195			
JTV 1371E	41	3.24390			
HBW 593F	41	3.53659	3.53659		
DXR 1368E	41	3.56098	3.56098		
XTD 1371F	41	3.60976	3.60976		
QWV 593E	41	3.80488	3.80488	3.80488	
SZW 593C	41		4.24390	4.24390	4.24390
XFR 1368C	41			4.56098	4.56098
HJY 1371C	41				4.97561
Sig.		.157	.134	.087	.098

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 41.000.

Sourness

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05		
		1	2	3
SZW 593C	41	3.87805		
XFR 1368C	41	4.29268	4.29268	
HJY 1371C	41	4.36585	4.36585	
DXR 1368E	41	4.70732	4.70732	4.70732
JTV 1371E	41		4.97561	4.97561
QWV 593E	41		5.12195	5.12195
XTD 1371F	41		5.26829	5.26829
HBW 593F	41		5.26829	5.26829
JHG 1368F	41			5.48780
Sig.		.125	.088	.170

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 41.000.

Swallowability

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05			
		1	2	3	4
JTV 1371E	41	4.14634			
JHG 1368F	41	4.90244	4.90244		
DXR 1368E	41		5.17073	5.17073	
QWV 593E	41		5.48780	5.48780	5.48780
HBW 593F	41		5.51220	5.51220	5.51220
SZW 593C	41		5.60976	5.60976	5.60976
XTD 1371F	41		5.63415	5.63415	5.63415
XFR 1368C	41			6.02439	6.02439
HJY 1371C	41				6.46341
Sig.		.113	.184	.119	.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 41.000.

Mouthfeel

Duncan[a]

SAMPLES	N	Subset for alpha = 0.05	
		1	2
JHG 1368F	41	4.39024	
JTV 1371E	41	4.41463	
XTD 1371F	41	4.56098	
HBW 593F	41	4.63415	
SZW 593C	41	4.78049	4.78049
XFR 1368C	41	5.00000	5.00000
QWV 593E	41	5.12195	5.12195
DXR 1368E	41	5.21951	5.21951
HJY 1371C	41		5.85366
Sig.		.158	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 41.000.

APPENDIX V: MICROBIOLOGICAL ANALYSIS

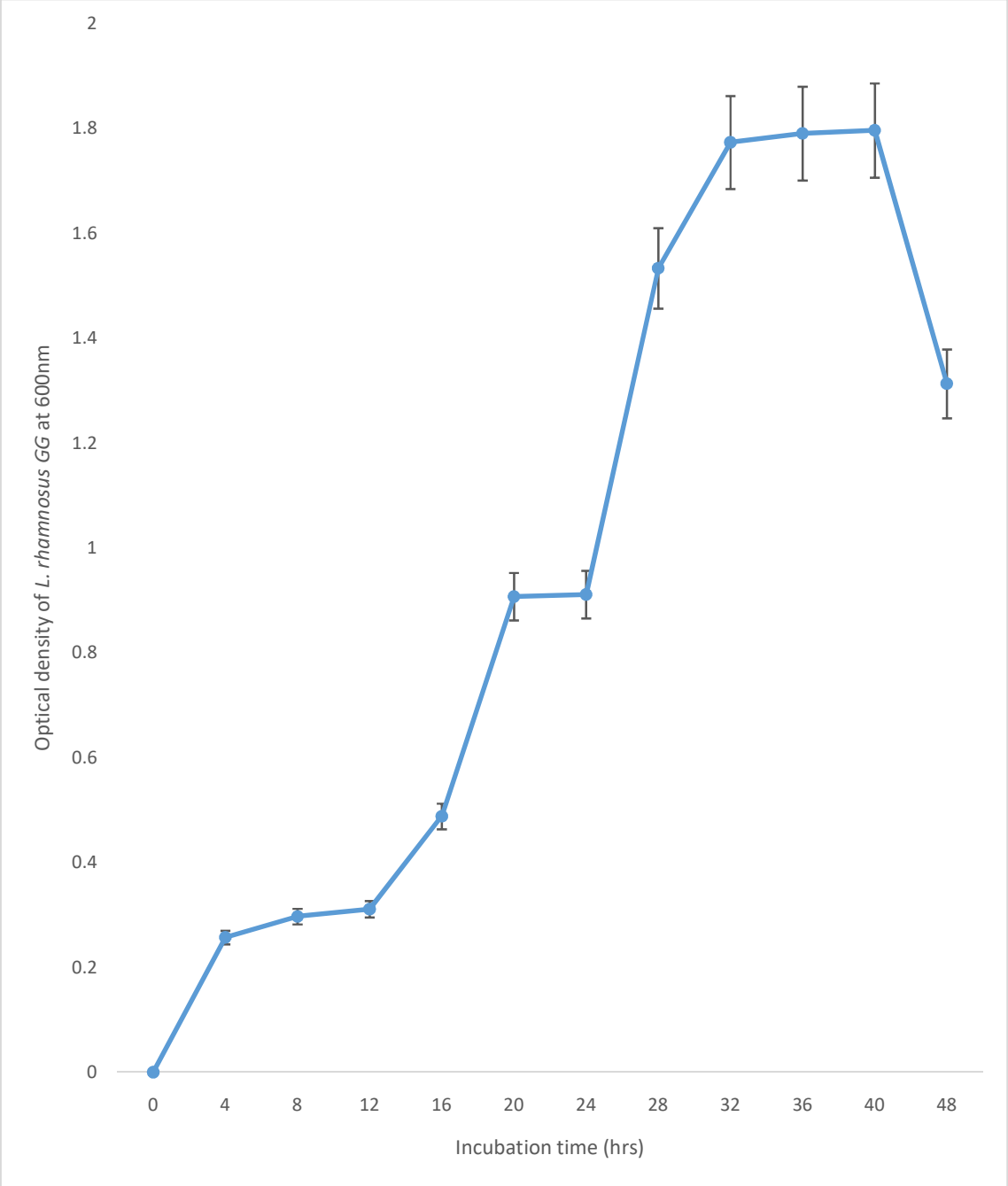
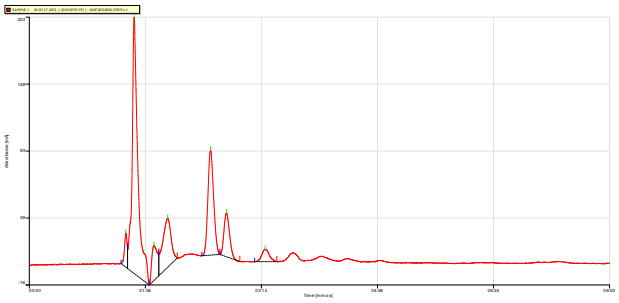
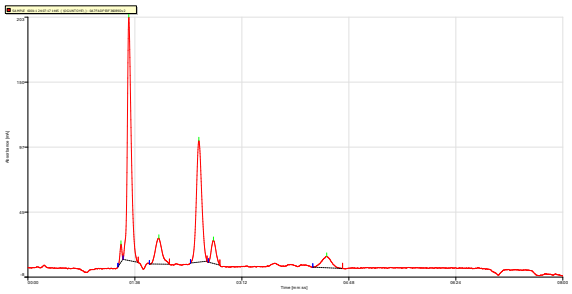


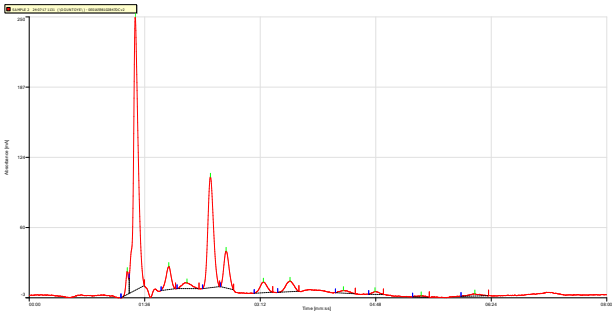
Figure showing the growth curve of *Lactobacillus rhamnosus GG*under the laboratory conditions used in this study

APPENDIX VI: HPLC Chromatogram for organic acids in provitamin A cassava hydrolysates

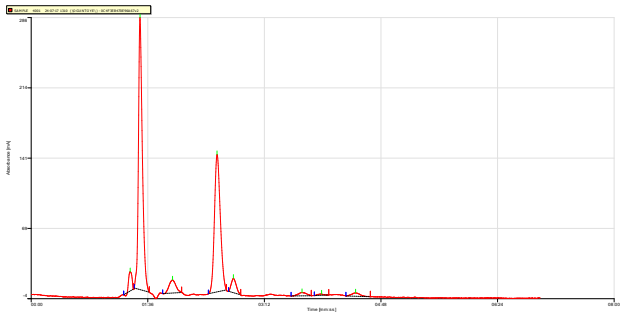




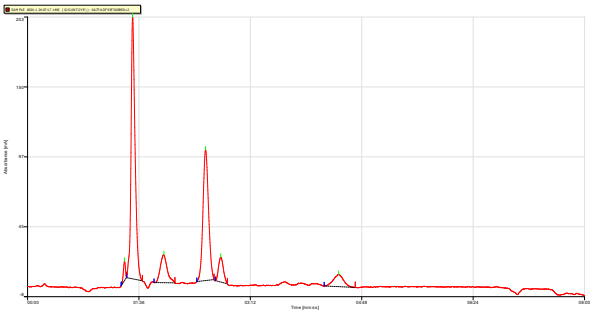
(a).TMS-I011371 hydrolysate without *L. rhamnosus GG*



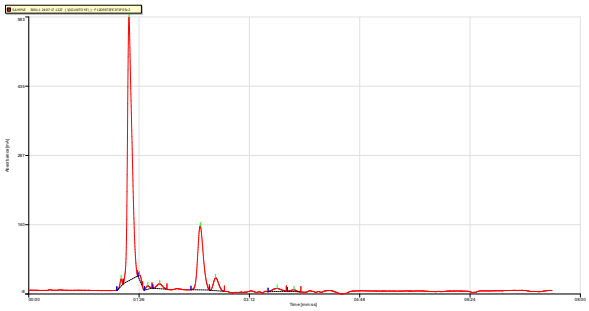
(c).TMS-I011368 hydrolysate without *L. rhamnosus GG*



(d). TMS-I011368 hydrolysate with free *L. rhamnosus GG*



(e). TMS-I011368 with encapsulated *L. rhamnosus GG*



(f). TMS-I070593 hydrolysate without *L. rhamnosus GG*

APPENDIX VII: Publicationsfrom this study

Journal articles

- **Oguntoye, M.A,** Ezekiel, O.O and Oridupa, O.A (2021): Viability of encapsulated *L. rhamnosus GG* in Provitamin A Cassava hydrolysate during fermentation, storage, under in-vitro and in-vivo gastrointestinal conditions. *Elsevier Journal of Food Bioscience*, 40 (100845).*https://doi.org/10.1016/j.fbio.2020.100845*

- **Oguntoye, M.A** and Ezekiel, O.O (2020): Provitamin A cassava hydrolysate with *Lactobacillus rhamnosus GG* improves serum lipid profile in Wistar rats. *Current Development in Nutrition, 4* (Suppl. 2): 445. *https://doi:10.1093/cdn/nzaa045_078.*

Conference Papers:

- **Oguntoye, M.A** and Ezekiel, O.O (2020): Provitamin A cassava hydrolysate with *Lactobacillus rhamnosus GG* improves serum lipid profile in Wistar rats. Abstract (P06-078-20) presented in *Nutrition 2020 Live Online* Virtual conference organised by American Society for Nutrition, 1st – 4th June, 2020.

- **Oguntoye, M.A** and Ezekiel, O.O (2019): Descriptive Sensory Analysis of Ready-to-drink Provitamin A cassava hydrolysate with *Lactobacillus rhamnosus GG.* In: Akinwande, *et al.,* (Eds.), Novelty in Food Science and Technology: A road map to food security and sustainability. Proceedings of the 5th Regional Food Science and Technology Summit held in Ilorin, Nigeria, 10th-11th June, 2019, pp 267-274. Masterprint Publisher, Ibadan.