ISOLATION, CHARACTERISATION AND MODIFICATION OF SOME YEAST STRAINS FROM CITRUS JUICE FOR ENHANCED FERMENTATIVE ABILITY

BY

BLESSING CHIDIOBASI

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY,ZARIA

NIGERIA.

DECEMBER, 2014.

ISOLATION, CHARACTERISATION AND MODIFICATION OF SOME YEAST STRAINS FROM CITRUS JUICE FOR ENHANCED FERMENTATIVE ABILITY

BY

Blessing Chidi OBASI
B.Sc. (ASUT, 1989), M.Sc. (A.B.U. 2005).
(Ph.D/Scien/11347/07-08)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF PHILOSOPHY DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

DECEMBER, 2014

DECLARATION

I declare that the work in the dissertation entitled**"ISOLATION, CHARACTERISATION AND MODIFICATION OF SOME YEAST STRAINS FROM CITRUS JUICEFOR ENHANCED FERMENTATIVE ABILITY"** was performed by me in the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria under the supervision of ProfessorsC.M.Z.Whong, S.A. Ado and Dr. I.O. Abdullahi.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____ _____ _____

Name of Student Signature Date

CERTIFICATION

This dissertation entitled **"ISOLATION, CHARACTERISATION ANDMODIFICATION OF SOME YEAST STRAINS FROM CITRUS JUICE FOR ENHANCED FERMENTATIVE ABILITY"**by Blessing Chidi Obasi meets the regulations governing the award of the degree of Doctor of Philosophy in Microbiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. C.M.Z. Whong Chairman. Supervisory Committee	Signature	Date
Prof. S. A. Ado Member. Supervisory Committee	Signature	Date
Dr. I. O. Abdullahi Member. Supervisory Committee	Signature	Date
Prof. S. A. Ado Head, Dept. of Microbiology	Signature	Date
Prof. A.Z. Hassan Dean, Postgraduate School	Signature	Date

DEDICATION

This dissertation is dedicated to the ALMIGHTY GOD, THE FATHER, THE SON AND THE HOLY SPIRIT without whom this work would not have been possible.

ACKNOWLEDGEMENT

My gratitude goes to my GOD for the gift of life up to the present day. It is hardly possible to list all those who have in one way or the other contributed immensely to the noble task of helping.However, while expressing my indebtness to all generally, it is worthwhile to mention a few whose contributions to the success of this study were remarkably outstanding.

I thank my supervisors,Professors C.M.Z. Whong, S.A. Ado and Dr. I.O. Abdullahi for their assistance whenever challenges arose. My sincere gratitude to the entire staff of Microbiology Department, Ahmadu Bello University, Zaria for their unalloyed assistance and support.I specially, thank Dr. E.E. Ella who assisted me immensely in the ordering of the kit used in the analysis of some aspect of this work, Mrs. E. T. Addai, Prof. E. D. Jatau, Prof.J.B.Ameh, Mr. J. Boman, Mr. A. M. Shittu, Mr. A. Shiabu and Mrs. D. O. Toryila.

I am grateful to my father Barrister, S.C.M. Obasi my step mother Mrs F.A. Obasi, Prof. E. Uyovbisere (Soil Science Department) and Dr. S.C. Egege my uncle for their financial support and encouragement. My appreciation also goes to Prof G.I. Ndukwe, Mr. G. A. Shallangwa, Mr.A. Omachi, and Mr. C. A. Ochigbo,(Chemistry Department), I also thank the fellowing,Mr. M. B. Odoba,(Department of Public Health Veterinary and Preventive Medicine),Prof. C.A. Okuofu, Mr. P.C. Alika, Mr. Y. Yakubu,(Department of Water Resources),Mr. S.A. Ekwuribe, and Mr. M. Bashir,(Multi-user Research laboratory), and Mr. O. Ogunleye(Biology Department) all inAhmadu Bello University, Zaria respectively.

Finally, I thank Miss Adamu Iyang Comfort who did the typing of the work.

ABSTRACT

Yeast species present in sweet orange juice were isolated from healthy fermented single strength orange juice (FSSOJ) and defective single strength orange juice (DSSOJ). The characterisation of the indigenous yeast flora was carried out using analytical profile index techniques (API AUX 20C kit), physiological characterisation and genetic modification by mutation (nitrous acid and UV light irradiation treatment). The physiological characterisation involved the testing of several parameters required for ethanol production and dough leavening ability for bakery products (bread). These parameters were temperature, ethanol concentration tolerance, flocculation rate and sugar fermentative activity. The isolation rate of different yeast strains obtained using (API kit) in this research showed that yeast varies in their morphological and physiological characteristics. A total of 51 species were identified, representing 4 genera; 32 from FSSOJ, 19 species from DSSOJ. The isolation and prevalence rate of the yeast strains were *Candida krusei* (29)(57%),*Rhodotorula minuta*(10)(20%), *Candida zeylanoides* (4)(8%), *Candida parapsilosis* (3)(6%), *Geotrichumcapitatum*(2)(4%), *Candida lusitaniae, Candida norvegensis* and *Kodamea ohmeri* (1)(1%) respectively. The ability of some of the isolates:*Candida lusitaniae, Candida parapsilosis* and *Rhodotorula minuta* to metabolise xylose showed that they possessed the genes responsible for xylose fermentation. Thus, these made them suitable for ethanolic fermentation of hydrolysates of cellulosic materials. All the wild yeast strains obtained in this research combined four attributes namely; flocculation ability, tolerance to ethanol 8-18% (v/v), growth at 37 - 42^{0}C, and ability to ferment 10-40% (w/v) glucose except *Geotrichum capitatum.* Yeast possessing such combination of attributes appeared to have considerable potential for industrial application. Based on the physiological attributes and mutagenicity of the wild yeast strains for ethanol, dough leavening ability and the evaluation of the bread quality made with them,the

following results were obtained. The mutants of nitrous acid-induced and UV-irradiated(NAM and UVM:2F36,2F8,2F9,DS6 and DS20) selected for ethanol production showed an appreciable increase in ethanol yields which ranged from 14.78-18.18% v/v within two to five days, while the wild yeast strains(2F36,2F8,2F9,DS6 andDS20)ranged from 10.93-17.05% v/v within five to seven days of fermentation period respectively. The ethanol yields were obtained from fermentation of 5% sorghum starch after distillation, using titrimetric dichromate method. The result obtained from the dough leavening capacity showed that the mutants of sweetened dough had a better leavening ability than that of the unsweetened dough. However, some species of the wild strains, *Kodamea ohmeri,Candida norvegensis,* and *Rhodotorula minuta* obtained in this research had an outstanding dough leavening capacity both in the sweetened and unsweetened dough when compared with the commercial baker's yeast. The results of the hedonic rating of the bread samples showed that the panelists preferred the wheat bread made using the mutant strains of yeast in the sweetened bakery product, while those samples made using the wild strains were preferred in the unsweetened bread samples. This study enabled the acquisition of data on the basic physiological and mutagenic pattern of yeasts required for the industrial production of higher ethanol concentration and dough leavening capacity with regard to their use in bakery products.

TABLE OF CONTENTS

Cover Page i

Fly Leaf iii

Title Page iii

Declaration iv

Certification v

Dedication vi

Acknowledgement vii

Abstract viii

Table of Contents x

List of Tables xxi

List of Figures xxiii

List of Appendices xxvii

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Statement of the Problem 3

1.2 Justification of the Study 4

1.3 Aim and Objectives 4

1.3.1 Specific objectives of the study 5

CHAPTER TWO 6

2.0 LITERATURE REVIEW 6

2.1 Yeast 6

2.2 History of Yeast 7

2.3 Nutrition and Growth of Yeasts 8

2.4 Ecology of Yeasts 9

2.5 Reproduction of Yeasts 10

2.6 Uses of Yeast 12

2.6.1 Alcoholic beverages 12

2.6.1.1 Beer 12

2.6.1.2 Wine 13

2.6.2 Baking(Bread and related products) 14

2.6.2.1 Types of baker's yeast 16

2.6.3 Industrial ethanol production 17

2.6.4 Bioremediation 18

2.6.5 Non alcoholic beverages 18

2.6.6 Nutritional supplements 19

2.6.7 Probiotics of yeasts 20

2.6.8 Aquarium hobby of yeasts 20

2.6.9 Yeast extract 21

2.6.10 Food spoilage 21

2.6.11 Pathogenic yeasts 22

2.6.12 Science of yeasts 23

2.7 Characteristics of Brewing Yeasts 23

2.7.1 Physiological characteristics of brewer's yeast 27

2.8 Nutritional Requirements of Brewer's Yeast 28

2.8.1 Carbohydrate compounds 28

2.8.1.1 Monosaccharide 28

2.8.1.2 Disaccharides 28

2.8.1.3 Trisaccharides 29

2.8.2 Nitrogenous compounds 29

2.8.3 Minerals (trace element) 30

2.9 Brewing Yeast Flavour Production 31

2.9.1 Aldehydes 32

2.9.2 Higher alcohol 32

2.9.3 Esters 32

2.9.4 Organic acids 32

2.9.5 Sulphur compounds 33

2.10 Yeast Flocculation 33

2.10.1 Classification of brewer's yeast strains 34

2.10.2 Factors affecting flocculation 37

2.11 Yeasts Species Associated with Orange Juice 37

2.12 Ethanol and Its Properties 38

2.12.1 History of ethanol production 39

2.12.2 Uses of ethanol 40

2.12.2.1 Transportation fuel and agriculture 40

2.12.2.2 Ethanol and the environment 41

2.13 Ethanol Production 41

2.13.1 Sugar 42

2.13.2 Starches 43

2.13.3 Cellulosic materials 43

2.14 Fermentation 44

2.14.1 History of fermentation 45

2.14.2 Chemistry of fermentation 46

2.14.3 Ethanol fermentation 46

2.14.4 The chemical process of fermentation of glucose 47

2.14.5 Glycolysis 48

2.14.6 Aerobic respiration 48

2.14.7 Lactic acid fermentation 49

2.14.8 Hydrogen gas production in fermentation 50

2.14.9 Effect of oxygen 51

2.14.10 Ethanol fermentation with yeast 51

2.14.11 Industrial ethanol fermentation 52

2.14.12 Effects and side effects of ethanol 54

2.15 Mutation 56

2.15.1 Classification of the mutation types by effect on fitness 56

2.15.1.2 Harmful mutation 57

2.15.1.3 Neutral mutation 58

2.15.2 Classes of mutation 58

2.15.2.1 Spontaneous mutation 58

2.15.2.2 Induced mutation 59

2.15.2.3 Radiation 61

2.15.2.5 DNA repair systems 63

2.15.3 Screening for nutritional mutation 64

2.15.3.1 Screening procedures 65

2.15.3.2 Quantitative positive auxanography for the screening of nutritional mutants 65

2.15.4 Application of mutations 65

2.16 Bread Production 66

2.16.1 Ingredients of bread 67

2.16.2 Processing stages 70

2.16.2.1 Recipes and formulations……70

2.16.2.2 Dough kneading 70

2.16.3 Bread making process 71

2.16.3.1 Sponge and dough method 71

2.16.3.2 Straight dough method 73

2.16.4 Sources of fermentable sugars in bread-making 74

2.16.5 Changes during fermentation 74

2.16.6 The fermentation speed 75

2.16.7 Proofing time and dough handling 75

2.16.8 Carbon(iv)oxide and gluten development 76

2.16.9 Dough leavening 76

2.16.10 The baking process 77

2.16.11 Bread crust 77

2.16.12 Characteristics of good bread 78

2.17 Meaning of Sensory Evaluation 81

2.17.1 Objectives of sensory evaluation 81

2.17.2 Sensory evaluation methods 81

2.17.2.1 Affective / hedonic tests 82

2.17.2.2 Analytical tests 82

2.17.3 Advantages of sensory evaluation methods 83

2.17.4 Sensory evaluation techniques 83

2.17.5 Uses of sensory techniques 88

CHAPTER THREE 89

3.0 MATERIALS AND METHODS 89

3.1 Sample Collection 89

3.2 Sample Preparation 89

3.4 Isolation of Yeast Strains 90

3.5 Maintenance of Yeast Strains 91

3.6 Characterisation of Yeast Strains 91

3.6.1 Morphological characteristics of yeasts 91

3.6.1.1 Colonial morphology of yeasts 91

3.6.1.2 Vegetative morphology of yeasts 91

3.6.1.3 Pseudomycelium or true mycelium observation in yeasts 92

3.6.1.4 Pellicle formation in yeasts 92

3.6.2 Biochemical characterisation of yeast species 92

3.6.2.1 Carbon assimilation test 92

3.6.2.2 API technique 93

3.6.3 Fermentative ability of the yeast strains 94

3.6.3.1 Flocculence test for yeasts 94

3.6.3.2 Ethanol tolerance test for yeasts 94

3.6.3.3 Fermentation of glucose at 37^0 and 42^0C by yeasts 95

3.6.3.4 Sugar utilisation test for yeasts 95

3.6.3.5 Growth in 3% ethanol test for yeasts 96

3.6.3.6 Growth in 50% glucose test for yeasts 96

3.6.3.7 Growth at elevated temperatures test for yeasts 96

3.6.3.8 Growth in 3 and 10% sodium chlorides test for yeasts 97

3.7 Genetic Modification of the Characterised Yeast Species. 97

3.7.1 Mutation of yeasts using nitrous acid 97

3.7.2 Mutation of yeasts using UV irradiation at 254nm 98

3.7.3 Screening for nutritional mutants 98

3.8 Ethanol Production using the Wild Strains,Modified and Brewer's Yeast. 99

3.8.1 Inocula preparation of the yeasts strains 99

3.8.2 Substrate preparation for ethanol fermentation 99

3.8.3 Preparation of the yeast fermentation medium 99

3.8.4.2 Determination of cell number 101

3.8.4.3 Ethanol concentration 101

3.8.4.4 Reducing sugars 102

3.9 Propagation of Yeast Species for Use in Dough Leavening Tests 103

3.9.1 Dough leavening test 103

3.10Bread Baking Process 104

3.11 Consumer Panel Sensory Evaluation 104

3.12 Statistical Analysis..... 104

4.0 RESULTS..... 106

4.1 Morphological and Physiological Characteristics of Yeast Strains Isolated from Fresh and Spoilt Single Strength Orange Juice. 106

4.2 Carbon Assimilation 109

4.3 Flocculation Rate..... 111

4.4 Ethanol Concentration Tolerance 111

4.5 Fermentation Activities..... 113

4.6 Mutagenic Treatments of the Yeast Isolates..... 116

4.7 Characterisation of the Nutritional Requirements of the Identified Mutant Yeast Strains for both UV- Irradiation and Nitrous Acid Treatments. 125

4.8 Fermentation of 5% Sorghum Starch with Wild and Brewing Yeast Strains for Ethanol Production. 127

4.9 Fermentation of 5% sorghum Starch with Mutants (nitrous acid) Treatment and Brewing Yeast Strains for Ethanol Production. 134

4.10 Fermentation of 5% Sorghum Starch with Mutant (UV light-Irradiated) Yeast Strains for Ethanol Production. 140

4.11 Fermentative Ability 154

4.12 Growth in 3 and 10% NaCl..... 154

4.13 Growth in 50% Glucose..... 154

4.14 Growth at Elevated Temperatures 155

4.15 Growth in 3% Ethanol 157

4.16 Dough Leavening Tests 157

4.16.1 Sweetened dough leavening test 157

4.16.2 Unsweetened dough leavening test. 163

4.16.3 Ultra Violet (UV) light-Irradiated Yeast Strains used for Sweetened and Unsweetened Dough Test 169

4.17 Sensory Evaluation 201

CHAPTER FIVE 206

5.0 DISCUSSION 206

5.1 Morphological and Physiological Characteristics of Yeast Strains Isolated 206

5.2 Carbon Assimilation Profile 208

5.3 Flocculation Profile 209

5.4 Ethanol Tolerance Profile 210

5.5 Fermentation Profile 211

5.6 Nutritional Requirements of Mutants 212

5.7 Genetic Modification of the Characterised Yeast Strains 213

5.8 Alcohol Producing Ability of the Wild, Modified and Brewers Yeast Strains 214

5.9 Dough Leavening Capacity of the Wild, Modified and Baker's Yeast Strains 217

5.10 Sensory Evaluation of Bread Made with Wild Strains, Modified and Baker's Yeast ……...220

CHAPTER SIX 221

6.0SUMMARY, CONCLUSION AND RECOMMENDATIONS..... 221

6.1 SUMMARY 221

6.2 CONCLUSION 222

6.3RECOMMENDATIONS..... 224

REFERENCES..... 226

APPENDICES……246

LIST OF TABLES

Table	Title	Pages
2. 1:	Differences between Laboratory and Brewing Strains of Yeast (*Saccharomyces*)	25
2. 2:	Differences between Ale and Lager Yeast	26
2. 3:	Flocculation Degree of Brewing Strains	35
4. 1:	Morphological and Physiological Characteristics of Yeast Strains Isolated from Fresh and Spoilt Single Strength Orange Juice.	107
4. 2:	Carbon Assimilation Test Using API20C AUX Kit (BIOMERIEUX)	110
4. 3:	The Performance of Identified Yeast Strains with respect to Flocculation Rate, Tolerance to Graded Ethanol Concentration and Ethanol Tolerance, Measured as % Cell Viability of the Yeasts in 10% (v/v) Ethanol	112
4. 4:	Fermentative Performance of Yeast Strains with Glucose at various Concentrations and Growth at Elevated Temperatures	114
4. 5:	Fermentative Pattern of Yeast Strains for Some Sugars	115
4. 6:	Characterisationof the Nutritional Requirements of the Wild and Identified Mutant Strains using Amino Acids	126
4.7a:	Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast for Ethanol Production at 4 minutes.	141
4.7b:	Fermentation of 5% sorghum starch with mutant (UV light) irradiation yeasts strains for ethanol production at 6 minutes.	143
4.7c:	Fermentation of 5% sorghum starch with mutant (UV light) irradiation yeast strains for ethanol production at 10minutes.	144
4.7d:	Fermentation of 5% sorghum starch with mutant (UV light) irradiation yeast strains for ethanol production at 15minutes.	146

4.7e:Fermentation of 5% sorghum starch with mutant (UV light) irradiation yeast strains for ethanol production at 20 minutes. 148

4.7f:Fermentation of 5% sorghum starch with mutant (UV light) irradiation yeast strains for Ethanol Production at 25 minutes. 149

4.7g:Fermentation of 5% sorghum starch with mutant (UV light) irradiation yeast strains for ethanol production at 30 minutes……...…….150

4.8Production rate/maximum yields of ethanol from brewing, wild and mutant yeast strains from5% sorghum starch fermentation. 152

4.9Production rate/maximum yield of ethanol %(v/v) wild, nitrous acid and UV light-irradiated (mutants) yeasts strains from fermentation of 5% sorghum starch. 153

4.10Performance of yeast strains with respect to the markers 156

4.11Assessment of leavening capacities of mutant yeast strains with UV- light at different time intervals in sweetened dough 170

4.12Assessment of leavening capacities of mutant yeast strains with UV- light at different time intervals inunsweetened dough …….171

4.13 Analysis of variance of organoleptic attributes of sweetened and unsweetened bread samples made with wild yeast and its mutant species and commercial baker's yeast..... 203

4.14Comparative analysis of the mean performance of the wild yeasts, its mutants and commercial baker's yeast on bread samples 204

4.15 Analysis of variance of organoleptic attributes of sweetened and unsweetened bread samples with mutant yeasts (Ultra Violet light) 205

LIST OF FIGURES

Figure	Title	Page
2.1	Yeast cells life cycle……..……11	
4.1	Prevalence rate of yeast strains isolated from fresh and spoilt single strength orange juice…….108	
4.2	Percentage Survival and kill rate of UV irradiated yeast strains at 4 minutes…….117	
4.3	Percentage Survival and kill rate of UV irradiated yeast strains at 6 minutes…….118	
4.4	Percentage Survival and kill rate of UV irradiated yeast strains at 10 minutes……...119	
4.5	Percentage Survival and kill rate of UV irradiated yeast strains at 15 minutes…..120	
4.6	Percentage Survival and Kill rate of UV irradiated yeast strains at 20 minutes……..121	
4.7	Percentage Survival and kill rate of UV irradiated yeast strains at 25 minutes…..122	
4.8	Percentage Survival and kill rate of UV irradiated yeast strains at 30 minutes…..123	
4.9	Percentage Survival and kill rate of nitrous acid at 20 minutes……..….124	
4.10	Fermentation of 5% starch with Brewing yeast (INS) for ethanol production……....128	
4.11	Fermentation of 5% sorghum starch with wild yeast strain (2F36) for ethanol production……..…..129	
4.12	Fermentation of sorghum starch with wild yeast strain (DS6) for ethanol production (pH 6.5)……...….130	
4.13	Fermentation of 5% sorghum starch with wild yeast strain (7F8) for ethanol production (pH6.5) ……..……..…131	

4.14 Fermentation of 5% sorghum starch with wild yeast strain (7F9) for ethanol production……..……..132

4.15 Fermentation of 5% sorghum starch with wild yeast strain (DS20) for ethanol production ……..……...…..133

4.16 Fermentation of 5% sorghum starch with mutant (nitrous acid treated) strain (2F36) for ethanol production at 20 minutes pH6.5)……...…….....135

4.17 Fermentation with of sorghum starch mutant (nitrous acid treated) yeast strain (7F9) for ethanol production at 20 minutes (pH 6.5)……...…..136

4.18 Fermentation of 5% sorghum starch with mutant (nitrous acid treated) yeast strain (DS20) for ethanol production at 20 minutes pH6.5)……...……...137

4.19 Fermentation of 5% Sorghum starch with mutant (nitrous acid treated) yeast strain (7F8) for ethanol production at 20 minutes (pH 6.5)……..……...…138

4.20 Fermentation of sorghum starch with mutant (nitrous acid treated) yeast strain (DS6) for ethanol production at 20 minutes (pH 6.5)..……139

4.21 Leavening profiles of wild strains and baker's yeast sweetened dough……..……159

4.22 Proof heights of wild strains and baker's yeast sweetened dough……. 160

4.23 Leavening profiles of wild strains, mutant (nitrous acid) treatment and bakers' yeast in sweetened dough…….....……...…….161

4.24 Proof heights of sweetened dough fermented wild strains, mutant (nitrous acid) treatment and bakers' yeast…….……..……...162

4.25 Proof heights of unsweetened dough fermented wild strains, mutant (nitrous acid) treatment and bakers' yeast……..……..…….165

4.26 Leavening profile of unsweetened dough fermented with wild strains, mutant (nitrous acid) treatment and bakers' yeast …….……....166

4.27a Leavening profile of unsweetened dough fermented with wild yeast and baker's yeast ……..……...…….167

4.27b Proof heights of unsweetened dough fermented with wild yeast and baker's yeast…....168

4.28a Proof heights of sweetened dough of UV mutants at 4mins……..……..……172

4.28b Leavening profile of sweetened dough for UV mutants at 4 mins……...……...173

4.28c Proof heights of sweetened dough of UV mutants at 6 mins ……..174

4.28d Leavening profile of sweetened dough for UV mutants at 6 mins……...……..175

4.28e Proof heights of sweetened dough of UV mutants at 10 mins…….176

4.28fLeavening profile of sweetened dough for UV mutants at 10 mins……177

4.28g Proof heights of sweetened dough of UV mutants at 15 mins……...……178

4.28h Leavening profile of sweetened dough for UV mutants at 15 mins……179

4.28i Proof heights of sweetened dough of UV mutants at 20 mins…….……180

4.28j Leavening profile of sweetened dough for UV mutants at 20 mins……181

4.28kProof heights of sweetened dough of UV mutants at 25 mins…….……182

4.28l Leavening profile of sweetened dough for UV mutants at 25 mins……183

4.28m Proof heights of sweetened dough of UV mutants at 30 mins……184

4.28n Leavening profile of sweetened dough for UV mutants at 30 mins……185

4.29aProof heights of unsweetened dough of UV mutants at 4mins…….……...186

4.29bleavening profile of unsweetened dough for UV mutants at 4 mins ……..……188

4. 29c Proof heights of unsweetened dough of UV mutants at 6mins…….……..189

4.29d Leavening profile of unsweetened dough for UV mutants at 6 mins…….…..…..190

4. 29e Proof heights of unsweetened dough of UV mutants at 10 mins……..…..……191

4.29f Leavening profile of unsweetened dough for UV mutants at 10 mins……...…….192

4.29g Proof heights of unsweetened dough of UV mutants at 15 mins…….193

4.29h Leavening profile of unsweetened dough for UV mutants at 15 mins……..……194

4.29i Proof heights of unsweetened dough of UV mutants at 20 mins……195

4.29j Leavening profile of unsweetened dough for UV mutants at 20 mins……196

4.29k Proof heights of unsweetened dough of UV mutants at 25 mins……197

4.29l Leavening profile of unsweetened dough for UV mutants at 25 mins……198

4.29m Proof heights of unsweetened dough of UV mutants at 30 mins……199

4.29n Leavening profile of unsweetened dough for UV mutants at 30 mins…….200

LIST OF APPENDICES

Appendix	Title	Page
I	Identity of yeasts isolated from fresh and spoilt single Strength orange juice.....	246
II	Isolation and prevalence rate of yeast strains isolated from fresh and spoilt single strength orange juice …….....	248
III	Panel rating score sheet, bread …….……..…	249
Iva	Effects of irradiation of yeast from fresh and spoilt single strength orange juice (Fermented) with UV light at 4 minutes …….	250
IVb	Effects of irradiation of yeast strains with UV light at 6 minutes ……..…	251
IVc	Effects of irradiation of yeast strains with UV light at 10 minutes ……	252
IVd	Effects of irradiation of yeast strains with UV light at 15 minutes ……	253
IVe	Effects of irradiation of yeast strains with UV light at 20 minutes ……	254
IVf	Effects of irradiation of yeast strains with UV light at 25 minutes …….……	255
IVg	Effects of irradiation of yeast strains with UV lightat 30 minutes …….	256
V	Effects of the treatment of yeast strains with nitrous acid at 20 minutes ……..…	257
VI	Fermentation of 5% sorghum starch with wild and brewing yeast strains for ethanol production (PH 6.5) …….….....	258
VII	Fermentation of 5% sorghum starch with mutant (nitrous acid treated) and brewing yeast strains of for ethanol production at 20 minutes …….……..….	259

VIIIAssessment of Leavening capacities of wild and baker's yeast strains in sweetened dough ……260

IXAssessment of leavening capacities of mutant yeast strains (nitrous acid) treatment in sweetened dough ……..……..……261

XAssessment of leavening capacities of wild and baker's yeast strains in unsweetened dough ……..……...…262

XIAssessment of leavening capacities of mutant yeast strains (nitrous acid) treatment in unsweetened dough ……....……263

XIIComparison of mean values of leavening capacities of sweetened and unsweetened dough of baker's yeast ……...…264

XIIIComparison of mean values of leavening capacities of sweetened and unsweetened dough of wild yeasts ……..265

XIVComparison of mean values of leavening capacities of sweetened and unsweetened dough of mutants yeast strains of nitrous acid treatment……...…267

XVComparison of mean values of leavening capacities of sweetened and unsweetened dough of mutant yeast strains (UVLight-Irradiated)……..269

XVIAssessment of the Mean performance of the leavening capacities of the mutant yeast strains (UV light- irradiated) of sweetened dough using analysis of variance (ANOVA) ……..…274

XVIIAssessment of the mean performance of the leavening capacities of the mutant yeast strains (UV light- irradiated) of unsweetened dough using analysis of variance (ANOVA)……275

XVIIIProof heights of UV mutants 4 to 30 mins of sweetened dough ……...……..…276

XIXProof heights of UV mutants 4 to 30 mins of unsweetened dough …….....…277

CHAPTER ONE

1.0 INTRODUCTION

Citrus is the most economically important tree crop in the world (Spiegel-Roy and Goldschmidt, 1996). The majority of citrus arrives the market in the form of processed products, such as single-strength orange juice and frozen juice concentrates. In Florida, one of the world's top producing regions, more than 96% of all oranges are processed into orange juice. In the 1999-2000 seasons, Florida produced more than four billion liters of single–strength orange juice (Anon, 2001).

In Nigeria, the fruit canning industry ,Lafia canning factory,established in Ibadan,in 1954and acquired by Fumman Agricultural Products Limited in 1994 with the sole aim of processing and marketing wholesome fruits and juices has greatly improved.The current status of the fruit juice industry has greatly increased in the citrus cultivation and in the volume of juice produced .From 1994-2008,fruit manufacturing in Nigeria has taken a giant leap.This quantum leap was facilitated by the government by the ban on the importation of fruit juice, a challenge to which the local manufacturers reacted positively by increasing their production,quality and quantity–wise(Orebe *et al*.,2008).

Citrus juices are acidic beverages (pH 3-4) with high sugar content (15^0 Brix). Under these conditions, acidolactic bacteria, molds and yeasts comprise the typical microbiota. Typical yeast species found in citrus juices are *Candida parapsilopsis, Candida stellata, Saccharomyces cerevisiae, Torulaspora delbrukii* and *Zygosaccharomyces rouxii,* Also included are species from the genus *Rhodotorula, Pichia, Hansenispora,* and *Metschickowia* (Hatcher *et al.,* 2000). Despite

the economic importance of citrus juices, there are few reports investigating yeasts species associated with them (Parish and Haggins,1989; Deak and Beuchat, 1992; Hatcher*et al.,* 2000). A detailed study of these yeast isolates from citrus juice is needed and a method developed to enhance their fermentative ability and alcoholic tolerance capacity. Yeast species are used in many industrial fermentation processes including alcoholic beverage production and leavening or rising of bread dough.(Aboaba and obakpolo,2010).

Advances in microbiology and fermentation technology have continued steadily up until the present. In the late 1970s, for example, it was discovered that microorganisms could be mutated with physical and chemical treatments to be higher-yielding, faster growing, tolerant of less oxygen, and able to use a more concentrated medium (Wang *et al.,* 1980). Strains selections and hybridization weredevelopedas well, thus, affecting most modern food fermentation(Wang *et al.,* 1980).

Various factors influence the fermentation process which determine the end products obtained. These include substrate related factors such as cultivar types, cultivation conditions, conditions at harvest and post harvest handling (Bell *et al.,* 1999; Jonathan and Errol, 2000; Joshi and Sandhu, 2000; Liu, 2002; Kourkoutas*et al.,* 2005).

The basic requirements for fermentative production of ethanol are an appropriate microbial strain, suitable substrate and process technology. Stewart *et al.,* (1984), outlined the desirable characteristics of an ideal microorganism used for ethanol production. These include rapid fermentative ability, flocculating ability (to facilitate sedimentation, cell recovery and recycle), osmotolerance (to allow fermentation of high concentration of carbohydrates), ethanol tolerance (to allow production of high ethanol concentrations) and thermotolerance (to minimise cooling

requirements).*Saccharomyces cerevisiae*, a yeast, has been the most preferred microorganism employed in the production of ethanol, wine and bread in most industries (Wiegel, 1980; Jones *et al.,* 1981; Govender *et al.,* 2008). The preference of this yeast to any other microorganism is due to its ability to produce ethanol unadulterated by other products. No strain has been found to possess the entire desirable characteristic but those with only one or two have been described by some workers (Panchal and Stewart, 1981; Panchal*et al.,* 1982; Hacking*et al.,* 1984; Anderson*et al.,* 1986;Lin and Tanaka, 2006).

Citrus juice concentrates are readily storable and can be used for production processes even when the fruit is out of season (Ramachandra and Arun, 2005; Saddiq *et al.,* 2006). In India alone,for example, an estimated loss of about 35,000 million rupees (638 million US$) worth of fruits and vegetables was recorded despite the fact that India produces around 60-65 million tons of fruits and vegetables (Ramachandra and Arun, 2005) while a report, indicates that about fifty percent of citrus fruits goes to waste in Nigeria (Jenifer, 1999). In an effort aimed at increasing the low industrial utilisation and reducing the high wastage of orange (*Citrus sinensis)* fruits in the developing world, there is need to investigate the possibility of exploiting the fermentativeability of yeast obtained from orange juice to determine its suitability for ethanol production and other fermentation products.

1.1 Statement of the Problem

Yeast have a low alcohol tolerance level at about 10-12% (Oke and Ijebor, 1997). Studies have shown that when the genetic structure of yeasts is altered, they can simultaneously convert glucose and xylose to ethanol. The ability to effectively ferment xylose to ethanol increases the yield of ethanol to about 40% (Ho *et al*, 2004). This shows that the alcohol tolerance level and

fermentative ability of yeasts from citrus can be greatly enhanced by genetic modification and employed in the on-going global fermentation ethanol programme.

1.2 Justification of the Study

Citrus juice harbours a large and divergent group of yeast species which could play important roles beneficial to many industrial processes involving fermentation. Examples of such processes include ethanol production, wine and bread making. However, only a very few of these yeasts are typical fermentative species, such as *S. cerevisiae, C. intermedia* or *T. delbrueckii,* and mild fermentative species mostly *H. uvarum* and *H. occidentalis* (Covadonga *et al.,* 2002). The quality of these products would greatly depend on the yeast strain (Kunkee, 1984; Okunowo *et al.,* 2005). Development of improved starter organisms for fermentation of citrus juice may offer a relatively simple avenue for reducing post harvest wastage of citrus fruits in low utilisation environment, and in places where the production of citrus concentrates is low or non-existent.

The yeast being single cell eukaryotic fungus can be easily modified using various physical or biological systems (genetics) and the new traits transferred to their offspring.The world is tending toward bioethanol production and this will require the utilisation of yeast with good fermentative ability.

1.3 Aim and Objectives

The aim of this study is to isolate, characterise and genetically modify some yeast strains from orange juice to enhance their fermentative ability.

1.3.1 Specific objectives of the study

1. To isolate and characterise yeast species present in citrus juice (*C. sinensis*).
2. To determine the fermentative ability of the yeast species (alcohol production and dough leavening capacity).
3. To carryout genetic modification of the characterised yeast species.
4. To compare the alcoholic producing ability and dough leavening capacity of the genetically modified yeast strains, wild strain with the brewers and baker's yeast.
5. To carry out sensory evaluation of bread made using the wild strain,modified and baker's yeasts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Yeast

Yeasts are eukaryotic micro-organisms classified in the kingdom fungi, with the 1,500 species currently described (Kurtzman and Fell, 2006), estimated to be only 1% of all yeast species (Kurtzman and Piskur, 2006). Most reproduce asexually by budding, although a few do so by mitosis.

Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae, as seen in most mold (Kurtzman and Fell, 2005). Yeast size can vary greatly depending on the species, typically measuring 3.0 – 4.0 µm in diameter, although some yeasts can reach over 40µm (Walker *et al*., 2002). The yeast species *Saccharomyces cerevisiae*has been used in baking and in fermentating alcoholic beverages (Bread, wine and Beer) for thousands of years (Jean-luc et *al*., 2007). It is also extremely important as a model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic micro-organisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology (Ostergaard *et al*., 2000).

Other species of yeast, such as *Candida albicans* are opportunistic pathogens and can cause infections in humans. Yeasts have recently been used to generate electricity in microbial fuel cells, Anon, (2007a) and produce ethanol for the biofuel industry.

Yeasts do not form a single taxonomic or phylogenic grouping. The term "yeast" is often taken as a synonym for *Saccharomyces cerevisiae*(Kurtzman, 1994), but the phylogenetic diversity of the yeasts is shown by their placement in two separate phyla, the Ascomycotaand the Basidiomyceta. The budding Yeasts ("true yeast") are classified in the order Saccharomycetales (Anon, 2009a).

2.2 History of Yeast

The word "yeast" comes to us from the old English gist, gyst and from the Indo – European root yes-, meaning boil, foam or bubble (Anon, 2008).

Yeast microbes are probably one of the earliest domesticated organisms. People have used yeast for fermentation and baking throughout history. Archaeologists digging in Egyptian ruins found early grinding stones and baking chambers for yeast bread, as well drawings of 4,000 – year – old bakeries and breweries (Philips, 1999). In 1680, the Dutch naturalist Anton Van Leewenhoek first microscopically observed yeast, but at that time did not consider them to be living organisms, but rather globular structures (Huxley, 2009).

In 1857, a French microbiologist Louis Pasteur proved in the paper "Memoire surla fermentation alcoolique" that alcohol fermentation was conducted by living yeasts and not by a chemical catalyst (Philips, 1999; Barnett, 2003).

Pasteur showed that by bubbling oxygen into the yeast broth, cell growth could be increased, but fermentation was inhibited – an observation later called the "Pasteur effect". By the late 18th century, two yeast strains used in brewing had been identified: *Saccharomyces cerevisiae*, also called top fermenting, and *Saccharomyces carlsbergensis*, bottom fermenting yeast. *S.*

*cerevisiae*has been sold commercially by the Dutch for bread making since 1780; while around 1800, the Germans started producing*S. cerevisiae*in the form of cream.

In 1825, a method was developed to remove the liquid so that yeast could be prepared as solid blocks (Klieger, 2004). The industrial production of yeasts blocks was enhanced by the introduction of the filter press in 1867. In 1872, Baron Max de Springer developed a manufacturing process to create granulated yeast, a technique that was used until the First World War (Anon, 2010a).

In the United States, naturally occurring air-borne yeasts were used almost exclusively until commercial yeast was marketed at the Centennial Exposition in 1876 in Philadelphia. There, Charles L. Fleischmann exhibited the product and a process to use it, as well as serving the resultant baked bread(Anon,2010a).

2.3 Nutrition and Growth of Yeasts

Yeasts are chemorganotrophs, as they use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, such as glucose and fructose, or disaccharides such as sucrose and maltose. Some species can metabolise pentose sugar like ribose, alcohols and organic acids(Barnett,2003). Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes), or are anaerobic, but also have aerobic species that grow only anaerobically that is the absence of oxygen (obligate anaerobes). Yeasts grow best in a neutral or slightly acidic pH environment. Yeasts vary in what temperature range they grow best. *Leucosporidium frigidium,* for example grows at -2 - 20^0C, *Saccharomyces tellurians* at 5 - 35^0C and *Candida slooffi* at 28 - 45^0C (Kurtzman,2005). The cells can survive freezing under certain condition with viability decreasing overtime.

Yeasts are generally grown in the laboratory on solid growth media or in liquid broths. Common media used for the cultivation of yeasts include potato dextrose agar (PDA) or potato dextrose broth, Wallerstein laboratories nutrient (WLN) agar, yeast peptone dextrose agar (YPD), and yeast mould agar or broth (YM). Home brewers who cultivate yeast frequently use dried malt extract (DME) and agar as a solid growth medium. The antibiotic cycloheximide is sometimes added to yeast growth media to inhibit the growth of *Saccharomyces* yeasts and select for wild/indigenous yeast species. This will change the yeast process(Kurtzman,2005).

2.4 Ecology ofYeasts

Yeasts are very common in the environment, but are usually isolated from sugar-rich materials. Some good examples include naturally occurring yeasts on the skins of fruits and berries (such as grapes, apples or peaches), and exudates from plants (such as plant saps or cact). Some yeasts are found in association with soil and insects (Slavikora and Vadkertiova, 2003, Suh *et al*., 2005). The ecological function and biodiversity of yeasts are relatively unknown compared to those of the other microorganisms (Herrera and Maria, 2010).

Yeasts, including *Candida albicans, Rhodotorula rubra, Torulopsis* and *Trichosporon cutaneum,* have been found living in between people's toes as part of their skin flora (Oyeka and Ugwu, 2002). Yeasts are also present in the gut flora of mammals and some insects (Martini, 1992), and even deep-sea environments host an array of yeasts (Bass *et al*., 2007; Kutty and Philip, 2008). An Indian study of seven bee species found 45 species from 16 genera colonise the nectarines of flowers and honey stomachs of bees. Most were members of the *Candida* genus: the most common species in honey stomachs was *Dekkera intermedia* and in flower nectarine, *Candida blankii* (Sandhu and Manjit, 1985).

Yeasts colonising nectarines of the stinking hellebore have been found to raise the temperature of the flower which may aid in attracting pollinators by increasing the evaporation of volatile organic compounds (Barley, 2010). A black yeast has been recorded as a partner in a complex relationship between ants, their mutualistic fungus, (a fungal parasite of the fungus) and bacteria that kills the parasite. The yeast have a negative effect on the bacteria that normally produce antibiotics to kill the parasite and so may affect the ant's health by allowing the parasite to spread (Little and Cameron,2008).

2.5 Reproduction of Yeasts

Yeasts can reproduce asexually through budding or sexually through the formation of ascospores. The most common mode of vegetative growth in yeast is asexual reproduction (Bala-subramania *et al.*, 2004). During asexual reproduction new bud or daughter cell grows out of the parent yeast when the condition is right, then after the bud reaches an adult size, it separates from the parent yeast cell forming a new cell (Yeong, 2005)(Fig.2.1). Some yeasts, including *Schizosaccharomyces pombe*, reproduce by mitosis instead of budding (Balasubramanian and Glotzer, 2004). Under low nutrient conditions, yeasts that are capable of sexual reproduction will form ascospores. Yeast that is not capable of going through the full sexual cycle is classified in the genus *Candida*. Under high stress conditions, haploid cells will generally die; under the same conditions, however, diploid cells can undergo sporulation, entering sexual reproduction (meiosis) and producing a variety of haploid spores, which can go on to mate (conjugate), reforming the diploid state (Neiman, 2005) (Fig.2.1).

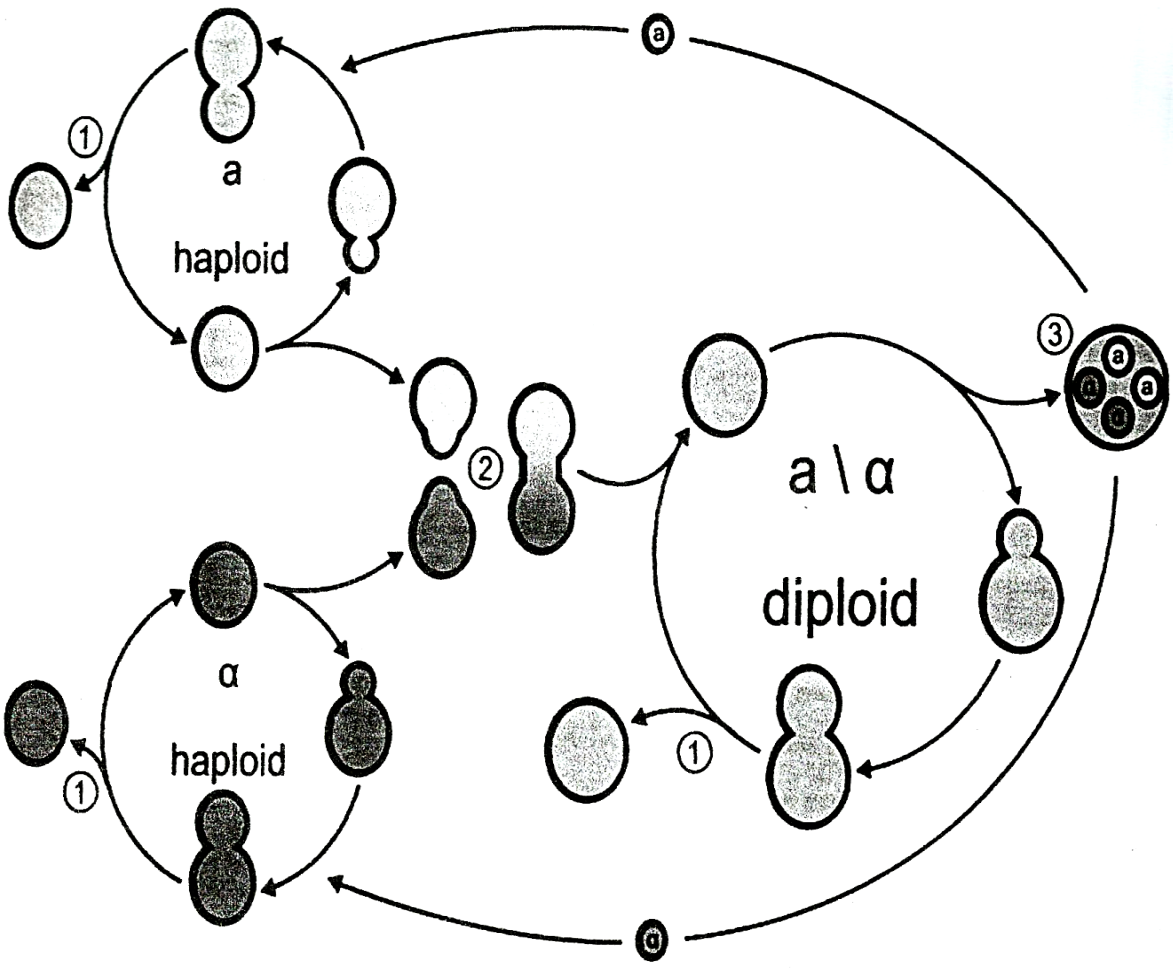


Figure 2.1: Yeast cell's life cycle: (1) Budding (2) Conjugation (3) Zygote formation

(Source:Neiman,2005)

2.6 Uses of Yeast

The physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Many types of yeasts are used for making many foods: baker's yeast in bread production: brewer's yeast in beer fermentation; yeast in wine fermentation and for xylitol production (Rao, *et al*., 2004). So-called "red rice yeast" is actually a mold, *Monascus purpureus.* Yeasts include some of the most widely used model organisms for genetics and cell biology.

2.6.1 Alcoholic beverages

Alcoholic beverages are defined as beverages that contain ethanol (C_2H_5OH). This ethanol is almost always produced by fermentation – the metabolism of carbohydrates by certain species of yeast under anaerobic or low-oxygen conditions. Beverages such as wine, beer, or distilled spirits all use yeast at some stage of their production. A distilled beverage is a beverage containing ethanol that has been purified by distillation.

Carbohydrate containing plant material is fermented by yeast, producing a dilute solution of ethanol in the process. Spirits such as whiskey and rum are prepared by distilling these dilute solutions of ethanol. Components other than ethanol are collected in the condensate, including water, esters and other alcohols, which account for the flavor of the beverages.

2.6.1.1 Beer

Brewing yeast is classified by brewers as "top cropping" (or "top fermenting") and "bottom cropping" (or "bottom-fermenting") (Anon, 2006a). Top cropping yeasts are so called because they form foam at the top of the wort during fermentation. An example of a top cropping yeast is *Saccharomyces cerevisiae*sometimes called an "ale yeast" (Anon, 2010a). Bottom cropping

yeasts are typically used to produce lager-type beers, though they can also produce ale-type beers. These yeasts ferment more sugars, creating a dryer beer, and grow well at low temperatures. An example of bottom cropping yeast is *Saccharomyces pastorianus*, formerly known as *S. carlsbergenensis.*

The most common top cropping brewer's yeast, *S. cerevisiae*, is the same species as the common baking yeast(Anon, 2002a).

However, baking and brewing yeasts typically belong to different strains, cultivated to favour different characteristics: baking yeast strains are aggressive, to carbonate dough in the shortest amount of time possible; brewing yeast strains act slower, but tend to produce fewer off-flavours and tolerate higher alcohol concentrations (with some strains, up to 22%)(Anon,2002a).

Brettanomyces is a genus of wild yeast important in brewing lambic, a beer produced not by the deliberated addition of brewer's yeasts, but by spontaneous fermentation by wild yeasts and bacteria. *Brettanomyces lambicus,B. bruxellensis* and *B. claussenii* are native to the Senne Valley region of Belgium, where lambic beer is produced (Verachetert and Iserentant, 1995).

2.6.1.2 Wine

Yeast is used in wine making, where it converts the sugars present in grape juice (Must) into ethanol. Yeast is normally already present on grape skins (the white powder called "the bloom"). Fermentation can be done with this endogeneous "wild yeast" (Ross, 1997), but this procedure gives unpredictable results, which depend upon the extract types of yeast species present. For this reason, a pure yeast culture is usually added to the must; this yeast quickly dominates the fermentation. The wild yeasts are repressed, which ensures a reliable and predictable fermentation (Gonzalez*et al.*, 2001).

Most added wine yeasts are strains of *S. cerevisiae,* though not all strain of the species are suitable (Gonzalez*et al.*,2001). Different strains of*S. cerevisiae*yeast have different physiological and fermentative properties, therefore the actual strain of yeast selected can have a direct impact on the finished wine (Dunn*et al*., 2005). Significant research has been undertaken into the development of novel wine yeast strains that produce a typical flavor profile or increased complexity in wines (Mcbryde *et al.*, 2006).

The growth of some yeast, such as *Zygosaccharomyces* and *Brettanomyces*, in wine can result in wine faults and subsequent spoilage (Loureiro and Malfeito-ferreira, 2003).

Brettanomyces produces an array of metabolites when growing in wine, some of which are phenolic compounds. Together, these compounds are often referred to as "*Brettanomyces character",* and are often described as "antiseptic" or "barnyard" type aromas.

Researchers from University of British Columbia, Canada, have found a new strain of yeast that has reduced amines. The amines in red wine and Charonnay produce off-flavours and cause headaches and hypertension in some people. About 30% of people are sensitive to biogenic amines, such as histamines (Anon, 2011a).

2.6.2 Baking(Bread and related products)

Baker's yeast is the common name for the strains of yeast commonly used as a leavening agent in baking bread and related products, where it converts the fermentable sugars present into carbon(iv)oxide and ethanol. The gas causes the dough to expand or rise as it forms pockets or bubbles. When the dough is baked, the yeast dies and the air pockets "sets", giving the baked product a soft and spongy texture. The use of potatoes, water from potato boiling, eggs or sugar in a bread dough accelerates the growth of yeasts. Salt and fats such as butter slow down yeast growth. The majority of the yeast used in baking is *Saccharomyces cerevisiae,* the same species

commonly used in alcohol fermentation. Additionally, *Saccharomyces exigus (S. minor)*awild yeast found on plants, fruits and grains, is occasionally used for baking. Sugar and vinegar provide the best conditions for yeast to ferment. In bread making, the yeast initially respires aerobically, producing carbodioxide and water. When the oxygen is depleted, anaerobic respiration begins, producing ethanol as a waste product; however, this evaporates during baking (Moore, 1996).

It is not known when yeast was first used to bake bread. The first records that show this use came from Ancient Egypt (Legras *et al*., 2007). Researchers speculate a mixture of flour meal and water was left longer than usual on a warm day and the yeasts that occur in natural contaminants of the flour caused it to ferment before baking. The resulting bread would have been lighter and tastier than the normal flat, hard cake.

A weak solution of water and sugar can be used to determine if yeast is expired. In solution, active yeast will foam and bubble as it ferments the sugar into ethanol and carbon(iv)oxide. Some recipes refer to this as proofing the yeast as it "proves" (tests) the viability of the yeast before the other ingredients are added. When using sour dough starter, flour and water are added instead of sugar; this is referred to as proofing the sponge(Legras *et al* ; 2007). When yeast is used for making bread, it is mixed with flour, salt and warm water or milk. The dough is kneaded until it is smooth, and then left to rise, sometimes until it has doubled in size. Some bread doughs are knocked back after one rising and left to rise again. A longer rising time gives a better flavor, but the yeast can fail to raise the bread in the final stages if it is left for too long initially. The dough is shaped into loaves, left to rise until it is the correct size, and then baked.

2.6.2.1 Types of baker's yeast

Baker's yeast is available in a number of different forms. Each version has certain advantages over the others, the choice of which form to use is largely a question of the requirement of the recipe at hand and the training of the cook preparing it.

Cream yeast:Cream yeast is the closest form to yeast slurries of the 19th century, being essentially a suspension of yeast cells in liquid, siphoned off from the growth medium. Its primary use is in industrial bakeries with special high-volume dispensing and mixing equipments, and it is not readily available to small bakeries or home cooks (Brookline, 2004).

Compressed yeast: This is essentially cream yeast with most of the liquid removed. It is best known in the form of cake yeast, which is essentially soft solid, beige in color, but is also available in crumbed form for bulk usage. It is highly perishable; though formally widely available for the consumer market, it has become less common in supermarkets in some countries due to its poor keeping properties, having been obsoleted in such markets by active dry and instant yeast. It is still widely available for commercial use, and is somewhat more tolerant of low temperatures than other forms of commercial yeast; however, even there, instant yeast has made significant market in- roads (Brookline, 2004).

Active dry yeast:This is the form of yeast most commonly available to non–commercial bakers as well as the yeast of choice for situations where long travel or uncontrolled storage conditions are likely. It consists of coarse oblong granules of yeast, with live yeast cells encapsulated in a thick jacket of dry, dead cells with some growth medium. Under most conditions, active dry yeast must be proofed or rehydrated first and, despite its better keeping qualities than other forms, is generally considered more sensitive when actually used in recipes, Active dry yeast also provides an alternative to butter and salt for seasoning pop corn. (Brookline, 2004).

Instant yeast:This form of baker's yeast appears similar to active dry yeast, but has smaller granules with live cells. It is more perishable than active dry yeast, but also does not require rehydration, and can usually be added directly to all but the driest dough. Instant yeast generally has a small amount of ascorbic acid added as preservative. Some producers provide two or more form of instant yeast in their product portfolio; for example Lesaffre's "SAF instant Gold" is described specifically for dough with high sugar contents (Brookline, 2004).

Rapid-rise yeast:This is a variety of yeast (usually a form of instant yeast) designed to provide greater carbon(iv)oxide output to allow faster rising at the expense of shortened fermentation times. There is considerable debate as to the value of such a product: while most baking experts believe it reduces the flavor potential of the finished product, others, feel that at least for direct-rise recipes, it makes little difference (Bookline,2004). Rapid-rise yeast is often marketed specifically for use in bread machine.

Flake Yeast:This is dead yeast, sold primarily as a nutritional supplement. It has little/no leavening power.

2.6.3 Industrial ethanol production

Fermentative yeasts produce their energy by converting sugar into ethanol (alcohol) and carbon(iv)oxide. This ability of yeast to convert sugar into ethanol has been harnessed by the biotechnology industry to produce ethanol fuel. The process starts by milling a feedstock, such as sugar cane, field corn, or other cereal grains, and then adding dilute sulfuric acid, or fungal alpha amylase enzymes,to break down the starches into complex sugars.A glucoamylase is then added to break the complex sugars down into simple sugars. After this, yeasts are added to convert the

simple sugar to ethanol, which is then distilled off to obtain ethanol up to 96% in concentration (Anon, 2009a). *Saccharomyces* yeasts have been genetically engineered to ferment xylose one of the major fermentable sugars present in cellulosic biomasses, such as agriculture residues, paper wastes, and wood chips (Ho *et al*., 1998; Brat *et al.*,2009).Such a development means ethanol can be efficiently produced from more inexpensive feedstock, making cellulosic ethanol fuel a more competitively priced alternative to gasoline fuels (Siegfried, 2009).

2.6.4 Bioremediation

Some yeasts can find potential application in the field of bioremediation. One of such yeast, *Yarrowia lipolytica*, is known to degrade palm oil mill effluent, (Oswal *et al.*, 2002),TNT (Tri Nitro Toluene, an explosive material) (Jain *et al*., 2004) and other hydrocarbons, such as alkanes, fatty acids, fats and oils. (Fickers *et al.*, 2005). It can also tolerate high concentrations of salt and heavy metals, (Bankar *et al.*, 2009), and is being investigated for its potential as a heavy metal biosorbent (Bankar *et al.*, 2009).

2.6.5 Non alcoholic beverages

Root beers and other sweet carbonated beverages can be produced using the same methods as beer, except the fermentation is stopped sooner; producing carbon(iv)oxide, but only trace amounts of alcohol and significant amount of sugar is left in the drink. **Kvass,** a fermented drink made from rye, is popular in Eastern Europe; it has a recognizable, but low alcoholic content. Yeast in symbiosis with acetic acid bacteria is used in the preparation of **Kombucha**, a fermented sweetened tea. Species of yeast found in tea can vary, and may include: *Brettanomyces bruxellensis, Candida stellata, Schizosaccharomyces pombe, Torulaspora delbrueckii* and *Zygosaccharomyces bailii* (Teoh *et al.*, 2004). Kombucha is a popular beverage

in Eastern Europe and some former soviet republics under the name **chainyi grib,** which means "tea mushroom". **Kefir** and **kumis** are made by fermenting milk with yeast and bacteria (Lopitz *et al.*,2006).

2.6.6 Nutritional supplements

Yeast is used in nutritional supplements popular with vegans and the health conscious, where it is often referred to as "nutritional yeast". It is a deactivated yeast, usually *S. cerevisiae.* It is an excellent source of protein and vitamins, especially the B-complex vitamins whose functions are related to metabolism, as well as other minerals and cofactors required for growth. It is also naturally low in fat and sodium. Some brands of nutritional yeast, though not all, are fortified with vitamin B_{12}, which is produced separately by bacteria. Nutritional yeast, though it has a similar appearance to brewer's yeast,is very different and has a very different taste. Brewer's yeast is a good source of B-complex vitamins but, contrary to some claims, it contains little or no vitamin B_{12}(Anon, 2011a). Nutritional yeast has a nutty, cheesy flavor which makes it popular as an ingredient in cheese substitutes. It is often used by vegans in place of Parmean cheese. Another popular use is as a topping for popcorn. It can also be used in mashed and fried potatoes, as well in scrambled eggs. It comes in the form of flakes, or as a yellow powder similar in texture to cornmeal, and can be found in the bulk aisle of most natural food stores. In Australia, it is sometimes sold as "savory yeast flakes" Though "nutritional yeast" usually refers to commercial products; inadequately fed prisoners have used "home-grown" yeast to prevent vitamin deficiency (Lee,2009).

2.6.7 Probiotics of yeasts

Some probiotics supplements use the yeast *S. boulardii* to maintain and restore the natural flora in the gastrointestinal tract. *S. boulardii* has been shown to reduce the symptoms of acute diarrhoea in children (Centina and Sierra, 1994; Kurugol and Koturoglu, 2005); prevent re-infection of *Clostridium difficile*(Mcfarland *et al.*,1994); reduce bowel movements in diarrhoea predominant IBS patients (Maupas *et al.,* 1983) and reduce the incidence of antibiotics, (Mcfarland *et al.,* 1995), travelers (Kollaritsch *et al.,* 1989) and HIV/AIDS (Saint-marc*et al.,* 1995) associated diarrhoeas.

2.6.8 Aquarium hobbyof yeasts

Yeast is often used by aquarium hobbyists to generate carbon(iv)oxide (CO_2) to nourish plants in planted aquariums (Pedersen *et al.,* 2007). A homemade set up is widely used as a cheap and simple alternative to pressurized CO_2 systems. While not as effective as these, homemade setup is considerable cheaper for less demanding hobbyists. There are several recipes for homemade CO_2, but they are variations of the basic recipe: Baker's yeast, with sugar, baking soda and water, are added to a plastic bottle. A few drops of vegetable oil at the start reduce surface tension and speeds the release of CO_2.

This will produce CO_2 for about 2 or 3 weeks; the use of a bubble counter determines production. The CO_2 is injected in the aquarium via a narrow hose and released through a diffuser that helps dissolve the gas in the water. The CO_2 is used by plants in their photosynthesis.

2.6.9 Yeast extract

Yeast extract is the common name for various forms of processed yeast products that are used as food additives or flavours. They are often used in the same way monosodium glutamate (MSG) is used, and like MSG, often contain free glutamic acid. The general method for making yeast extract for food products such as vegemite and marmite on a commercial scale is to add salt to a suspension of yeast making the solution hypertonic, which leads to the cell shriveling up. This triggers autolysis where the yeast's digestive enzymes break their own proteins down into simpler compounds, a process of self-destruction. The dying yeast cells are then heated to complete their breakdown, after which the husks (yeast with thick cell walls which would give poor texture) are separated.The yeast autolysates are used in "Vegemite" and "promite" (Australia); "Marmite","Bovril" and "Oxo" (the United Kingdom, Republic of Ireland and South Africa); and Cenovis (Switzerland)(Pedersen,*et al*,2007).

2.6.10 Food spoilage

Yeasts are able to grow in foods with low level of pH (5.0 or lower), water activity and in the presence of sugars, organic acids and other easily metabolised carbon sources (Kurtzman, 2006). During their growth, yeasts metabolise some food components and produce metabolic end products. This causes the physical, chemical, and sensory properties of a food to change, and the food is spoiled. (Fleet and Praphailong, 2001).The growth of yeast within food products is often seen on their surface as in cheese or meats or by the fermentation of sugars in beverages, such as juice, and semi liquid products, such as syrups and jams (Kurtzmann, 2006). The yeast of the *Zygosaccharomyces* genus has had a long history as spoilage yeast within the food industry. This is mainly due to the fact that these species can grow in the presence of high sucrose, ethanol, acetic acid, ascorbic acid, benzene acid, and sulfur dioxide concentrations (Loureiro and

Malfeito, 2003).These are some of the chemical agents commonly used food preservation methods.

Other preservative resistant spoilage yeasts include *Candida krusei, Pichia membranifaciens, Schizosaccharomyces pombe* and certain strains of *Saccharomyces cerevisiae* (Pit and Hocking, 1997).

2.6.11 Pathogenic yeasts

Some species of yeast are opportunistic pathogens which can cause infection in people with compromised immune systems.

Filobasidiella neoformans is a significant pathogen of immunocompromised people causing the disease termed *Cryptococcosis*. This disease occurs in about 7-9% of AIDS patients in the USA, and a slightly smaller percentage (3-6%) in Western Europe (Anon, 2006a). The cells of the yeast are surrounded by a rapid polysaccharide capsule, which helps to prevent them from being recognised and engulfed by white blood cells in the body.

Yeasts of the *Candida* genus are another group of opportunistic pathogens which causes oral and vaginal infection in humans, known as Candidiosis. *Candida* is commonly found as commensal yeast in the mucus membranes of humans and other warm-blooded animals. However, sometimes these same strains can become pathogenic. Here the yeast cells sprout a hyphal outgrowth, which locally penetrate the mucosal membrane, causing irritation and shedding of the tissues. (Anon, 2006b).The pathogenic yeasts of Candidiasis in probable descending order of virulence for humans are: *C. albicans, C. tropicalis, C. stellatoidea, C. glabrata, C. krusei, C. parapsilosis, C. guilliermondii, C. viswanathii, C. lusitaniae* and *Rhodotorula mucilaginosa.* (Hurley, *et al.,* 1987).*Candida glabrata* is the secoundary most common Candida pathogen after

C. albicans, causing infections of the urogenital tract, and of the blood stream (Candidemia) (Stoyan and Carbon, 2004).

2.6.12 Science of yeasts

Several yeasts, particularly *S. cerevisiae*have been widely used in genetics and cell biology. This is largely because *S. cerevisiae is* a simple eukaryotic cell, serving as a model for all eukaryotes, including humans, for the study of fundamental cellular processes such as the cell cycle, DNA replication, recombination, cell division and metabolism. Also, yeasts are easily manipulated and cultured in the laboratory, which has allowed for the developments of powerful standard techniques, such as yeast two-hybrid, synthetic genetic array analysis and tetrad analysis. Many proteins important in human biology were first discovered by studying their homologues in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes.

On 24th April, 1996, *S. cerevisiae*was announced tobe the first eukaryote to have its genome, consisting of 12 million base pairs, fully sequenced as part of the Genome Projects (Williams, 1996). At the time, it was the most complex organism to have its full genome sequenced, and it took seven years and the involvement of more than hundred laboratories to accomplish (Henahan, 2009). The second yeast species to have its genome sequenced was *Schizosaccharomyces pombe,* which was completed in 2002 (Wood *et al.,* 2002). It was the sixth eukaryotic genome sequenced and consists of 13.8 million base pairs.

2.7 Characteristics of Brewing Yeasts

According to Hutkins (2006), brewing yeasts are drastically different from the yeast strains found in a typical laboratory(Table 2.1). They differ physiologically, biochemically, and genetically, and these differences play a significant role in beer fermentation (Table 2.2). Two

genera of yeast are commonly used in the brewing industry, each responsible for one of the major categories of beer i.e. ale and lager. Ale is made using the top fermenting yeast, *S. cerevisiae*, while lager beer is produced by bottom fermenting Yeast, *S. pastorians* (formally known as *S. carlbergensis*).

The major differential characteristics between ale and lager yeasts are their mode of flocculation, fermentation and the temperature required for fermentation processes. Top or bottom fermentation does not mean that the yeast is confined to a specific area of the fermentor. Rather, upon growth, top-fermenting yeasts tend to form low density lumps or flocculation that trap carbon(iv)oxide and rise to the surface.Lager yeast, in contrast, flocculates and settles to the bottom of the fermentor. Ale Yeasts typically ferment from 18 - 27^0C, whereas lager yeast ferment around or below 15^0c. For this reason, ales are historically more prominent in warmer region such as Great Britain, and lager yeast are more common in colder region such as Germany.In Nigeria,lager yeast is more commonly used for the production of lager beer for example *S.pastorians*(formally known as *S.carlbergensis*).

Table 2. 1:Differences between Laboratory and Brewing Strains of Yeast (*Saccharomyces*)

Laboratory Strains	**Brewing strains**
Haploid	Polyploid and Aneuploidy
Sporulating	Sporulate poorly
Spore viable	Spores mostly non-viable
Able to mate (δ and α)	Mating rare

(Source:Hutkins,2006)

Table 2. 2: Differencesbetween Ale and Lager Yeast

Saccharomyces cerevisiae	***Saccharomyces pastorianus* (lager)**
Flocculated Yeast rises to the top	Flocculated Yeast settles to the bottom
Optimum growth temperature greater than 30^0C	Optimum growth temperature less than 30^0C
Minimum growth temperature is 15^0C	Minimum growth temperature is 7^0C
Maximum growth temperature is 40^0C	Maximum growth temperature is 34^0C
Cannot metabolise melibiose	Able to metabolise melibiose
Slow assimilation of maltotriose	Efficient assimilation of maltotriose
Sporulating (at low frequency)	Non-sporulating

(Source:Hutkins,2006)

There is much diversity in beer despite the use of only two major (ale or lager) categories. Both ale and lager have colors ranging from pale to black, alcohol content ranging from1% to 10% (v/v), and both can be lightly or strongly flavoured. Ale brewing strains constitute a broad spectrum of *Saccharomyces* strains, some of which are similar to laboratory strains. Lager yeast strains are more conserved and are likely to be closely related to the first lager strains, *S carlbergensis* CBS1513 (IFO11023), which was discovered in 1883 by Carlsberg's laboratory (Kodama *et al.,* 2005). Yeasts are a rich source of the vitamin B complex, especially thiamine (vitamin B1), riboflavin (vitamin B2), and nicotinic acid (niacin). Yeasts also contain ergosterol or pro-vitamin D which is converted to commercial vitamin D by exposure to ultra-violet light (Neiman,2005).

2.7.1 Physiological characteristics of brewer's yeast

The fundamental physiological characteristics of brewer's yeast is their ability to degrade carbohydrates, usually six-carbon molecules (C_6) and some diasaccharides such as sucrose and maltose into two-carbon (C_2) component like ethanol without completely oxidizing them to carbon(iv)oxide, even in the presence of oxygen. Yeast which produces or accumulates ethanol in the presence of oxygen is called crab tree-positive yeast. The crab tree effect is an alcoholic fermentation where the degradation of hexose sugars in the presence of oxygen is due to insufficient capacity, saturation or pyruvate overflow. Thus during ethanol production, the energy for growth is provided by the glycolysis and fermentation pathways rather than by the respiration pathways. In the case of *S. cerevisiae,* the crab tree effect relies largely on a glucose repression circuit, which in the presence of glucose or other carbohydrate, represses respiration (Piskur *et al.,* 2006).

2.8 Nutritional Requirements of Brewer's Yeast

Although yeast can assimilate ethanol, the main nutrient classes impacting brewing yeast performance are carbohydrates and nitrogenous compounds. Brewing strains of *Saccharomyces* can utilise various carbohydrates (glucose, sucrose, fructose, maltose, galactose, raffinose and maltotriose), with a major distinguishing characteristic of lager yeast being the ability to ferment melibiose. The uptake of carbohydrate follows the simplest route i.e. monosaccharides (glucose and fructose) are utilised first, followed in order of complexity by disacharides (maltose) and trisaccharides (maltotriose), excluding larger dextrins (glucose chains longer than three monomers are refered to as dextrins and commonly given the annotation, "DP" such as DP4 and so on) (Lodolo *et al.,* 2008).

2.8.1 Carbohydrate compounds

2.8.1.1 Monosaccharide

The monsaccharides are transported across the cellular membrane by common membrane transporter which facilitates diffusion and active transport process. Among the four putatively identified for glucose uptake, the most important glucose uptake system recognised are low affinity (constitutively expressed) and a high affinity transporter, which is repressed in the presence of high glucose concentrations (Bisson *et al.,* 1993). Catabolite repression (the high affinity transporter) is a trait only found in fermentative yeast strains.

2.8.1.2 Disaccharides

The disaccharide maltose, which accounts for 50-55% of the total carbohydrates found in wort, has an uptake mechanism that involves two systems (Stewart, 2006). These include an energy-

dependent maltose permease (ATP◄~~ADP~~►P)system which transports the maltose intact across the cellular membrane and a maltose (α-glucosidase)system which hydrolyses maltose internally to yield two glucose units.

2.8.1.3 Trisaccharides

The trisaccharide, maltotriose, accounts for 10-14% of the total carbohydrate in wort and has an independent energy-dependent permease for intact transport, but shares α-glucosidase to hydrolyse maltotriose to three units of glucose. Thus three maltose utilisation genes (MAL genes) are involved in the operation of the high-affinity maltose transporter (Lodolo *et al.*, 2008).

2.8.2 Nitrogenous compounds

The main sources of nitrogen in wort are amino-acids, ammonium ion and some 'di and tripeptides' which originate from the malt used in wort production. In most wort, the free amino nitrogen (FAN) is utilised by yeast for protein formation (structural and enzyme) required for yeast growth and other functions such as osmoregulation. The level and composition of FAN in wort have a great impact on higher alcohol, ester, vicinal diketone (VDK), and on hydrogen sulphide (H_2S) formation due to the role of amino-acid metabolism in the formation of these flavours compound (Lodolo *et al.*, 2008). Two VDKs diacetyls (2,3-butanedione) and 2,3-pentanedione, are fermentation by- products that possess very low flavor threshold, 0.1 and 0.9 mg/L respectively, and impact a buttery flavor. VDKs are undesirable in most beers, especially lagers which are supposed to have a cleaner organoleptic profile, and these are caused by the biosynthesis of valine, leucine and isoleucine (Cyr*et al.*, 2007).

Aminoacids required by yeast are classified into four (4) groups, namely A, B, C and D which are utilised in a sequence that is independent of fermentation conditions (Pierce, 1987). The group A amino-acids (arginine, asparagines, aspartate, glutamate, glutamine, lysine, serine, and threonine) are utilised before group B amino-acids (histidine, Isoleucine, methionine and valine) (Perpete *et al.,* 2005).

Group C aminoacids (alanine, glycine, phenylalanine tyrosine and tryptophan) are only absorbed after the complete depletion of the group A amino acid while group D amino acid (proline) are poorly utilised because it has no free amino group and thus cannot be transmitted (Lodolo *et al.,* 2008).

2.8.3 Minerals (trace element)

Minerals (trace elements) are also necessary for adequate fermentation performance. Calcium availability is important in the multifunction roles as a second messenger in the modulation of growth and metabolic responses of yeast cells to external stimuli. Calcium is also required for yeast flocculation, which is commonly used to differentiate between ale and lager yeasts(Lodolo *et al,2008*).

Trace amounts of copper and iron are also necessary for growth; the elementsact as cofactors in several enzymes involved in the respiratory chain. Iron also serves as the building block for heme formation, and is involved in cellular redox homeostasis and oxidative stress resistance. Magnesium, the most abundant intracellular divalent cation;acts primarily as an enzyme cofactor while zinc is essential for the structure and function of over 300 enzymes including alcohol dehydrogenase (Lodolo *et al.,* 2008).

Furthermore, dissolved oxygen (DO) is necessary at the initial stage of a yeast fermentation process to achieve a balanced fermentation which results in the desired end products. Oxygen has many roles in yeast physiology and various genes are expressed based on the oxygen in the environment to regulate cellular metabolism (Lodolo *et al.,* 2008). The primary role of oxygen in the initial stages of fermentation is the generation of sufficient amounts of biomass to efficiently carry out the fermentation. Oxygen is also essential for biosynthesis of ergosterol and unsaturated fatty acid (UFAs).

Ergosterols and UFA's are integrated into the membrane bilayer structure of growing cells which are very important in the uptake of nutrients across the membrane barrier. Yeast denied ofan adequate oxygen supply will have inferior membrane with a reduced transport capability and a reduced ability to withstand osmotic stresses and high exterior ethanol levels. The most common way to provide oxygen for fermentation and to avoid negative metabolic processes is to aerate the wort or yeast slurry before pitching (Lodolo *et al.,* 2008).

2.9 Brewing Yeast Flavour Production

The biochemical pathways that are active during fermentation contribute significantly to the unique flavours associated with beer. Flavour compounds are intermediates in the pathways starting from the catabolism of wort compounds (Sugar, nitrogenous compounds and sulphur Compounds) to the synthesis of compounds necessary for yeast growth (amino acids, proteins, nucleic acids, lipids e.t.c). Yeast-derived flavour-active compounds include ethanol, carbon(iv)oxide (CO_2), carbonyls (aldehydes/ketones), higher alcohols, esters, fatty and organic acids, and sulphur compounds, with the primary by-products being ethanol and carbon(iv)oxide.

2.9.1 Aldehydes

Aldehydes are among the higher alcohol produced during fermentation either through anabolic or catabolic pathways (Lodolo *et al.*, 2008). Acetaldehyde is a major aldehydes and it act as an intermediate in the formation of ethanol and acetate which causes a "grassy" off-flavour at a threshold of 10-20mg/L(Cyr *et al*;2007).

2.9.2 Higher alcohol

Higher alcohol contributes to the overall beer flavour which is synthesised through two routes either anaerobic or catabolic. The anabolic pathway involves the synthesis of pyruvate from the wort sugars while the catabolic process is by the Ehrlich pathway, which involves the conversion of branched amino acids to higher alcohols by transamination, decarboxylation and reduction reactions (Lodolo *et al.*, 2008).

2.9.3 Esters

Esters are responsible for beer flavours such as fruity, (ethyl acetate), banana (isoamyl acetate) and apple/aniseed (ethyl caproate). Ester formation is linked to lipid metabolism, alcohol acetyl transferases (ATF genes) and cellular growth (Lodolo *et al.*, 2008).

2.9.4 Organic acids

Organic acids (pyruvate, citrate, malate, acetate, e.t.c.) impart sour flavours and contribute to pH reduction during fermentation. In particular α-acetolactate and α-acetohydroxy acids are interesting due to their roles as precursors of diacetyl and 2,3-pentanedione (Lodolo *et al.*, 2008).

2.9.5 Sulphur compounds

The primary sulphur components imparting beer flavour are sulphur dioxide (SO_2), hydrogen sulphide (H_2S), dimethyl sulphide (DMS), mercaptans, reduced sulphur (sulphur-containing amino acids such as cysteine and methionine), coenzymes (COA, biotin, thiamine and pyrophosphate) and other cellular metabolites (glutathione, sulphides and thiols) (Lodolo *et al.,* 2008).

2.10 Yeast flocculation

The magical art of yeast coming together, dropping to the bottom of a fermentor, is called flocculation. Flocculation is a well known example of natural, active and reversible aggregation of cells into flocs (Calleja, 1987). The ability of yeast cells to flocculate is very important in industrial processes like brewing or bioconversion. Yeast flocculation is an important process for the production of beer that causes the yeast to sediment to the bottom of the fermenter and used for the next fermentation, while the beer may be matured without the need of centrifugation step (Stewart and Russel, 1995). The aggregation of the cells in flocs generally occur during the late exponential or early stationary growth phase (Straver *et al.,* 1993). As a result, the majority of the cells are separated from the culture medium. However, an interesting yeast strain showed an inverse flocculation pattern (Strauss *et al.,* 2003).The variability in the occurrence of flocculation is a problem (Straver *et al*., 1993). Premature flocculation hampers complete fermentation of the growth medium, whereas, failure of the cell to flocculate at the end of fermentation requires the use of expensive techniques such as centrifugation or filtration to remove cells (Stratford, 1989,1991;Sadosky and Schawrz, 2002). Yeast flocculation is a complicated process that currently only partly understood. It requires the presence of at least two types of molecules on

the yeast cell surface. Mannans is one of the molecules (carbohydrate chains), which are the gene products of the MNN genes and are always present on the cell surface. The other type is flocculins (sugar binding proteins), which are the gene products of the FLO genes, that are activated only after depletion of nutrients (Speers *et al*., 2006)

2.10.1 Classification of brewer's yeast strains

Brewing strains may be classified in four classes according to their ability to flocculate (Hussain *et al*., 1986): Non flocculent strains (powdery yeasts, completely dispersed), Standard ale strains (flocculating into small, loose lumps late in fermentation).Highly flocculent yeasts (flocculating into dense masses late in fermentation and producing beer under attenuated and sweet) and Very highly flocculent yeasts (flocculating very early in fermentation owing to non-separation of daughter cells).

Yeast flocculation can also be classified according to Christopher (2006), as high, medium or low flocculatinggroup (Table 2.3). Ale yeast strains are found in each category, while lager yeasts are predominantly medium flocculators. A Hefeweizen strain is an example of a low flocculator.

Table 2. 3:Flocculation Degree of Brewing Strains

Flocculation degree	Notes/Descriptions
High flocculation	Start to flocculate by 3-5 days. Sometimes need to rouse the yeast. High level of diacetyl, lower attenuation and good for malty ales.
Medium flocculation	Start to flocculate by day 6-15. Ideal for ales, clean, balanced flavor production and it is also called "Powdery".
Low flocculation	Not start to flocculate by day 15. Most wild yeasts are low flocculators, good for Henfeweizen, Belgians and make a headache for filtering.

(Source:Christopher, 2006)

High flocculation yeast strains will begin to clump in 3-5 days, and drop to form a compact yeast cake at the bottom of the fermentor. The yeast will often need to be "roused" back into the beer by gently swirling the carboy, or with a commercial fermentor. High flocculation is usually associated with low attenuation and increased level of diacetyl and esters (Christopher, 2006).

Medium flocculators tend to produce "cleaner" beers. They stay in suspension longer, and reduce diacetyl and esters to a greater degree. The attenuation is also greater. In a brewery, they are slightly more difficult to work with than high flocculators because of the increased work and filtering materials required. Medium flocculators will flocculate best at cold temperature, where high flocculators can flocculate well at 18^0C (Christopher, 2006).

Low flocculators are rarely used in brewing. This is because they do not settle out of the beer; they create filtering problems and haze. But some beers are intended to have yeast in suspension. For example, German Hefeweizans and Belgian Wit beers are fermented with yeast strains that do not flocculate well, leaving the desired cloudy appearance. The more contact the yeast has with the beer, the better able the yeast is at reducing the diacetyl and ester level (Christopher, 2006).

Low temperatures promote flocculation. More yeast will drop out of solution at 4^0C than 21^0C and more yeast will drop out at 3^0C compared to 4^0C. Some yeast strains require two weeks at 4^0C to completely clear. The flocculation character of a yeast strain has a direct impact on flavor and performance. Highly flocculent yeast generally is less attenuative. The ideal yeast strain would be highly flocculent, with good attenuation, and provide the desired flavour contribution (Christopher, 2006).

2.10.2 Factors affecting flocculation

A number of factors influencing flocculation process have been reported (Jin and Speers, 1998). The genetic aspects have been recognised since 1950's (Esser *et al.,* 1987), and flocculation is under genetic control (Statford, 1996; Teusissen and Steensma, 1995; Hidekatsu *et al*., 2001). The yeast cell wall, the cell age, pitching rate of the yeast, temperature and other factors of the growth medium can influence the process (Soares *et al.,* 1994; Christopher, 2006).

The flocculation depends on brewery conditions (Johnson *et al.,* 1988) and the nature of the strain used (Statford,1996; Versteepen *et al.,* 2000). It is well established that the wort components influence the ability of the yeast cells to flocculate (Benabdesselam,2010). The kinetic approach shows that the calcium content, for example, has influence on the flocculation process (Stan, 2002), the original gravity and initial oxygen content of the wort (Christopher, 2006), and premature flocculation of yeast is induced by some wort constituents (Benabdesselam,2010).

2.11 Yeasts Species Associated with Orange Juice

Typical yeast species found in citrus juices are *Candida parapsilosis, Candida stella, Saccharomyces cerevisiae, Torulaspora delbrucekii, Saccharomycopsis crataegensis,* and *Zygosaccharomyces rouxii,* although species from the genus *Rhodotorula, Pichia, Hanseniaspora and Metschnikowa* are also common (Hatcher *et al*., 2000).

Yeast species in orange juice have been identified by some workers from a variety of citrus juice source. Distribution of these species was considerably different depending on the type of the sample. Fourteen different species have been identified from pasteurized single-strength orange

juice that had been contaminated after pasteurization (PSOJ), while only six species were found in fresh-squeezed, unpasteurized orange juice (FSOJ). Among PSOJ isolates, *Candida intermedia* and *Candida parapsilosis* were the predominant species, *Hanseniaspora occidentalis* and *Hanseniaspora uravum* representing up to 73% of the total FSOJ isolates (Covadonga *et al.*, 2002).Others include *Clavisapora lusitaniae,* and *Geotricum citri-aurantii* using restriction pattern of the internal transcribed spacer region (5.8S-ITS) analysis.

2.12 Ethanol and Its Properties

Ethanol or ethyl alcohol, has been described as one of the most exotic synthetic oxygen-containing organic chemicals because of its versatility as a chemical intermediate for other organic chemicals. Ethanol under ordinary condition is a volatile, flammable, clear, colorless liquid. Its odor is pleasant, familiar, and characteristic, as is its taste when it is suitably diluted with water. The physical and chemical properties of ethanol are primarily dependent upon the hydroxyl group. This group imparts polarity to the molecule and also gives rise to intermolecular hydrogen bonding. In the liquid state, hydrogen bonds are formed by the attraction of the hydroxyl hydrogen of one molecule and the hydroxyl oxygen of a second molecule (Najafpour and Lim, 2002).

The effect of this bonding is to make liquid alcohol behave as though it were largely dimerised. This behaviour is analogous to that of water, which however, is more strongly bonded and appears to exist in liquid clusters of more than two molecules.

The chemistry of ethanol is largely due to the hydroxyl group, namely, reactions of dehydration, dehydrogenation, oxidationsand esterification. The hydrogen atom of the hydroxyl group can be

replaced by an active metal, such as sodium, potassium, and calcium, to form a metal ethoxide (ethylate) with the evolution of hydrogen gas (Najafpour and Lim, 2002).

2.12.1 History of ethanol production

Ethanol is not a new product (fuel). In the 1850's, nearly 90 million gallons were produced every year and it was a major lighting fuel. During the Americiancivil war, a liquor tax was placed on ethanol to raise money for the war. The tax increased the price of ethanol so much that it could no longer compete with other fuels such as kerosene in lighting devices and it disappeared from the market.

In 1906, the Federal Liquor Tax in America,was repealed and ethanol became competitive as fuel. In 1908, Henry Ford designed his model T ford to run on a mixture of gasoline and alcohol, calling it the fuel of future. During World War 1, the use of ethanol increased rapidly and, by the end of the war, production had risen to 50 million gallons a year. It was used not only as a fuel, but in the manufacture of war materials as well (Bernton *et al.*, 1982).

In 1919, the ethanol industry received another blow when the era of prohibition began in America. Since ethanol was considered liquor, it could only be sold when it was mixed with petroleum to make it undrinkable. In a process called denaturing, ethanol was rendered poisonous by the addition of 35% petroleum components (Bernton *et al*., 1982).

By the 1920's, ethanol was no longer thought of as an alternative to gasoline; it was considered a gasoline extender or octane enhancer that boosts the power of a car's engine. With the end of prohibition in 1933, interest in the use of ethanol as fuel was revived. During World War II, production of ethanol rose dramatically to 600 million gallons a year. While some ethanol was

used as fuel, most was used in the production of synthetic rubber, since supplies of natural rubber had been cut off by the war in Asia (Bernton *et al*., 1982).

In the 1970's, interest in ethanol as a transportation fuel was revived when embargoes by major oil producing countries cut gasoline supplies. Since that time ethanol use has been encouraged by offering tax benefits for producing ethanol into gasoline.In 1988, ethanol began to be added to gasoline for the purpose of reducing carbon monoxide emissions.

2.12.2 Uses of ethanol

2.12.2.1 Transportation fuel and agriculture

Ethanol can be used as a total or partial replacement for gasoline. Gasoline containing ten percent ethanol-E10-is used in many urban areas that donot meet clean air standards. Some states promote more widespread use of E10. Minnesota, for example, required almost all gasoline sold in the state to contain ethanol. All vehicles that run on gasoline can use E10 without making changes to their engines. Over 99% of the ethanol produced in the United States is mixed with gasoline to make E-10 (Anon, 2007a).

Ethanol can be used in other types of engines. Small, four cycle gasoline engines, for example, found in small scale agricultural equipment (e.g. tillers small tractors) can often burn 80 to 85 percent ethanol as a direct replacement for gasoline (Anon, 2007a).

E85 is an alternative fuel that is about 85% ethanol and 15% gasoline used mainly in the mid west and south of the United States. Ethanol can be used in supercharged diesel engines for up to about 25% of the total fuel. This is done by carrying the ethanol in a separate fuel tank and injecting it into the diesel engine through a super charger air stream. Ethanol can also replace

aviation fuel in aircraft engines. Ethanol is a renewable fuel because it is made from plant(Anon, 2007a).

Ethanol can be used in a variety of cooking, heating, and lighting appliances. In some cases, ethanol can be used in modified appliances designed for conventional fuels. In other cases, appliances designed specifically for ethanol fuel are required (Anon, 2007a).Ethanol is alsouseful as a solvent for many substances and in making perfumes,paints, lacquers and explosives.

2.12.2.2 Ethanol and the environment

Unlike gasoline, ethanol is non-toxic (safe to handle) and biodegradable; it quickly breaks down into harmless substances if spilled. Ethanol reduces carbon monoxide and other toxic pollution from the tailpipes of vehicles, making the air cleaner. It keeps engine running smoothly without the need for lead or chemical additives. Because ethanol is made from crops that absorb carbon dioxide and give off oxygen, it has the potential to reduce greenhouse gas emissions and helps maintain the balance of carbon dioxide in the atmosphere. This process is called the carbon cycle(Anon, 2007a).

2.13 Ethanol Production

There are several ways to produce alcohol (ethanol) from various plant forms of biomass. The two most commonly used processes involve using yeast to ferment the sugar and starch in the feedstock (sorghum or wheat) to create ethanol. This is how wine, beer & liquor are made. Cider for example, is made by fermenting apple juice.

Ethanol demand is growing as a “clean” substitute for direct use as fuel, which can ease both natural resources limitation and environmental pollution (Roehr, 2001).

Ethanol is made by fermenting the sugars from a variety of agricultural products such as grains (barley, sunflower, wheat, sorghum) , as well as potato wastes, rice straw, cheese whey, corn flour, molasses, fruit, saw dust, urban waste, and yard clippings(Roehr, 2001). The many and varied raw materials used in the manufacture of ethanol via fermentation are conveniently classified under three types of agricultural raw materials: sugar, starches and cellulose material. Sugar (from sugar cane, sugar beets, molasses, fruits) can be converted to ethanol directly. Starches (from grains, potatoes, root crops) must first be hydrolysed to fermentable sugars by the action of enzymes from malt or mold. Starch-rich grains such as maize and sorghum are viable renewable resources for ethanol production (Turhollow and Heady, 1986; Lezinous *et al.,* 1995; Dien *et al.,* 2002).

Cellulose from (wood, agricultural residues, waste sulfite liquor from pulp and paper mills) must likewise be converted to sugars, generally by the action of mineral acids.Once simple sugars are formed, enzymes from yeast can readily ferment them to ethanol.

2.13.1 Sugar

The most widely used sugar for ethanol is black strap molasses which contains about 35-40 wt% of non-sugar solids. Blackstrap (Syrup) is collected as a by-product of cane manufacture. The molasses is diluted to a mash containing 10-20 wt% sugar. After the pH of the mash is adjusted to about 4-5 with mineral acid, it is inoculated with the yeast and the fermentation is carried out non-aseptically at 20-32^{o}C for about 1-3 days. The fermented beer, which typically contains 6-10 wt% ethanol, is then sent to the product recovery in purification section of the plant (James, 2006).

2.13.2 Starches

All potable alcohol and most industrial alcohol is currently made principally from grains. Fermentation of starch from grain is somewhat more complex than fermentation of sugars because starch must first be converted to sugar and then to ethanol. Starch is converted enzymatically to glucose either by diastase present in sprouting grain or by fungal amylase. The resulting dextrose is fermented to ethanol with the aid of yeast, producing carbon(iv)oxide as co-product. A second co-product of unfermented starch, fibre, protein and ash known as distillers grain (a high protein cattle feed) is also produced (James, 2006).

2.13.3 Cellulosic materials

This is a new experimental process which breaks down cellulose in woody fibers and is called "cellulosic ethanol". With this process ethanol can be made from trees, grasses and crop waste. Trees and grasses need less energy than grains, which must be replanted every year. Scientists have developed fast-growing trees that grow to size(i.e mature) in ten years. Many grasses can produce two harvests a year for many years (Anon, 2002b).

Each step in the process of the conversion of cellulose to ethanol proceeded with 100% yield; almost two-thirds of the mass would disappear during the sequence, most of it as carbon(iv)oxide, in the fermentation of glucose to ethanol.

This amount of carbon(iv)oxide leads to a disposal problem rather than to a raw material credit. Another problem is that the aqueous acid used to hydrolyse the cellulose in wood to glucose and other simple sugars destroys much of the sugars in the process. However, one way of making cellulose waste more susceptible to hydrolysis is by subjecting them to a short burst of high

energy elecstron beam radiation. An alternative to acid hydrolysis is the use of enzymes(Anon,2002b).

2.14 Fermentation

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, and using an endogenous electron acceptor, which is usually an organic compound (Klein, *et al.,* 2004). In contrast, respiration is where electron is donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (Adenosine triophosphate) by glycolysis. During fermentation, pyruvate is metabolised to various different compounds. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the conversion of pyruvate into ethanol and carbon(iv)oxide; and heterolactic fermentation is the production of lactic acid as well as other acids and alcohols. Fermentation does not necessarily have to be carried out in an anaerobic environment. Yeasts cells for example, greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (Dickinson, 1999).

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon(iv)oxides. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid (Voet,*et. al*., 1995).

2.14.1 History of fermentation

The word fermentation is derived from the Latin verb **fervere** which means to boil. It is thought to have been first used in the late fourteenth century in alchemy, but only in a broad sense. It was not used in the modem scientific sense until around 1600 (Anon, 2011c)

The first solid evidence of the living nature of yeast appeared between 1837 and 1838 when three publications appeared by Cagniard de la tour, C., Swan,T. and Kuetzing, F., each of whom independently concluded as a result of microscopic investigations that yeast is a living organism that reproduces by budding. It is perhaps because wine, beer, and bread were each basic foods in Europe that most of the early studies on fermentation were done on yeasts, with which they were made. Some bacteria were also discovered; the term fermentation was first used in English in the late 1840's, but it did not come into general use until the 1870s, and then largely in connection with the new germ theory of disease (Anon, 2011c).

Louis Pasteur (1822-1895), during the 1850s, and 1860s, showed that fermentation is initiated by living organisms in a series of investigations (Anon,2011c).

In 1857, Pasteur, in a series of investigations, showed that fermentation is initiated by living organisms. In 1857, Pasteur showed that lactic acid fermentation is caused by living organisms (Anon, 2011d). In 1860, he demonstrated that bacteria cause souring in milk, a process formally thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization (Anon,2009c). In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, **Etudes sur la Bieve**, which was translated into English in 1879 as **Studies onFermentation** (Anon,2011e). He

defined fermentation (incorrectly) as “life without air”, but correctly showed that specific types of microorganisms cause specific types of fermentations and specific end-products.

Although showing fermentation to be the result of the action of the living microorganisms was a breakthrough, it did not explain the basic nature of the fermentation process, or prove that it is caused by the microorganisms that appear to be always present. Many scientists, including Pasteur, had attempted unsuccessfully to extract the fermentation enzyme from yeast (Anon,2011e). Success came in 1897 when the German Chemist Edward Buechner ground up yeast, extracted a juice from them, then found to his amazement that this “dead” liquid would ferment a sugar solution, forming carbon dioxide and alcohol much like living yeasts (Edward and Cornish, 1997). The “unorganized ferments” behaved just like the organized ones.From that time on, the term **enzymes** came to be applied to all ferments. It was then understood that fermentation is caused by enzymes that are produced by microorganisms (Lagerkvist, 2005).

2.14.2 Chemistry of fermentation

Fermentation products contain chemical energy (they are not fully oxidised), but are considered waste products, since they cannot be metabolised further without the use of oxygen (or other more highly-oxidised electron acceptors). A consequence is that the production of adenosine triophosphate (ATP) by fermentation is less efficient than oxidative phosphorylation, whereby pyruvate is fully oxidised to carbon-dioxide (Purves *et al.,* 2004).

2.14.3 Ethanol fermentation

Ethanol fermentation, also referred to as alcoholic fermentation, is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby

produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, ethanol fermentation is classified as anaerobic.

Ethanol fermentation (performed by yeast and some types of bacteria) breaks the pyruvate down into ethanol and carbon(iv)oxide.

Ethanol fermentation occurs in the production of alcoholic beverages (Brewing and wine-making), ethanol fuel, and in the rising of bread dough (Bread-making). Usually only one of the products is desired; in bread-making, alcohol is baked out, and, in alcohol production, the carbon(iv)oxide is released into the atmosphere or used for carbonating the beverage. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced.

Ethanol fermentation produces unharvested by-products such as heat, food for livestock, and water.

2.14.4 The chemical process of fermentation of glucose

The chemical equation below summaries the fermentation of glucose whose chemical formula is $C_6H_{12}O_6$ (Purves *et al.,* 2004). One molecule is converted into two ethanol molecules and two carbon(iv)oxide molecules:

$C_6H_{12}O_6$ + Zymase => $2C_2H_5OH + 2CO_2$
Glucose Ethanol Carbon(iv)oxide

C_2H_5OH is the chemical formula for ethanol.

Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules. This is known as gylcolysis (Stryer, 1975).

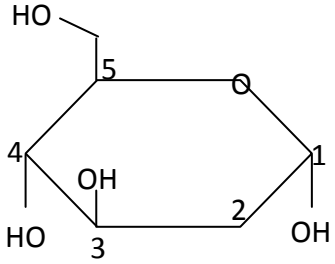
2.14.5 Glycolysis

To extract chemical energy from glucose, the glucose molecule must be split into two molecules of pyruvate (Berg and Cengage,2007). This process also generates two molecules of adenosine triophosphate as an immediate energy yield and two molecules of NADH (Anestis, 2006).The chemical equation of gylcolysis is summarised below:

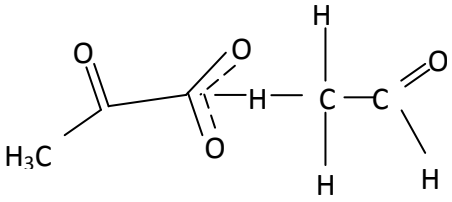
$$C_6H_{12}O_6 + 2+ 2ADP+ 2P_i+ 2NAD^+ \longrightarrow 2CH_3COCOO^- + 2ATP\ 2NADH + 2H_2O + 2H^+$$

Glucose — Adenosine diphosphate — Nicotinamide adenine dinucleotide — Pyruvate — Adnosine triophosphate — Nicotinamide adenine dinucleotide reduced form — water — Hydrogen ion

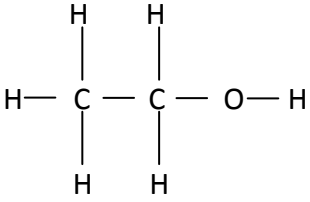
The chemical formula of pyruvate is CH_3COCOO^-. P_i stands for the inorganic phosphate. As shown by the reaction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.



Glucose depicted in Harworth Projection



Pyruvate



Acetaldehyde

Ethanol

2.14.6 Aerobic respiration

In aerobic respiration, the pyruvate produced by gylcolysis is further oxidised completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and are not required by facultative anaerobic organisms. In the absence of

oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways (Anestis, 2006).

2.14.7 Lactic acid fermentation

Lactic acid fermentation is the simplest type of fermentation. In essence, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces-transfers electrons to NAD^+, forming NADH. However there is only a limited supply of NAD^+ available in a cell. For glycolysis to continue, NADH must be oxidised-have electrons taken away-to regenerate the NAD^+. This is usually done through an electron transport chain in a process called oxidative phosphorylation; however, this mechanism is not available without oxygen ((Voet,*et al.,* 1995). Instead, the NADH donates its extra electrons to pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD^+ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate ((Voet,*et. al*., 1995).

In heterolactic acid fermentation, one molecules of pyruvate is converted to lactate: the other is converted to ethanol and carbon(iv)oxide. In homolactic acid fermentation, both molecules of pyruvate are converted to lactate the other is converted to ethanol and carbon(iv)oxide. In homolactic acid fermentation, both molecules of pyruvate are converted to lactate. Homolactic acid fermentation is unique because it is one of the only respiration processes that do not produce a gas as by product(Voet,*et. al*., 1995).

Homolactic fermentation breaks down the pyruvate into lactate. It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some

kinds of bacteria (such as lactobacilli) and some fungi. The lactobacilli, for example, convert lactose into lactic acid in yoghurt, giving it its sour taste. These lactic acid bacteria can be classed as homofermentative, where the end-product is mostly lactate, or heterofermentative, where some lactate is further metabolised and results in carbon(iv)oxide, acetate, or other metabolic products(Anestis, 2006).

The process of lactic acid fermentation using glucose is summarized below (Anestis, 2006). In homolactic fermentation, one molecule of glucose is converted to two molecules of lactic acid:

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Lactic acid}}{4CH_3CHOHCOOH}$$

In heterolactic fermentation, the reaction proceeds as follows: one molecule of glucose is converted to one molecule of ethanol, and one molecule of carbon(iv)oxide (Anestis, 2006).

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow[\text{Lactic acid}]{} \underset{\text{Ethanol}}{CH_3CHOHCOOH} + \underset{\text{Carbon(IV)oxide}}{C_2H_5OH} + CO_2$$

Before lactic acid fermentation can occur, the molecules of glucose must be split into two molecules of pyruvate. This process is called glycolysis (Berg and Cengage, 2007).

2.14.8 Hydrogen gas production in fermentation

Hydrogen gas is produced in many types of fermentation (Mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, glyoxylate fermentation), as a way to regenerate NAD^+ from NADH. Electrons are transferred to ferredoxin, which in turn is oxidisedby hydrogenase, producing hydrogen (Purves *et al.*, 2004). Hydrogen gas is a substrate

for methanogens and sulfate reducers, which keep the concentration of hydrogen sufficiently low to allow the production of such an energy-rich compound (Madigan *et al.,* 1996).

2.14.9 Effect of oxygen

The fermentation process does not require oxygen. If oxygen is present, some species of yeast (*Kluyveromyces lactis, Kluyveromyces lipolytica*) oxidise pyruvate completely to carbon(iv)oxide and water. This process is called respiration. Thus these yeasts produce ethanol only in an anaerobic environment.

However, many yeasts such as the commonly used baker's yeast *Saccharomyces cerevisiae*and *Schizosaccharomyces pombe,* prefer fermentation to respiration. These yeasts will produce ethanol even under aerobic conditions given the right sources of nutrition (Purves *et al.,* 2004).

2.14.10 Ethanol fermentation with yeast

The organisms of primary interest to industrial operations in fermentation of ethanol include *Saccharomyces cerevisiae, S. uvarum (S. carlbergenesis), Schizosaccharomyces pombe*, *Zymomonas mobilis,*and *Kluyveromyces* species.

Yeasts, under anaerobic conditions,metabolise glucose to ethanol primarily by way of the Embden-Meyerhof pathway. The overall net reaction involves the production of 2 molecules of ethanol, but the yield attained in practical fermentations however does not usually exceed 90-95% of theoretical. This is partly due to the requirement for some nutrient to be utilised in the synthesis of new biomass and other cell maintenance related reactions (Anon,2011a).

A small concentration of oxygen must be provided to the fermenting yeast as it is a necessary component in the biosynthesis of polyunsaturated fats and lipids. Typical amounts of oxygen maintained in the broth are 0.05-0.10mmHg oxygen tension.

The relative requirements for nutrients not utilised in ethanol synthesis are in proportion to the major components of yeast cells. These include carbon, oxygen, nitrogen and hydrogen. To lesser extent, quantities of phosphorous, sulfur, potassium, magnesium must also be provided for the synthesis of minor components. Minerals (that is manganese, cobalt, copper, zinc) and organic factors (amino acids, nucleic acids and vitamins) are required in trace amounts.

2.14.11 Industrial ethanol fermentation

Fermentation, one of the oldest chemical processes known to man, is used to make a variety of products including foods, flavourings, beverages, pharmaceuticals, and chemicals. The future of the fermentation industry, therefore, depends on its ability to utilize the high efficiency and specificity of enzyme catalysis to synthesis complex products and on its ability to overcome variations in quality and availability of raw materials (Najafpour and Lim, 2002).

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol is still made by this process. Simple sugars are the raw materials. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon(iv)oxide. The fermentation reaction, represented by the simple equation

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(ethanol)}}{2C_2H_5OH} + \underset{\text{(carbon(iv)oxide)}}{2CO_2}$$

is actually very complex, and an impure culture of yeast produces varying amounts of other substances, (including glycerine) and various organic acids. In the production of beverages, such

as whiskey and brandy, the impurities supply the flavour. Starches from potatoes, corn, wheat and other plants can also be used in the production of ethanol by fermentation.

However, the starches must first be broken down into simple sugars. An enzymereleased by germinating barley, diastase, converts starches into sugars. Thus, the germination of barley, called malting, is the first step in brewing beer from starchy plants, such as corn and wheat (Shakhashiri and Kevin, 2009).

The ethanol produced by fermentation ranges in concentration from a few % up to about 14%. Above about 14%, ethanol destroys the zymase enzymes and fermentation stops. This happens at different levels for different strains of yeast. It has been reported to damage mitochondrial DNA in yeast cells and to cause inactivation of some enzymes such as hexokinase and dehydrogenase (You *et al*., 2003).

Brewer's yeast tolerates up to about 5 or 6 % alcohol by volume. Beyond this alcohol level, the yeast cannot continue fermentation. Wine yeast is more tolerant at a range of 10-15%. The level of alcohol tolerance by yeast varies from 5 - 21% depending on the yeast strains and environmental conditions. The fermentation process has limits such as temperature.Temperaturegreater than 27^0C kills the yeast, less than 15^0C results in yeast activity which is too slow (Shakhashiri and Kevin, 2009).

Ethanol is normally concentrated by distillation of aqueous solutions, but the composition of the vapour from aqueous ethanol is 96% ethanol and 4% water. Therefore pure ethanol cannot be obtained by distillation. Commercial ethanol contains 95% by volume of ethanol and 5% of water. Dehydrating agents can be used to remove the remaining water and produce absolute ethanol (Shakhashiri and Kevin, 2009).

Much ethanol not intended for drinking is now made synthetically, either from acetaldehyde made from acetylene, or from ethylene made from petroleum. Ethanol can be oxidised to form acetaldehyde and then acetic acid. It can be dehydrated to form ether. Butadiene, used in making synthetic rubber, may be made from ethanol, as can chloroform and many other organic chemicals (Shakhashiri and Kevin, 2009).

Most industrial ethanol is denatured to prevent its use as a beverage. Denatured ethanol contains small amounts, 1 or 2% each, of several different unpleasant or poisonous substances. These denaturants render ethanol unfit for some industrial uses. Alcoholic solutions of non volatile substances are called tinctures; if the solute is volatile, the solution is called a spirit (Shakhashiri and Kevin, 2009).

2.14.12 Effects and side effects of ethanol

Ethanol is toxic, and the body begins to dispose of it immediately upon its consumption. Over 90% of it is processed by the liver. In the liver, the alcohol dehydrogenase enzyme converts ethanol into acetaldehyde, which is itself toxic (Shakhashiri and Kevin, 2009).

$$\underset{\text{Ethanol}}{C_3H_2OH} \longrightarrow \underset{\text{Acetyaldehyde}}{CH_3 - \overset{\overset{O}{||}}{C}- H} + 2H$$

Acetyaldehyde is destroyed almost immediately by the aldehyde dehydrogenase enzymes, which converts it to acetate ions.

$$\underset{\text{Acetyaldehyde}}{CH_3 - \overset{\overset{O}{||}}{C}- H} + 2H_2O \longrightarrow \underset{\text{Acetate ions}}{CH_3-\overset{\overset{O}{||}}{C}-O^-} + 3H$$

The hydrogen atoms in these equations are not unattached, but are spicked up by another biologically important compound, nicotinamide-adenine dinucleotide (NAD), whose function is to carry hydrogen atoms. NAD is involved in both of the above processes, being converted to NADH.

$$NAD + H \longrightarrow NADH$$

NADH must be recycled to NAD for the disposal of ethanol to continue. If the amount of ethanol consumed is not great, the recycling can keep up with the disposal of ethanol. The ethanol disposal rate in a 150-pound($95.6\times10^{3}{}_{m/s}{}^{2}$) human is about 0.5 ounce (1.75×10^{8}cm3) of ethanol per hour, which corresponds to 12 ounces($42.23\times10^{6}cm^{3}$) of beer, 4 ounces ($14.07\times10^{6}cm^{3}$)of wine or 1 ounce($3.51\times10^{6}cm^{3}$) of hard liquor (Shakhashiri and Kevin, 2009).

. Ethanol acts as a drug affecting the central nervous system. Its behavioral effects stem from its effect on the brain and not on the muscles or senses themselves. It is a depressant and depending on dose, can be a mild tranquilizer or a general anesthetic. It suppresses certain functions. At very low doses, it can appear to be a stimulant by suppressing certain inhibitory brain functions. However, as concentration increases, further suppression of brain functions produce the classic symptoms of intoxication: slurred speech, unsteady walk, disturbed sensory perceptions, and inability to react quickly. At very high concentrations, ethanol produces general anesthesia; a highly intoxicated person will be asleep and very difficult to wake, and if awakened, unable to move voluntarily (Shakhshiri and Kevin, 2009).

2.15 Mutation

Mutations are changes in the Deoxyribonucelic acid (DNA) sequence of a cell's genome and are caused by radiation, viruses, transpons and mutagenic chemicals, as well as errors that occur during meiosis or DNA replication (Bertran, 2000; Burrus and Walder, 2004; and Aminetzach *et al.,* 2005). They can also be induced by the organism itself, by cellular processes such as hypermutation.

Mutation can result in several different types of change in DNA sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Based on studies, on the fly *Drosophila melanogaster*, it has been suggested that if a mutation changes a protein produced by gene, this will probably be harmful, with about 70% of these mutations having damaging effects, and the remainder being either neutral or weakly beneficial (Sawyer *et al.,* 2007). Due to the damaging effects that mutations can have on cells, organisms have evolved mechanisms such as DNA repairs to remove mutations (Bertram, 2000). Therefore, the optimal mutation rate for species is a trade-off between costs of a high mutation rate such as deleterious mutations, and the metabolic costs of maintaining systems to reduce the mutation rate, such as DNA repair enzymes (Sniegowski, *et al.,* 2000). Viruses that use RNA as their genetic material have rapid mutation rates (Drake and Holland, 1999), which can be an advantage since these viruses will evolve constantly and rapidly, and thus evade such defensive responses,as the human immune system (Holland, *et al.,* 1982).

2.15.1 Classification of the mutation types by effect onfitness

Mutation is divided into beneficial, harmful and neutral, based on the effects.

2.15.1.1 Beneficial mutation

A beneficial mutation isa mutation that has a positive effect on an organism; increase fitness of organism, or which promotes traits that are desirable. It may also enable the mutant organisms to withstand particular environmental stresses better than wild type organism, or reproduce more quickly. Examples of beneficial mutation that promotes the fitness of the organisms:

- **Nylonase: Nylon Bacteria**

The nylonase bacteria can destroy short molecules of nylon (nylon-6). The mutation in these bacteria involves insertion of a single nucleotide in the genetic material. It is estimated that this frame shift mutation might have occurred in the 1940s when nylon was invented. Nylonase can be used in waste water treatment plants.

- **Antibiotic Resistance: Bacteria**

Antibiotics are used for the treatment of disease caused by bacteria. Constant use of antibiotics leads to the development of resistance among the targeted bacteria. Many times, the antibiotic resistance reduces the fitness of the particular bacteria population when they are exposed to non-antibiotic environment. These resistant bacteria do not have the ability to reproduce as fast as those without mutation, hence slowing the disease progression.

2.15.1.2 Harmful mutation

A harmful mutation is mutation that decreases the fitness of an organism. Changes in DNA caused by mutation can cause errors in protein sequence, creating partially or completely non-functional proteins. To function correctly, each cell depends on the thousands of proteins to

function in the right places at the right times. Some mutations alter a gene's DNA base sequence but do not change the function of the protein made by the gene. However, studies in yeast have shown that only 7% of mutations that are not in genes are harmful (Doniger, *et al.*, 2008).

2.15.1.3 Neutral mutation

Neutral mutations are defined as mutations whose effects do not influence the fitness of an organism or individual. In order words, it has no harmful or beneficial effect. These can accumulate over time due to genetic drift. It is believed that the "overwhelming" majority of mutation have no significant effect on an organism's fitness. Also DNA repair mechanism are able to mend most changes before they become permanent mutations, and many organisms have mechanism for eliminating otherwise permanently mutated somatic cells.

2.15.2 Classes of mutation

Two classes of mutations are spontaneous mutations (molecular decay) and induced mutations caused by mutagens.

2.15.2.1Spontaneous mutation

Spontaneous mutations arise from several potential sources. This type of mutations may result from errors in DNA from factors such as gamma radiations and heat, or even tranposons. It may also arise from frameshifts, usually caused by the deletion of DNA segment resulting in an altered codon reading frame. These mutations occur where there is a short repeated nucleotide sequence. Spontaneous mutations also originated from lesions in DNA (Prescott *et al.*, 2008).Spontaneous mutations on the molecular level can be caused by (Beth, 1998):

a. Tautomerism

A base is changed by repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base resulting in incorrect base pairing during replication.

b. Depurination

Loss of a purine base (A or G) to form an apurinic site (AP site).

c. Deamination

Hydrolysis changes a normal base to a typical base containing a keto group in place of the original amine group. Examples include C ⟶ U and A ⟶ HX (hypoxanthine), which can be corrected by DNA repair mechanism; and 5mec (5-methylcytosine) ⟶ T, which is less likely to be detected as a mutation because thymine is a normal DNA base.

d. Slippped strand mispairing

Denaturation of a new strand from the template during replication is followed by renaturation in a different spot ("slipping"). This can lead to insertion or deletions.

2.15.2.2 Induced mutation

Induced mutation occurs as a result of exposure of organisms to some chemical or physical agents called mutagens.

Mutagen:Mutagen is a natural or human made agent (physical or chemical) which can alter the structure or sequence of DNA (Anon, 2007b). Mutagens are classified according to their mode of action.

A. Chemical mutagens

Four common types of chemical mutagens are available; these are base analogs, DNA-modifying agents or specific mispairing, intercalating agents, and agents altering DNA structure.

i. Base analogs

These are chemical structures which closely resemble a nucleic acid base and are erroneously inserted into newly synthesised DNA strands such as bromouracil and amino purine (Ado, 2004).

ii. DNA- modifying agents (specific mispairing)

These change a base's structure and therefore alter its base pairing characteristics. Some mutagens in this category are fairly selective; they preferentially react with some bases and produce a specific kind of DNA damage. Methyl-nitrosoguanidine, for example is an alkylating agent that adds methyl group to guanine, causing it to mispair with thymine. Others include ethyl methane sulfonate & hydroxylamine. Hydroxylamine hydroxylates the C-4 nitrogen of cytosine causing it to base pair like thymine. Nitrous acid also causes cytosine (C) to uracil (U), adenine (A) to hypoxanthine, and adenine (A) to tyrosine (T) transitions (Prescott *et al.*, 2008).

iii. Intercalating agents

These are flat, multiple ring molecules which interact with bases of DNA and insert between them (intercalate). This insertion causes a 'stretching' of the DNA duplex and the DNA

polymerase is “fooled” into inserting an extra base opposite an intercalated moleculethereby resulting in frame shifts. Intercalating agents include acridine orange, proflavin and ethidium bromide (Prescott, *et al*; 2008).

iv. Agents altering DNA structure

These may be large molecules which bind to bases in DNA and cause them to be non-coding. These include N-acetoxyl-2-acetylamine fluorine (NAAF), agents causing intra and inter-strand crosslink such as psoralens(found in some vegetables and used in treatments of some skin conditions) and chemicals using DNA strand breaks. An example is peroxide (Anon, 2007b).

B. Physical mutagens

These include: Radiation for example UV (non ionising radiation), X-rays and gamma rays.

2.15.2.3 Radiation

Radiation was the first mutagenic agent known.Itseffects on genes were first reported in the 1920s. Visible light and other forms of radiation are all types of electromagnetic (EM) radiation which consists of both electric and magnetic waves. The length of EM waves (wavelength) varies and is inversely proportional to the energy they contain. This is the basis of the EM spectrum. Long waves (such as AM radio)contain least energy while, short waves (such as FM radio, TV, microwaves, infrared, ultraviolet (UV), X- and gamma radiation) contain higher energy (Anon, 2007b, 2009d). X- and gamma rays are energetic enough that they produce reactive ions (charged atoms or molecules) when they react with biological molecules. They are

therefore referred to as ionising radiation. UV radiation is not ionising but can react with DNA and other biological molecules. It is important as a mutagen and it causes the formation of thymine dimers of adjacent DNA strands (Anon, 2009d). UV radiation particularly longer wave UVA, can cause oxidative damage to DNA (Ellis *et al.*, 2001).

Measurement of radiation:Units used for ionising radiation of all types are rems (roentgen equivalent man). One (1) rem of any ionising radiation produces similar biological effects. Previously used unit was the rad (radiation absorbed dose). The dose rate is taken into account as the same number of rems given in a brief intense exposure (low dose rate) which would only increase the risk of mutation andcancer (Anon, 2007b, 2009d).

The sources of radiation:This could be natural (e.g. cosmic rays from sun and outer space); radioactive elements in soil and terrestrial products (wood, stone) and in the atmosphere (radon). They produce the so-called background radiation and one's exposure varies with geographic location. Artificial sources include medical testing equipment such as diagnostic x-rays and equipment, nuclear testing and power plants and various other products such as television sets, smoke detectors and airport x-rays equipment. Generally, a person's overall total average exposure from all sources is about 350m rem/year (Anon, 2007b).

Biological effects of radiation:Ionising radiation produces a range of damage to cells and organisms primarily due to the production of free radicals of water (the hydroxyl or OH radical). Free radicals possess unpaired electrons and are chemically very reactive.They will interact with DNA, proteins, lipids in cell membranes, e.t.c. The severity of the effects depends upon the dose received,viz: sublethal dose (100-250 rems), lethal dose (350-450rems) and supralethal dose (>450rems) (Anon, 2007b, 2009d).

Genetic effects of radiation:Ionising radiation produces a range of effects on DNA both through free radical effects and direct action through breaks in one or both strands, damage to / loss of base (mutation) and cross linking of DNA to itself or proteins. UV (Ultraviolet) radiation is less energetic and therefore non-ionising but its wavelength is preferentially absorbed by bases of DNA and by aromatic amino-acid of proteins. Purine and pyrimidine bases of nucleic acids absorb UV radiation strongly and the absorption peak UV for DNA and RNA is 260nm. Killing of cells by UV irradiation is due primarily to its action on DNA, so that UV irradiation at 260nm is most effective as a lethal agent (Anon, 2007). UV light may cause two adjacent pyrimidine residues (cytosine or thymine) to form a dimer (Ado, 2004). In a normal cell, the dimer can then trigger the repairing process. However, if it becomes non-functional, the pyrimidine dimer may lead to mutation (Anon, 2007b).

2.15.2.5 DNA repair systems

As DNA damage occurs spontaneously and also as a result of ubiquitous environmental agents, most organisms possess some capacity to repair the DNA. DNA is the only macromolecule which is repaired by cells (Prescott *et al.*, 2008). There are mechanisms for DNA repairs- (i) **Damage reversal** in which simple, enzymatic action restores normal structure without breaking backbone structure of the DNA. This is a photoreactivation process, where photolyase enzyme catalyzes the splitting of pyrimidine dimers in the presence of light; (ii) **Ligation of single strand** breaks where DNA ligase repairs simple breaks in one strand caused by x-rays and some chemicals like peroxides and (iii) **Damage removal** which involves cutting out and replacing a damaged or misappropriate base or section of nucleotides.

(iv) **Base excision repair**occurs when a damaged or inappropriate base is removed from its sugar linkage and replaced. The enzymes involved are glycosylase which cuts the sugar-base bond (Ado, 2004; Anon, 2007b). (v) **Mismatch repair** is a process which occurs after DNA replication as a check on its accuracy. It is carried out by a group of proteins which scan the DNA for incorrectly paired bases (or unpaired bases). The incorrect nucleotide is removed and the DNA polymerase replaces it with the right sequence (Anon, 2007b).

(vi)**Damage tolerance** is not truly a repair but a way of coping with damage so that life can go on. Recombination (daughter-strand gap) repair for example, which promotes recombination to fix the daughter-strand gap and not the dimer as a way to cope with the problems of non-coding lesion persisting in DNA (Anon, 2007b). (vii) **Mutagenic repair** (trans-lesion synthesis) occurs as an alternative response for a DNA polymerase blocked at a dimer which then changes its specificity such that it can insert any nucleotide opposite the dimer and thus continue replication- a kind of 'mutate or die' situation (Anon, 2007b).

2.15.3 Screening for nutritional mutation

A nutritional or biochemical mutation affects an organism's ability to produce a molecule (for example, an amino-acid) essential for growth. Nutritional mutations are commonly studied in haploid organisms such as *E. coli*, *Chlamydomonas*, *Neurospora*, and yeast, which are grown in the laboratory on well-defined media. Mutant strains that require a nutritional supplement for growth are termed auxotrophic in contrast to prototrophic wild-type or revertant strains. The replica-plating technique is commonly used for detecting auxotrophs in these organisms (Prescott *et al.*, 2008).

2.15.3.1 Screening procedures

Screening procedures are essential for two related types of study:

1. The geneticist wishes to isolate a mutant strain with a particular phenotype. This is because mutation process is random, the geneticist is unable to control which gene will become mutant. Techniques must therefore be devised to screen a large number of potentially mutant organisms for the mutant phenotype of interest.
2. The geneticist wishes to determine whether a given chemical or agent is mutagenic, the geneticist, therefore exposes organisms of one phenotype to the putative mutagen and screen their descendents for mutants (Ursula, 1978).

2.15.3.2 Quantitative positive auxanography for the screening of nutritional mutants

Afteridentification, isolation and genetical tests, a mutant has to be characterised, that is a search is made for the simplest growth factors or combination of growth factors, which will make growth possible when added to minimal medium (Ponk,2002). The great advantages of positive auxanography as a method of microbiological assay lies in the possibility of using moulds and especially mutants of moulds which are at present being produced in large numbers in laboratories all over the world, without having to weigh the mycelium to estimate the amount of growth.

2.15.4 Application of mutations

Mutations are invaluable to the process of evolution since they provide the raw material required for its occurrence. Even though most mutations make the organism less efficient and are thus

disadvantageous, the possibility of developing new traits through induced mutations has intrigued many plant breeders.

Many plant strains were improved for higher yield, higher resistance against disease and pest by using mutations. One application of induced mutations comes from the concentrated efforts to improve the yield of penicillin by the mould,*Penicillium* .When penicillin was first discovered, the yield was low and production was seriously limited. Then millions of spores were irradiated and selected for higher yields.Such mutants who produce penicillin more than the average have proven invaluable in the commercial production of this important antibiotic.

Mutations have been used extensively to elucidate the pathways by which biological processes occur. Metabolisms occur via sequences of enzyme-catalysed reactions. By isolating and studying mutations in the genes coding for the enzyme involved, the sequence of steps in a pathway can often be determined.

Morphogenesis frequently involves the sequential addition of proteins in the formation of specific 3-D structures. Again, the sequences of protein additions can often be determined by isolating and studying mutant organisms with mutation in the genes coding for the proteins involved.(Eyre-Walker and Keightley, 2007).

2.16 Bread Production

Bread is a staple food prepared by cooking dough of flour and water and often additional ingredients. Doughs are usually baked, but in some cuisines, bread is steamed (e.g. mantou), fried (e.g puri) or baked on an unoiled skillet (e.g. torlillas). It may be leavened or unleavened.Salt, fat and leavening agents such as yeast and baking soda are common ingredients though bread may contain other ingrdients, such as milk, egg, sugar, spice, fruit (e.g. raisins),

vegetables (e.g. onions), nuts (e.g. walnuts) or seeds (e.g. poppy). Referred to colloquially as the "staff of life", bread has been prepared for at least 30,000 years. The development of leavened bread can probably also be traced to prehistoric times. Sometimes, the word bread refers to a sweetened loaf cake, often containing appealing ingredients like dried fruits, chocolate chips, nuts or spices, such as pumpkin bread, banana bread or ginger (Kaplan, 2006). Fresh bread is prized for its taste, aroma, quality, appearance and texture. Retaining its freshness is important to keep it appetising. Bread that has stiffened or dried past its prime is said to be stale. Modern bread is sometimes wrapped in paper or plastic film or stored in a container such as a bread box to reduce drying. Bread that is kept in warm, moist environments is prone to the growth of mold. Bread kept at low temperatures, in refrigerator for example, will develop mold more slowly than bread kept at room temperature, but will turn stale quickly due to retrogradation (Kaplan, 2006).

The soft, inner part of bread is known to bakers and other culinary professionals as the crumb, which is not to be confused with small bits of bread that fall off, called crumbs. The outer hard portion of the bread is called the crust (Kaplan, 2006).

2.16.1 Ingredients ofbread

The basic ingredients are flour, yeast, salt and water. Others include fats or shortenings, bread improvers, and preservatives.

1. Flour

Flour is a product made from grains that have been ground to a powdery consistency. Flour provides the primary structure to the final baked bread. Wheat flour is the key ingredient in most breads and it is higher in protein/gluten. The quantity of the protein contained in the flour, serves as the best indicator of the quality of the bread dough and the finished bread. If one uses a flour

with a lower (9-11%) protein content to produce bread, a longer mixing time will be required to develop gluten strength properly (Plyer,1988).

2. Yeast

Many breads are leavened by yeast. The yeast used for leavening bread is *Saccharomyces cerevisiae*, the same species used for brewing alcoholic beverages. Yeast functions (France and Wragg, 1998) in bread making are to:

- ferment carbohydrates in flour, including sugar, producing carbon(iv)oxide gas to enable the dough to rise.
- Promote"ripening" of the dough as a result of the stretching effect of expanding pockets of CO_2 produced during fermentation and also on dough.
- form the cellular network found in bread crumb.
- give bread its characteristic "aroma"

3. Salt

Salt is an essential ingredient in bread. It is used in small amounts to give bread flavor and to restrict yeast activity. It also helps to control fermentation to produce bread of good volume,and affects the crumb and the overall texture by stabilising and strengthening the gluten (Nancy, 1996).

4. Water

Water is used to form the flour into a paste or dough. The volume of water required varies between recipes, but a ratio of 1 part water to 3 parts flour is common for yeast breads, while

recipes that use steam as the primary leavening method may have a water content in excess of one part water to one part flour by volume. Correct temperature and quantity of water used when making bread are important because water affects the dispersal of the other ingredients. Instead of water, other types of liquids, such as dairy products, fruit juices, or beer, may be used; they contribute additional sweeteners, fats or leavening components, as well as water(Plyer,1988).

5. Fats or Shortenings

Fats such as butter, vegetable oils, lard, or that contained in eggs affects the development of gluten in breads by coating and lubricating the individual strands of protein.Fats/shortenings also help to tenderize or keep the bread soft, enable the dough pass more easily through the production process, and keep the bread fresh long after baking(Plyer,1988).

6. Bread Improvers

These are often used in producing commercial bread to reduce the time needed for rising and to improve crumb texture, volume and softness of the bread. Chemical substances commonly used as improvers include: ascorbic acid, hydrochloride, sodium metabisulfate, ammonium chloride, various phosphates, amylase and protease. (Plyer,1988).

7. Preservatives.

Preservatives such as acetic acid (vinegar), are often used in commercial baking to ensure the freshness of the poduct and prevent staling.

2.16.2 Processing stages

The basic stages of conventional bread making process are recipes and formation, and dough kneading.

2.16.2.1 Recipes and formulations

A recipe is the amount of ingredients and the processing method. The recipes for bread differ all over the world because of the availability, the price and the quality of ingredients. Professional bakers use a system of percentages known as bakers percentage in their recipes formulations. Baker's percentages is the bakers notation method indicating the flour-relative proportion of ingredients used when making breads, cakes, muffins and other pastries (Griffin and Gisslen, 2005; Corriher, 2008; Paula, 2010). They measure ingredients by weight instead of volume, because measurement by weight is much more accurate and consistent than measuring by volume especially for dry ingredients (Stanley, 2009).

The proportion of water to flour referred to as the "hydration" is the most important measurement in bread recipes, as it affects texture and crumb most. The amount of flour is always stated as 100% and the ingredients are expressed as a percent of that amount by weight. Common formulations for bread include 100% flour, 60% water/liquid, 1% yeast, 2% salt and 1% vegetable oil, lard or butter (Reinhart, 2009). Calcium propionate is commonly added by commercial bakeries to retard the growth of molds.

2.16.2.2 Dough kneading

Dough kneading is the mixing and kneading of the raw materials in a way that a good quality bread can be obtained. Kneading is a process that develops and strengthens the gluten in dough. The kneading involves:

- Scaling flour and other ingredients
- Sieving the flour to remove impurities
- Activating yeast, dissolve yeast in water and add
- Instant yeast, add dry yeast to flour
- Add other ingredients like salt, sugar, milk, powder, fat e.t.c.
- Mix the ingredients (Pyler, 1988).

During dough kneading air is incorporated which is the basis for the structure of the bread. Kneading time is between 12-20minutes. During kneading, frictional heat causes the dough temperature to rise. When the ingredients are thoroughly mixed and the gluten network is sufficiently elastic and extensible the dough is ready(i.e when a piece of dough is stretched up to a thin film without breaking).

2.16.3 Bread making process

There are two main methods of making bread, namely; the sponge and dough method and the straight dough method. Each of these methods is capable of expansion; that is, produce carbon(iv)oxide and produce dough which is able to retain gas and expand (France and Wragg, 1998) Both methods also have their advantages and disadvantages.

2.16.3.1Sponge and dough method

A sponge can be defined as a stiff ferment or a slack dough and is made by mixing a proportion of the flour, with varying proportions of the total yeast and water. Some or all of salt can be used, depending on the size of the sponge and the length of fermentation. The rate or speed of

fermentation is controlled by the amount of yeast, salt (if any), the consistency of dough and temperature.

Sponge fermentation is allowed for approximately 1-2 h and is termed a "flying-sponge". The fermentation process is slower and more time is necessary for maximum ripening. Due to the higher flour content, more gluten is ripened and the acidity of the sponge also assists in promoting rapid dough ripening. Sponges may be classified according to the approximate flour used. Thus, there are quarter, half, three quarter and full sponges (i.e. 25, 50, 75, and 100% of flour content).

Advantages

- more tolerance during processing
- a weaker, cheaper flour can be used
- less yeast is usually required
- long sponge fermentation and short processing times
- a well developed gluten
- better flavoured/ good taste bread is obtained
- the long overnight sponge is safer than a long straight dough process because the effect of
- the over fermentation can be corrected in the final dough.

Disadvantages of sponge and dough method

- more bowls for the sponges are necessary.
- more space is needed for storage
- difficult planning (i.e. the extra work entailed by a two stage process)(France and Wragg,1998).

2.16.3.2Straight dough method

This is the process of bread making in which all the ingredients are mixed into a dough in a single batch or operation. The straight dough can be timed to ferment from 30 minutes to 14-16 h, according to the amount of yeast, salt and water used, the type and quality of flour, the temperature of the finished dough, and the room in which it is stored. The process of straight dough fermentation is dividedinto three periods, according to the length of fermentation time: short(30minutes-2h), medium,(3-6 h), and long (8-16 h) (France andWragg,1998).

Advantages

- The process is short
- Good quality bread with fine structure
- Easy planning
- Less space required
- Less bowls needed

Disadvantages

- Labour Intensive
- Skilled bakers needed
- Intermediate prover required
- Temperature and humidity control
- Less tolerance (France andWragg,1998).

2.16.4 Sources of fermentable sugars in bread-making

There are three sources of fermentable sugars in bread manufacture. First, there is the sugar present in dough at the start of bread-making process (including glucose, fructose, sucrose and maltose naturally present in flour) and secondly, any fermentable sugars such as sucrose added by the baker. The amount of fermentable sugar added by baker varies, but can reach to 25% w/w in some sweet dough (Nagodawittana and Trivedi, 1990).The third source of sugar is maltose produced by the amylolytic breakdown of starch (Evans, 1990). If bread is made without added sugars (plain dough), the pre-existing free sugars are completely fermented within the first hour, leaving only the starch-derived maltose to sustain fermentation (Oda and Ouchi, 1990; Higgins *et al*., 1999).

2.16.5 Changes during fermentation

At the commencement of bulk fermentation, the dough feels quite short and tough having practically no extensibility. The dough begins to increase in volume as carbon(iv)oxide, produced by the chemical breakdown of sugars by yeast, inflates it. The carbon(iv)oxide formed remains in solution, until saturation point is reached and then diffuses into air pockets incorporated during mixing. It is the quality of the gluten network which prevents the escape of gas. The chemical reactions cause a release in energy and consequent rise in dough temperature. If the dough is tested during the period of the fermentation, it will be found that it becomes progressively more extensible, due to the combined effects of chemical and physical changes. At the same time, the colour gradually brightens and the dough becomes more pungent in aroma (France and Wragg,1998; Phillips,2013).

2.16.6 The fermentation speed

Yeast produces carbon(iv)oxide out of the sugars present in the dough. More yeast produces more carbon(iv)oxide and the dough will be “faster”. Less yeast will make the dough slower. Sugar will affect the fermentation speed, the colour and taste. Amounts up to 3% will increase carbon(iv)oxide production. More sugar will reduce the fermentation speed and will give a noticeable sweeter taste.

Salt will slow down the fermentation speed and harden the gluten. In common bread dough, 1-2% salt is added.

Other ingredients like fat, milk powder, malt e.t.c. will require more yeast to maintain the same level of carbon(iv)oxide production (Reinhart, 2001;Phillips ,2013).

2.16.7 Proofing time and dough handling

The term proofing in bread making has two meanings: One having to do with yeast and the other having to do with dough. Yeast is proofed in water and a small amount of sugar is added to determine whether it is active before using. Proofing also denotes a stage in the rising of the dough. After its first rise, the dough is punched down and shaped in its final form and then set for its final rise known as “proofing” (Ulfstahl and Donalies, 2008).

During the bread making process the dough is given several fermentation periods interrupted by dough handling, to get a nice bread of good volume, fine structure, good taste and good digestibility. The effect on the different proofing periods is in an increased carbon(iv)oxide development, a better dough development resulting in increased gas retention ability. The following proofing periods can be indentified: sponge fermentation, 1^{st} bulk fermentation, 2^{nd} bulk fermentation, intermediate proof, and final proof and oven spring. The effect of all dough

handling is to stop temporarily the stretching of the gluten by pressing out the gas, to get an equal distribution of the dough cell throughout the dough and to increase the number of the dough cells causing a fine structure. The fermentation time is influenced by the quality of the flour, the dough temperature, the type of product, the bakery temperature and humidity, the stiffness of the dough, the amount of other ingredients in the recipe and the activity of the enzymes (Reinhart, 2009).

2.16.8 Carbon(iv)oxide and gluten development

The whole process is based on an optimum carbon(iv)oxide and gluten development. The dough temperature, baking temperature and humidity, the amount of sugar and the amount of water affect carbon(iv)oxide production. Gluten development is affected by the flour quality, the kneading time, the stiffness of the dough and the amount and type of bread improver. Gluten development improves, if the dough is allowed to autolyse, that is, foregoing early addition of salt and waiting until after 20minutes “rest” (Reinhart, 2001). An optimum carbon(iv)oxide and gluten development results in a good quality bread with a high volume and a fine structure. When the fingers are pressed into the dough, and very little resistance is felt,then the bread is ready for baking.

2.16.9 Dough leavening

Dough is a paste made of any cereals (grains) or leguminous crop by mixing flour with a small amount of water and/ or milk. This process is a precursor to making a wide variety of foodstuffs, particularly breads and bread-based items such as quick breads (Pan cakes), flatbreads, noodles, pastry e.t.c. Dough leavening is a process of incorporating gas through the use of ingredients such as yeast, bacteria, baking powder or soda, steaming and aeration into a bread dough which

causes the dough to rise through the release of carbon(iv)oxide, This is done before or during baking to produce a lighter, more easily chewed bread (Ulfshtahl, and Donalies, 2008).

2.16.10 Thebaking process

During baking, the dough will be changed from a pliable shape into a fixed shape, the taste will be developed and the crust will be formed. When dough is put into the oven, water will evaporate from the crust. The crust formation will start after the loaf has reached its maximum volume. The yeast has died at 50^0C and the gelatinisation of the starch begins. At 70^0C the protein coagulates releasing water which will be absorbed by the starch. The volume increases, the skin will crack as well as the cell walls. The water starts evaporating, the protein is coagulated, the starch gelatinises, the structure and the shape fixed. At 110^0C, the crust becomes thicker, the temperature increases and colouring starts. During baking the oven temperature and baking time are very important. In general small products have to be baked at a high temperature for a short period, big products have to be baked at a lower temperature for a longer period. The heat is penetrating small products much faster than big products. The type of bread, the addition of other ingredients like sugar and milk powder are other factors influencing the baking temperature and time. During baking steam will be developed by the evaporation of the water from the dough and will give bloom to the crust. Some products like crispy bread require the addition of steam before baking to increase the volume and make crust crispy (Reinhart, 2009)

2.16.11 Bread crust

The bread crust is formed from surface dough during the baking process. It is hardened and browned process through the Maillard reaction using the sugars and amino acids and the intense heat at bread surface. The nature of bread's crust differs depending on the type of bread and the

way it is baked. Commercial bread is baked using jets that direct steam towards the bread to help produce a desirable crust. The crust of most bread is less soft, and more complexly and intensely flavored, than the rest, and judgments vary among individuals and cultures as to whether it is therefore the less palatable or the more flavourful part of a particular style of bread. The first and the last slices of a loaf are sometimes referred to as the heel or the crust of the loaf (Kaplan, 2006).

2.16.12 Characteristics of good bread

In order to define a good loaf, one must have some knowledge of the desirable qualities of a particular type of bread and how these qualities are produced. The desirable features of a good loaf can be listed under two headings, external and internal(Pyler, 1988).The features are:External qualities:volume, symmetry of shape, bloom, crust, colour, eveness of bake, oven break moistness and cleanliness. Internal qualities: Colour, structure, sheen, texture, flavour and aroma, crumb clarity and elasticity.

A good loaf is the result of:

- good quality ingredients
- good processing
- good workmanship

i. Volume

Afair volume with a structure, which is not too open, is required for common bread.

ii. Symmetry of shape

This is defined as a beauty resulting from right properties, or a harmony between the parts. It is brought about by correct dough fermentation and moulding.

iii. Bloom

Natural bloom is the glow that denotes excellent fermentation, the use of good raw materials and fine workmanship.

iv. Crust colour

The right baking temperature, good raw materials and correctly fermented dough will give the right colour. Pale dull coloured bread is mainly caused by the absence of sugar. A harsh red-brown colour is caused by an under fermented dough. A dried out skin will give a poor crust colour.

v. Evenness of bake

Depends on the quality of the oven and the way the oven is operated like loading, heating e.t.c.

vi. Oven break

Properly processed dough will “break” properly during the first period of the baking process. When an oven break is not required, give the dough a longer final proof.

vii. Internal colour

The type of flour used and the structure of the crumb influence the colour. The right fermentation, manipulation and proving and baking condition cause a fine regular crumb structure.

viii. Structure

The structure of an ordinary tin loaf should have round fairly small dough cells and they should be regularly and evenly distributed.

ix. Sheen and texture

The way a cut surface reflects light will indicate the condition on the structure, which is called sheen. Over fermented dough will give a "woolly" and tight dough will give a "drummy" texture.

x. Flavour and aroma

The use of yeast, proper processing and baking will give a well-developed flavor and aroma. Longer fermentation processes are characterised by a flat taste.

xi. Crumb clarity and elasticity

The crumb, when pressed, should return when the pressure is raised, this is elasticity.

xii. Moistness

The water content, the fermentation, the salt, other ingredient like fat and malt and the baking conditions determine the moistness of a loaf.

2.17 Meaning of Sensory Evaluation

"Sensory evaluation" is the description and evaluation of food products using human senses (senses of sight, smell, taste, touch and hearing) (Manson and Nothingham, 2002).

Accordingly, sensory evaluation is an analytical method in which the human senses serve as a measurement tool to determine the quality and/or to describe the condition of food product.

Prerequisites for the success of this analytical process include: Standardisation of methods, regular training and performance measurement of testers, a statistical evaluation of test results,and a standardisation of terms (Manson and Nottingham, 2013).

2.17.1 Objectives of sensory evaluation

- One main objective of sensory evaluation is the measurement of sensory attributes and the quantification of the influence of these attributes on consumer acceptance. This aspect is becoming increasingly important because of interrelationship between enjoyment values and repurchases behaviour. Sensory attributes are directly linked to the concept of quality and thereby ultimately contribute to the success or failure of a product (Anon, 2013). Four variables affect sensory evaluation: food, the people, the testing environment and methods (Manson and Nottingham, 2013)

2.17.2 Sensory evaluation methods

There are two main methods; affective/hedonic tests and analytical tests, (Mason and Nottingham, 2013).

2.17.2.1 Affective / hedonic tests

These are tests which involve consumer preference or acceptance, that is, it serves to characterise consumer'sbehaviour. Preference infers a preference for one product over another; either over or in relation to a particular parameter. Acceptance infers utilisation/purchase of the products.

2.17.2.2 Analytical tests

These are sensory tests which are involved with analysing specific product attributes. They are divided into two categories: Comparative / discriminative and descriptive tests. These sensory tests on the other hand are referred to as "expert tests" because they may be carried out by trained persons and can give very detailed information about individual parameters.

1. Comparative / discriminative tests

These tests are further divided into two types:

- **Difference tests**

These are tests to find adifference between the control and other products.

- **Sensitivity tests**

These are used to test the ability of panelists to detect sensory characteristics

2. Descriptive tests

Descriptive tests are carried out and among the panelists to measure their ability to evaluate qualitative and quantitative characteristics of the product.

2.17.3 Advantages of sensory evaluation methods

- It gives real answer regarding consumer quality.
- It is relatively a cheap process (depending on how it is done).
- It gives a morerapid result.
- It is used for many applications in food.
- The methods are objective, more reliable, accurate and reproducible.

2.17.4 Sensory evaluation techniques

The following are sensory techniques as described by (Irfan, 2007).

- **Consumers**

Today's consumers are discerning, demanding and more knowledgeable about foods and expect products which are safe, good value and of high sensory quality. Therefore, knowing consumer's preferences and perceptions of the sensory characteristics of food products is very important to food manufacturers and retailers alike.

- **Sensory science**

Sensory science is the study of the reactions of the five senses- sight, hearing, smell, taste and touch to the characteristics of physical matter. The discipline does not just deal with "likes and dislikes", but scientifically evokes, measures, analyses and interpretes psychological responses to physical stimuli, and thus belongs to the specialised field of psychophysics.

- **Unique approach**

"Sensory science" adapts a unique approach to identification of the attributes that matter most to the end-consumer. Its emphasis is on "trained panelists", and where possible sensitive instruments, to provide the right guidance to the food manufacturers on what perceived attributes need to be incorporated into their products for their marketing success(Irfan,2007).

- **Measurement**

A person's sense of taste, smell, touch, sound and sight form his perception of food. In a taste panel, these senses can be measured scientifically to obtain information about particular aspects of a food (Irfan,2007).

- **Statistical techniques**

When conducting a taste panel, food is uniformly prepared and presented to panelists in isolated booths. Judges record evaluations of the product on a sensory evaluation sheet that is decodedand analysed by statistical procedures (Irfan, 2007)

- **Failure**

Without appropriate sensory analysis, there is a high risk of market failure; sensory analysis is too frequently often overlooked as a requirement before product launch, and is often carried out to a poor standard.

- **Requirement**

➢ Sophisticated sensory booth that conform to ASTM (American Society for Testing and Materials) standard (separate air conditioning maintained at 20 ± 2^0C)

- Glassware / containers for sensory evaluation of varieties of foods
- Specialised software for chemical / physical analysis of raw materials and processed foods

- **Selection of panelists**

- Development of sensory evaluation techniques is to be based on the selection of sensory panels
- Sensory panel should be chosen (among the employees of the company) screened and trained

- **Expectations from panelists**

- To produce reliable and valid data, the sensory panel must be treated as a scientific instrument.
- It is therefore, necessary that panelists are free from any psychological features and physical conditions which might affect human judgement.
- Panelists must have an ability to perform the task and to repeat their judgements.

- **Sensory ability**

It is necessary that each panelist must be free from the following defects:

- Taste perception disorders
- Odour perception disorders
- Colour blindness
- Denature defects

- **Health**

It is necessary that each panelist must be free from the following defects

- Allergies
- Use of those medications that affect the ability to taste
- Prone to minor infections of nose and throat

- **Attitude and interest**

It is necessary that each panelist must have the following:

- Motivation
- Availability

- **Training of the sensory panel**

It should be expected that since majority of the panelists will have no previous experience in product attributes, it will therefore be necessary to train them about the products first and then to take into account their differing quality expectations

- **Psychological factors affecting sensory panel**

Some of the psychological factors that might influence the sensory measurements of the panelists include:

a. Expectation error

- This occurs when panelists are given too much information about the samples
- Therefore the panelist should not be informed about the types of ingredients used in the sensory testing.

b. Stimulus error

- This occurs when panelists are influenced by some characteristics of the samples (that is shape, size, colour e.t.c.)

- Therefore the panelists will be instructed and trained not to impose the marking of one quality parameter on others.

c. Suggestion error

- This occurs when panelists are aware of the reactions of others during the sensory evaluations.
- This should be addressed by providing panelists with individual sensory booths.

d. Halo effects

- Sometimes panelists evaluate more than one quality characteristics at a time
- They should therefore be trained and instructed to evaluate each quality parameter separately.

e.Lack of motivation

- Lack of motivation may be among the panelists due to a number of reasons.
- This can be handled by the management support, proper sensory schedules and the keen interest of all the panelists.

f. Central tendency error

- Panelist may choose the mid range to avoid extremes
- All Panelists should therefore be advised to choose the correct scale for each quality characteristics rather than just selecting the mid range of the scale to avoid extremes.

g. Order effect

- This may affect the panelists if the sensory samples are provided in a defined order.
- All samples are to be presented in a random order with a three digit number assigned to each sample to avoid the order effect.

h. Sensory team

- Based on the individual performance of each trained panelist, 5 should finally be chosen for the sensory evaluation work and 2 should be kept as reserved panelists.
- Separate sensory teams are to be selected for different types of products based on the individual abilities of the panelists.

2.17.5 Uses of sensory techniques

- To improve the sensory quality of a product or to ensure interbatch consistency
- To ensure the sensory characteristics of the products and how they influence consumer preferences.
- To understand how the products perform against competitors products in relation to consumers perception and or sensory characteristics.
- To determine whether or not consumer can detect differences between products e.g. between two products or changes in the products due to recipe modifications
- To influence product listing with retailers by presenting independent research demonstrating that the company has a greater understanding of their products profile and consumers.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection

Mature ripe sweet oranges (*Citrus sinensis*) (i.e. sound and spoilt) and sorghum grains or guinea corn (*Sorghum guineese*),and baker's yeast (instant dry yeast) were purchased from a local market in Zaria.The spoilt oranges were soft and had wounds on the skin.Brewers yeast was obtained from the Nigerian Breweries, Kaduna plant, Kaduna State .

3.2 Sample Preparation

i. Extraction of Fresh Single-Strength-Orange Juice (FSSOJ) and Juice from Spoilt Orange Fruits or Spoilt Single Strength orange Juice (SSSOJ)

One hundred and fifty (150) medium sized ripe fruits of sweet oranges (sound and spoilt)were thoroughly washed and surface decontaminated by placing the whole fruits in 80^0C water bath for 2 minutes. The oranges were then peeled, sliced and juice extracted using the stainless steel blender. The juice, (2000ml) was then clarified or filtered by the use of sterile muslin cloth into sterile bottles under aseptic condition. The samples of FSSOJ were stored for two and seven day's interval at room temperature (26-28^0C) before analysis. The same procedure for extraction of the sound oranges was also used for the spoilt ones as described above. The sample of SSSOJ was analysed immediately after extraction.

ii. Sorghum Starch Preparation

Two (2 Kg) of sprouted sorghum sweet grains or guinea corn (*Sorghum guineese*) were washed, dried and milled into flour of particle size less than 2.0 mm sieve. It was then stored on a sterile container until when needed for analysis of ethanol fermentation.

3.3 Media Preparation

The medium for the isolation of pseudomycelium and pellicle formation was prepared according to the manufacturer's instructions as follows:

Corn meal agar (Oxoid), 1.7% (w/v), sterilised by autoclaving at 121^0C for 15 minutes at 15psi(pounds per square inch) and aseptically poured into plates, was used for the observation of pseudomycelium or true mycelium.

Malt extract broth (Oxoid) was used for the observation of pellicle formation. The broth was prepared using 1.7% (w/v) malt extract and dispensedinto test tubes plugged with cotton wool and sterilised by autoclaving.

3.4 Isolation of Yeast Strains

The yeast strains were isolated from the fresh single-strength orange juice (FSSOJ) and spoilt single- strength orange juice (SSSOJ) using the techniques of Teramoto,*et al*.,(2005) with some modification. This involved using a medium containing potato dextrose agar (Oxoid), 3.5% (w/v), streptomycin 1.0% (w/v), and sodium propionate, 0.5% (w/v). The use of streptomycin and sodium propionate was to discourage the growth of bacteria and moulds respectively.

Serial dilution of each of the various samples of orange juice (sound and spoilt) was made. The stock culture was prepared by taking 50ml of the orange juice sample, and mixing it with 450ml of sterile peptone water. Each sample was serially diluted (10 fold dilution of 1:10, 1:100,

1:1000, 1:10,000) in sterile peptone water and 0.1ml of each dilution was plated out in duplicate on potato dextrose agar (PDA). The medium was prepared according to specification by the manufacturers (Oxoid). The duplicate plate was incubated at ambient temperature (26-28^{0}C) for 48-72 h.

3.5 Maintenance ofYeast Strains

The discrete isolated colonies (pure cultures) were picked out, purified by re-streaking on PDA, and maintained/ stocked on slants of the same medium at 5^{0}C in the refrigerator.

3.6 Characterisation of Yeast Strains

The yeast strains were identified based on their morphological features and biochemical characteristics according to the method used by Barnett,(2003) as follows:

3.6.1 Morphological characteristicsofyeasts

3.6.1.1 Colonial morphologyof yeasts

The yeast isolates were purified by subculturing on fresh potato dextrose agar (PDA). The plates were incubated at room temperature (26-28^{0}C) for three to five days. Observations were made on the appearance and the pigmentation of the colonies of each isolate.

3.6.1.2 Vegetative morphologyof yeasts

After the incubation period,a slide was prepared using a wet mount method by placing a drop of methylene blue dye on a pre-cleaned glass slide, some scrap of the colony was placed on the glass slide, covered with a cover slip and observed microscopically for polar or multipolar budding yeast cells (vegetative cells) at magnification of X40.

3.6.1.3 Pseudomycelium or true mycelium observationin yeasts

Pseudomycelium or true mycelium was observed by growing the purified isolates on corn meal agar (Oxoid) prepared according to manufacturer's instruction. After cooling, the plates were streaked towards oneside of the petri dish with a small inoculum using a sterile loop. Two point inoculations were made on either side of the streak; sterile cover slip was placed over one point inoculations and another one over the centre of the streak. The plates were incubated for four days at room temperature (26 – 28^0C) before examination. The areas of the inoculum under the cover slips were observed for pseudomycelium or true mycelium formation. The pseudomycelia were identified by their enlongated, equal sized cells, or elongated cells with a characteristic arrangement on the axis of pseudomycelium. The true mycelia were differentiated from pseudomycelia by continuous growth of hypha tips followed by the formation of septa.

3.6.1.4 Pellicle formationin yeasts

Formation of pellicle was observed using malt extract broth. The yeast cultures were inoculated into the sterile medium and incubated for seven days. The Formation of film on the surface of the broth indicated pellicle formation.

3.6.2 Biochemical characterisation of yeast species

3.6.2.1 Carbon assimilation test

The carbon assimilation activities of yeast species on carbohydrates were carried out by the use of Analytical Profile Index (API 20 C) kit method as described by Espinel *et al., (*1998) and Moghaddas *et al.,* (1999). The API 20 C AUX strips (Biomerieux,Lyon,France) comprises of D-glucose, glycerol, 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose , Inositol, D-sorbitol, Methyl-αD-glucopyranosides, N-acetyl-glucosamine, D-celloboise, D-

trehalose, D-melezitose and D-raffinose respectively, API C medium, API C strips, API NaCl 0.85% medium, Incubation box (Tray & lid) and the result sheet.

The API 20 C AUX strip consists of 20 cupules containing dehydrated substrates which enable the performance of the 19 assimilation tests as stated above. The cupules are inoculated with a semi-solid minimal medium. The yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source. The reactions are read by comparing them to growth controls. Identification is obtained by referring to the analytical profile index or using the identification software(Biomerieux).

3.6.2.2 API technique

An ampule of API NaCl 0.85% medium (2ml) was inoculated with a 24 h old yeast culture and the turbidity of suspension was compared with McFarland standard no. 2. One- hundred microlitre(100µl)(0.1ml) of the previous suspension was transferred into an ampule of API C medium and homogenized gently using sterile micropipette to avoid bubble formation.Five milliliters (5) of distilled water was dispensed into the honey-combed wells of the incubation box tray in order to create humid atmosphere.The API strips with 20 cupules containing 19 dehydrated substrates and 1 negative control were placed in the tray and filled with the suspension obtained in ampule of API C medium avoiding the formation of bubbles by placing the tip of the micropipette against the side of the cupule. Precautions were taken not to overfill or under-fill the cupules, the surfaces were either flat or slightly convex in order to avoid wrong results. The tray was covered with the lid and incubated at $29^0 \pm 2^0$C for 48-72 h according to the manufacturer's manual. The reactions were read by comparing the growth in each cupule with the control.Cupules more turbid than the control indicated a positive reaction meaning that the yeast was capable of utilising the substrate as the sole carbon source. The reactions were

recorded in the result sheet provided and the numerical profile constructed from the reaction patterns was used for identification of the yeast species by referring to Analytical Profile Index.

3.6.3 Fermentativeability of the yeast strains

The following parameters were used to characterise the yeast for attributes important for ethanol production and dough-leavening capacity.

3.6.3.1 Flocculencetest foryeasts

The flocculating ability of the yeast strains was tested by using a method described by Ameh *et al., (*1989) with some modifications. The test yeast was grown on PDA for 48 h harvested by centrifugation at 1500rpm for 3 minutes, and washed twice with sterile distilled water. The washed yeast cells (3.0 X 10^9 cell/ml) were suspended in 10ml of sterile distilled water and 1 ml of acetate buffer (pH 4.5) was added and mixed thoroughly with the suspension and allowed to stand for 10 minutes. The amount of sediment formed after 10 minutes indicates whether or not the yeast was flocculent (0.5-1.0 ml flocculent; 0.0-0.4 non-flocculent),using calibrated centrifuged tubes.

3.6.3.2 Ethanol tolerance test for yeasts

The ethanol tolerance was tested by qualitative and quantitative method. The qualitative test was carried out by using the method described by (Aguilera *et al*., 2006). This was based on visual assessment of turbidity and sediment in a tube of basal medium containing graded concentrations (8, 10, 12, 14, 16, 18, 20% (v/v) of absolute ethanol inoculated with fresh yeast cells at 3 X 10^9 cell/ml. The assessment was made after 48 h of incubation at room temperature (26 -28^{0}C). The quantitative test was described as stated by Nigam et al., (1998). It was based on viable counts

determined at selected intervals of incubation time (0, 24, 48 hours) at room temperature (26^0-28^0C). The medium used was made up of 2% (w/v) glucose (Analar), 0.3% yeast extract (Oxoid), 0.5% of peptone (Oxoid), 1.5% agar (Oxoid) and 10% (v/v) absolute ethanol. Percent viability was calculated for each yeast strain after the respective incubation period.

3.6.3.3 Fermentation of glucose at 37^0 and 42^0C by yeasts

The fermentative ability of the yeast was determined using glucose as the carbon source in broth (glucose in basal medium). The medium used contained graded concentrations of glucose (10, 20, 30, 40% (w/v)), 0.2% yeast extract and oxidative fermentative (O/F) medium 1.0%. A portion (8ml) of the basal medium was added into test tubes with inverted/ Durham tubes and autoclaved at 121^{0C} for 15 minutes. After cooling 2ml of filter sterilised 2% (w/v) glucose solution was added to each of the tubes. The test tubes were then inoculated with 0.1ml yeast suspension (3,0 X 10^9 cell/ml) obtained by McFarland method of the wild, mutant and standard strains. The set up was incubated at 37^0 and 42^0C respectively to determine the glucose utilisation after 48 hours. After 48 h the approximate ratio of gas-filled and unfilled Durham tubes was also determined (i.e. green- yellow indicates utilisation of glucose and green, no action on glucose).

3.6.3.4 Sugar utilisation testfor yeasts

The isolated strains were tested for their ability to ferment other sugars apart from glucose. The basal medium contained 0.2% yeast extract (Oxoid), and 1.0 % oxidative fermentative medium (O/F). This was dispensed in 8ml amounts each into test tubes containing Durham tubes and sterilised by autoclaving at 121^0C for 15 minutes. The carbon sources that were used included membrane filter-sterilised 6.0% (w/v) solution of glucose, maltose, sucrose, galactose, lactose

and 12% (w/v)raffinose. A standard suspension (0.1ml) of washed yeast cells (3.0 X 10^9 cells/ml) was introduced into each of the test tubes containing the 8ml basal medium and 2ml of each of the various carbon sources. The test tubes were incubated at room temperature for 48 hours. A basal medium without any of the sugar was also inoculated with the test organisms to serve as control. After 48 hours the test tubes were observed for gas production and colour change, which indicated fermentative ability of the yeast strains (Ameh *et al*., 1989; Oke and Ijebor, 1997).

3.6.3.5 Growth in 3% ethanol test for yeasts

The inhibition of yeast growth by 3% ethanol or utilisation of ethanol as a sole carbon source was tested using the method of Akinyemi, (1990). Aliquots (0.1) ml of 24 h washed yeast suspension (3.0 X 10^9 cells/ml) of wild, mutant and standard strains were inoculated in sterile normal saline (NaCl) 0.85% (w/v) and 1.0% (w/v) yeast extract (Oxoid) broth media containing 3% of ethanol in each case.An inoculated sterile medium without ethanol was used as control. The tubes were incubated for 48-72 h at room temperature (26-28^{0}C). Occurrence of turbidity or sedimentation was taken to indicate growth in ethanol.

3.6.3.6 Growth in 50% glucose test for yeasts

The yeast isolates were grown on a basal agar medium of 50% (w/v) glucose, 1.0% (w/v) yeast extract (Oxoid), 0.75% (w/v) peptone (LABM) and 2% (w/v) agar (Oxoid), incubated at room temperature (26-28^{0}C) and examined for growth within four to seven days.

3.6.3.7 Growth at elevated temperatures test for yeasts

The following temperatures, 30, 35, and 42^{0}C, were chosen and used for the observation of growth of the yeast isolates. Yeast strains were streaked on potatoes dextrose agar plates and

incubated at the different temperatures indicated above. Observation for growth was made after 48-72 hours incubations.

3.6.3.8 Growth in 3 and 10% sodium chloride test for yeasts

The medium that was used to observe growth on sodium chloride (NaCl) was composed using 3% and 10% (w/v) sodium chloride, also 3.0% PDA, 1.0% (w/v) yeast extract (Oxoid),0.75% (w/v) peptone and 2.0% (w/v) agar (Oxoid) was added to make up the medium. The sterile medium was dispensed into sterile Petri dishes and allowed to set. Yeast strains were streaked on agar surface and examined for growth after four to seven days of incubation at room temperature (26^0-28^0C).

3.7 Genetic Modification of the Characterised Yeast Species.

A 2-stage modification mutation treatment as reported by Ado (2004) using nitrous acid and UV irradiation at 254 nm was carried out on the yeast species isolated, thus:

3.7.1 Mutation of yeasts using nitrous acid

A small quantity (0.15ml) of sterile freshly prepared 2.0M sodium nitrite was added to 5ml of the yeast suspension of the organism (3.0 X 10^9cells/ml) in 10ml acetate buffer (0.2M, pH 4.4). The mixture was allowed to stand at room temperature for 20minutes and thereafter the reaction was stopped by serial dilution in tris/HCl buffer (0.2M, pH 8.0).An aliquot(1ml) of the diluted suspension was plated on potato dextrose agar and incubated at room temperature (26-28^0C) for 72-96 h. After incubation, the total viable counts of the organisms were determined.

3.7.2 Mutation of yeasts using UV irradiation at 254nm

A suspension of 3.0 X 10^9 cells/ml yeast population of the actively growing yeast, 10ml each in Petri dishes was exposed to 254nm UV irradiation at a distance of 6cm for 4, 6, 10, 15, 20, 25, and 30minutes.This was used to determine the exposure time that effected mutagenesis on the yeast cells. After the intervals of exposure, the organisms were recovered and kept in the dark room to prevent photoreactive action. A postirradiation treatment was carried out with2% (w/v) caffeine for 5 hours to prevent dark repairs.This was centrifuged at 1500rpm for 5 minutes to remove the caffeine and thereafter re-suspended (i.e.the dark repair treatment) in 0.85% (w/v) sterile saline (normal saline). Then the tubes, after the irradiation treatment were kept in the dark at 18^{0C} for 12-16hours and thereafter, the organism serially diluted (10^{-1} to 10^{-6}).Then,an aliquot of 0.1ml of each dilution was plated on potato dextrose agar and incubated at room temperature (26-28^0C) for 72-96 h to allow for expression of the mutants (i.e. replication of the modified gene). The mutants (yeasts cells) was then used for the next stage that isthe ethanol production and determination of the dough-leavening/rising capacity of the yeast strains.

3.7.3 Screening for nutritional mutants

The qualitative positive auxanography technique as described by Ursula, (1978) and Pronk ,(2002)with some modification was used for the screening of UV and nitrous acid mutants thus: it involved visual assessment of turbidity and sediment in a tube of minimal basal medium (broth) containing (w/v) glucose25%, (w/v) 15% $NaNO_3$; (w/v) 1.3% $MgSO_4.7H_2O$; (w/v) 1.3% KCl; (w/v) 3.8% KH_2PO_4; (w/v) 10mg $FeSO_4.7H_2O$, (w/v) 1.0mg $ZnSO_4.7H_2O$ and 2.5 litres of distilled water. The pH was adjusted to 6.5 and sterilised by autoclaving at 121^0C for 15 minutes. Eight essential aminoacidsleucine, lysine, methionine, histidine, cysteine, tryptohan, isoleucine and valine were used.An aliquot (1ml) of each of these aminoacids was added to the test

organisms of both UV and nitrous acid mutants. A control was prepared containing each of the aminoacids. The inoculated tubes and the control were all incubated at (26-28^{0C}) for 24 - 72 h and the broths were observed for growth. Cloudy broths were recorded as positive utilisation of the test amino-acids.

3.8 Ethanol Production using the Wild Strains,Modified and Brewer's Yeast.

3.8.1 Inocula preparationof the yeasts strains

Yeast inocula were prepared by using slant cultures to inoculate 20ml of sterile YM agar medium which contained yeast extract 3g, malt extract 3g, peptone 5g, glucose 10g and 20g agar (per litre). Yeast cells was cultured in a rotatory shaker set at a speed of 150 revolution per minute (rpm) at 30^{0}C for 72 h in a pre-culture medium containing 2% (w/v) glucose, 0.5%(w/v) peptone, 0.3% (w/v) yeast extract, 0.1% (w/v) potassium hydrogen phosphate (KH_2PO_4), and magnesium tetraoxosulphate (IV) ($MgSO_4.7H_2O$) at pH 5.5 (Corredor, 2006).

3.8.2 Substrate preparationfor ethanol fermentation

The raw biomass(sprouted sorghum)(2kg) was washed, dried and milled into flour of particle size less than 2.0mm sieve as described by Gibbons and Westby (1986). The flour was stored in sterile container until needed for analysis.

3.8.3 Preparation of theyeast fermentation medium

Ethanol fermentation was carried out in500ml Erlenmeyer flasks each containing 200ml of medium as described below. Five grammes (5g) of starch substrate (sprouted sorghum flour), was weighed into 400ml basal medium composed of peptone, 0.4g, malt extract 0.4g, yeast extract 0.8g, calcium carbonate, 0.8g ammonium phosphate, 0.8, ferrous sulphate, 0.004g and

magnesium sulphate 0.8g. The pH of the medium was adjusted to 6.5 with 1.0N hydrochloric acid . The flasks with basal medium were sterilised by autoclaving at 121^0C for 15minutes and inoculated with 5% yeast inocula from wild, modified and standard strains containing 3.0 X 10^9 cells /ml. The flasks were incubated on a shaker with an agitation rate of 150 rpm at 30^0C for 7 days. The ethanol yield was determined at the end of fermentation period using the titrimetric dichromate method (Pilone, 1985; Ameh *et al.,* 1989).

3.8.4 Analyticalprocedures

Twenty millilitres (20ml) samples from each fermentation flask were withdrawn after thorough shaking of the fermentation flask in order to homogenize the contents at 24 hours interval during fermentation.The withdrawn sample was used for the analysis of cell biomass, cell number, ethanol and sugar concentrations.

3.8.4.1 Analysis of cell biomass

The biomass concentration was determined by the dry weight methodas decribed by Association of Official Analytical Chemicals(A.O.A.C.)(2000). The cells were separated by centrifuging at 1500rpm for 3minutes, repeatedly washed with sterile distilled water and then used for determination of cell growth. The cells were dried at 70^0C for 2 h and weighed. The constant weight was expressed per 100ml of fermentation medium.

$$\text{Dry Weight} = \frac{W_2 - W_1}{W_3} X\ 100$$

Where: W_1 = Weight of filter paper

W_2 = Weight of filter paper + Sample after drying

W_3 = Volume of Sample used.

3.8.4.2 Determination of cell number

The samples, 0.1ml each of fermented broth of wild, modified and standard strains were measured into a curvette and the optical density (absorbance) was measured with spectrophotometer(6405 UV/VIS Jenway,England) at 560nm using distilled water as blank.

3.8.4.3Ethanol concentration

The ethanol yield was determined by the use of titrimetric dichromate method after distillation as described by (Pearson,1981; Pilone, 1985; Ameh *et al*,. 1989).

Qualitative test for ethanol:Five millilitres (5ml) of the fermented broth was measured into a beaker; 0.1g of potassium dichromate crystal and 5drops of concentrated sulphuric acid were added.Acolour changefrom yellow to green would indicate the presence of ethanol (Pilone, 1985).

Sample preparation:Thirty (30ml) samples of the fermented broth from each of the culture medium ofwild, modified and standard yeast strains were taken every 24 h and centrifuged at 1500rpm for 5 minutes to remove cells. The cell-free supernatant recovered was distilled using a 50ml distillation unit at 78^0_C over uniformtime duration for all the samples.

Alcohol contentof ethanol:The distillate obtained was used to determine the ethanol yield by the titrimetric dichromate method as described by (Pearson, 1981; Pilone, 1985;Ameh,*et al*;1989) as follows:

Two millilitres (2ml) each of the distillate from the various samples of wild, modified and standard yeasts species were pipetted into 2ml of 0.05mol.L^- of the acidified potassium dichromate solution. The mixture was heated at 60^0C for 20minutes to complete the oxidation of

ethanol. The excess dichromate remaining in the solution was titrated with 0.3mol.L^{-} of standard solution of Iron II ammonium sulfate. When the brown colour of the solution gets a green tinge, 5 drops of 1, 10-0-phenanthroline indicator was added, the titration was continued until the colour changed from a green tinge to intensely green. The ratio of volume of titrant consumed by the sample (VA) and the blank (VB) was used in computing the percentage ethanol content (V/V) as fellows:Ethanol (%v/v) = 25-25 (VA/VB).

3.8.4.4 Reducing sugars

The liberated glucose in the culture fermentation broth of the wild, modified and standard species of yeasts was analysed by optical density measurement at 540nm by dinitrosalicylic acid (DNSA) method of Miller *et al.* (1959).

Two millilitres (2ml) of the withdrawn fermented broth and centrifuged samples were added to 2ml of DNSA solution in a test tube and heated at 60^0C in a water bath for 5 minutes for colour development. After cooling at room temperature (26^0-28^0C), 4ml of each of the samples were introduced into a curvette and measured against distilled water as blank using spectrophotometer(6405UV/VIS Jenway England) at 540nm. The glucose level was read against a standard curve prepared earlier.

Preparation of DNSA reagent: DNSA reagent was prepared by dissolving 1g of DNSA, 2.0M sodium hydroxide (i.e. 8g) in 30ml distilled water.Thirty grammes (30g) of sodium potassium tartarate (Rochelle salt) was also dissolved in 30ml distilled water. The two mixture were mixed, heated with constant stirring until it dissolved completely, and then was diluted up to 100ml with distilled water. The optical density of the solution was standardised from 0.40-0.50 at 540nm using fructose.

3.9 Propagation of Yeast Species for Use in Dough Leavening Tests

The yeast isolate was propagated using the procedure of Ameh and Umaru (2000) with some modifications. The propagation was carried out in 250ml Erlenmeyer flasks each containing 150ml basal medium composed of glucose, 2% (w/v); yeasts extract (Oxoid) 0.5% (w/v); peptone (LABM) 1% (w/v); ammonium tetraoxosulphate VI ($(NH_4)_2SO_4$) 0.1% (w/v) and magnesium tetraoxosulphate VI ($MgSO_4$) 0.1% (w/v). The pH of the medium was adjusted to 5.6 using few drops of 1.0N HCl prior to autoclaving. The sterilised medium was inoculated with 3.0 X 10^9 cells/ml wild and modified yeasts strains and incubated on a shaker at an agitation rate of 150rpm at 30^0C for 7days. The cells were harvested by centrifugation and rinsed twice with sterile distilled water. The cream yeast obtained after decanting supernatant was stored in refrigerator for approximately 24 hours before use.

3.9.1 Dough leavening test

The test was carried out in two phases: first, sweetened and second unsweetened dough leavening test. The method used was as described by Ricon *et al.,(*2001) and Brookline, (2004).The test material consisted of 50%(w/v) wheat flour, 15%(w/v) sucrose, 1%(w/v) sodium chloride (salt), 1%(w/v) vegetable oil, 30% and 40%(v/v) sterile distilled water, 2%(w/v) laboratory harvested cream yeast (wild and modified) and commercial bakers instant dry yeast. Thirty percent (30%) sterile distilled water was used for mixing thesweetened dough. The ingredients were mixed and the dough kneaded until it was smooth. Then it was deposited at the bottom of 250ml sterile measuring cylinders whose sides had been smeared with a layer of vegetable oil to prevent the dough from sticking to the sides of the cylinders. The cylinders were labeled, sealed with foil paper and incubated at 30^0C for 3, 24 and 48 h respectively. The volumes of the dough were recorded at zero hour and thereafter for every 30 minutes for 3,24

and 48 h (1440 and 2880 minutes) respectively. The fermention time was extended from 3 -48h because the laboratory yeast did not leaven the dough at 3h interval as was observed in the control strain(commercial baker's yeast).The unsweetened dough was prepared as stated above except that sucrose was not added. The dough leavened with baker's yeast served as the control Proof eights of each isolate are taken as the difference between the final dough volume and the initial reading taken at zero minute.

3.10 Bread Baking Process

The dough (sweetened and sweetened) was fermented at 27 ± 1^0C for proofing for 3,24, and 24h interval.At the end of proofing the bread dough was baked for 45 minutes at 180^0C in a hot air oven. (France andWragg, 1998). The bread was cooled for 1hour at 27 ± 1^0C after baking, then placed in apolythene bag and stored at27 ± 1^0C for 18-24h before sensory evaluation.

3.11 Consumer Panel Sensory Evaluation

The sensory quality of the bread was analysed using a30-semi- trained consumer panelists using the approved AACC(American Association of Cereal Chemists,2000)method.The panelists received encoded samples and questionnaires as well as instruction for the evaluation of the samples.Panelists used hedonic scale of 1(lowest) to 5 (highest) to judge the following sensory attributes:texture,visual,appearance (colour),aroma and overall acceptability (Appendix III).

3.12 Statistical Analysis

Data obtained from the different experimentations were subjected to statistical analysis where applicable using statistical package social sciences (SPSS) version 20. The dough leavening test was analysed using ANOVA and paired t-test to determine the mean performance between the baker's yeast strain, the wild strains and the mutants of nitrous acid-induced and UV light irradiation

onsweetened and the unsweetened dough. The sensory evaluation results were also analysed using Analysis of Variance (ANOVA) and the means were separated using Duncan multiple Range Test (DMRT) for the bread samples (sweetened and unsweetened). All tests were conducted at 5% significant level. (Appendices XII - XVII).

CHAPTER FOUR

4.0 RESULTS

4.1 Morphological and Physiological Characteristicsof Yeast Strains Isolated from Fresh and Spoilt Single Strength Orange Juice.

A total of 51 yeast isolates were obtained based on their morphological and physiological characteristics and were identified to the species level using API20C AUX kit (BIOMERIUX). Morphological characteristics of the yeast isolates such as colonial morphology (colony pigmentation, size and surface appearance), microscopic morphology (cell shape and arrangement), and presence of pseudomycelium or true mycelium and pellicle formation are as shown in Table 4.1. The colonies of the yeast strains isolated were spherical, creamy, smooth, raised and large. Some appeared pinkish, smooth, shiny and flat. Microscopically, the vegetative cells were spherical, oval, elongated with uni or multipolar budding.

The prevalence of the yeasts isolated and identified showed that *Candida krusei* 29 (56.86%) had the highest isolation rate followed by *Rhodotorula minuta* 10 (19.60%). Others include *Candida zeylanoides* 4(7.84%), *Candida parapsilosis* 3(5.88%), *Geotrichum capitatum* 2(3.92%), *Candida lusitaniae* 1(1.96%), *Candida norvegensis* 1(1.96%) and*Kodamaea ohmeri* 1(1.96 %) (Fig 4.1and Appendix II).

Table 4. 1:Morphological and Physiological Characteristics of Yeast Strains Isolated from Fresh and Spoilt Single Strength Orange Juice.

CP	CSA	CS	AR	PTM	PF	GG	GE	GN_1	GN_2	30^0C	35^0C	37^0C	42^0C	Suspected Organism
White to creamy	Smooth, raised and large	Spherical and oval	Singly, budding	+/-	+/-	+	+	+	+/-	+	+	+	+	*Candida spp*
Creamy	Smooth, and large	Spherical cells	Multipolar, budding	+/-	-	-	+	+	-	+	+	+	+	*Geotrichum spp*
Creamy	Smooth, and large	Ellipsodial cells and oval	Singly, budding	-	-	+	+	+	-	+	+	+	+	*Kodamaea spp*
White and pinkish red	Smooth, large and shiny	Spherical cells	Singly, budding	-	+/-	+	+	+	+	+	+	+	+	*Rhodotorula spp.*

Key: **CP** = Colony pigmentation **AR** = Arrangement **GG** = Growth on 50%w/v glucose

CSA = Colony surface appearance **PTM** =Pseudomycelium/true mycelium **GE** =Growth on 3% (v/v) ethanol

CS = Cell shape **PF** = Pellicle formation **GN_1** =Growth on 3% (w/v)NaCl **GN_2**=Growth on 10% (w/v) NaCl

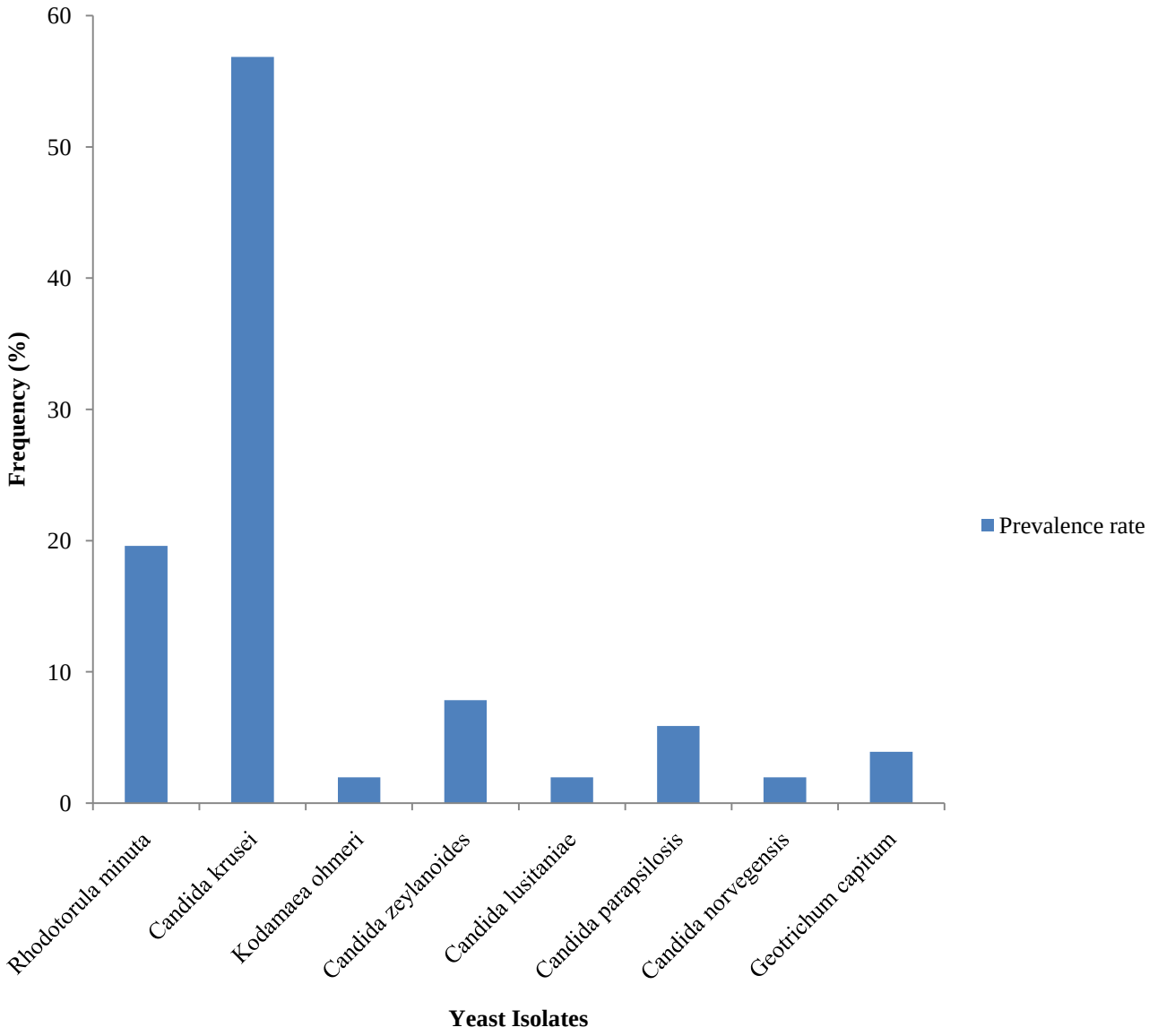


Fig 4.1: Prevalence Rate of Yeast Strains Isolated from Fresh and Spoilt Single Strength Orange Juice

Physiological properties such as growth on 50% (w/v) glucose, ethanol 3% (v/v), sodium chloride 3% and 10% (w/v) and growth at elevated temperatures are presented in Table 4.1. All the strains utilised glucose for growth except *Geotrichum* species. They showed growth at various elevated temperatures of 30, 35, 37 and 42^0C. Some species of *Candida* and *Geotrichum* did not grow at 10% (w/v) sodium chloride (Table 4.1). Other physiological properties such as carbon assimilation, flocculation rate, tolerance to ethanol concentration, ethanol concentration and fermentative potentials of yeast strains isolated are presented in Tables 4.2, 4.3, 4.4, and 4.5 respectively.

4.2 Carbon Assimilation

D-glucose was assimilated by all the yeast strains except *Geotrichum* species, while *Candida norvegensis* did not assimilate any of the other sugars except D-glucose. The following strains *Candida lusitaniae, Candida parapsilosis, Kodamaea ohmeri,* and *Rhodotorula minuta* assimilated glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, sorbitol, methyl-α D-glucopyranoside, N-acetyl-glucosamine, D-maltose, D-sucrose, D-trehalose and D-melezitose respectively. *Candida zeylanoides* assimilated, calcium 2-keto-gluconate and D-cellobiose . *Candida krusei* assimilated only glycerol and N-acetyl-glucosamine (Table 4.2).

Table 4. 2:Carbon Assimilation Test Using API20C AUX Kit (BIOMERIEUX)

GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF	IDENTITY
+	+/-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	***Candida krusei***
+	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	***Candida lusitaniae***
+	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	-	***Candida parapsilosis***
+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	***Candida zeylanoides***
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	***Geotrichum capitatum***
+	+	+	-	-	+		+	-	-	+	+	-	-	+	+	+	-	-	***Kodamaea ohmeri***
+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+/-	+/-	-	***Rhodotorula minuta***
+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	***Candida norvegensis***

Key: + = Carbon assimulated (turbid), - = Carbon not assimulated (non-turbid), +/- =variation

GLU = D-glucose ADO =adonitol MDG =methyl-α-D-glucopyranoside SAC =D-sucrose
GLY = glycerol XLT = xylitol NAG =N-acetyl-glucosamine TRE = D-trehalose
2KG = calcium 2-keto-gluconate GAL = D-galactose CEL = D-cellobiose
ARA = L-arabinose INO = inositol LAC =D-lactose
RAF = D-raffinose XYL = D-xylose SOR =sorbitol
MAL =D-maltose MLZ = D-melezitose

4.3 Flocculation Rate

Among the yeast strains analysed, all were found to be flocculent except one that showed poor flocculation rate. Their flocculation rates for the flocculent yeast strains ranged from 0.6 - 1.1ml/10minutes for the following strains *Candida krusei/inconspicua, Candida parapsilosis, Geotrichumcapitatum* and *Kodamaeaohmeri*. *Candida zeylanoides* and *Candida lusitaniae* had a flocculation rate of 0.6ml and 0.8ml/24hours respectively. The only strain that showed poor flocculation rate was *Candida norvegensis*, which was 0.4ml per 16 h. The flocculation rate for the brewing strain of *Saccharomyces carlbergensis* was 0.5ml per 10 minutes (Table 4.3).

4.4 Ethanol Concentration Tolerance

Six out of the eight strainsisolated from four genera tolerated ethanol from 8 to 18%v/v. These are *Candida krusei, Candida lusitaniae, Candida zeylanoides, Geotrichum capitatum, Kodamaea ohmeri* and*Rhodotorulaminuta.*Two strains,*Candida norvegensis* and *Candida parapsilosis*, tolerated ethanol from 8-12% and 16%v/v and one strain*Candida lusitaniae*tolerated ethanol up to 20%v/v. The brewing strain *Saccharomyces carlbergensis*toleratedethanol (8 - 16%v/v) (Table 4.3).The result obtained when tolerance to 10%(v/v) ethanol was tested quantitatively in terms of the relative viabilities of the yeast strains after various incubation periods (0, 24 and 48 hours), showed *Geotrichumcapitatum, Rhodotorulaminuta* and *Candida krusei* had a high tolerance of 1.76, 1.63 and 1.2% viability respectively. The rest of the strains exhibited various degrees of poor ethanol tolerance at 10% (v/v) concentration ranging from 0.5-0.9% cell viability (Table4.3).

Table 4. 3:The Performance of Identified Yeast Strains with respect to Flocculation Rate, Tolerance to Graded Ethanol Concentration and Ethanol Tolerance, Measured as % Cell Viability of the Yeasts in 10% (v/v) Ethanol

Yeast strain	Flocculation rate (ml/10mins)	Ethanol concentration tolerance							Ethanol tolerance, measured as viability of yeast in 10% (v/v)		
		8	10	12	14	16	18	20	0 hour	Viable count at 48 hours (CFU/ml)	% cell viability ***
C. norvegensis	**0.4**	+	+	+	-	-	-	-	**3.0×10^9**	**2.5×10^7**	**0.83**
Saccharomyces carlbergensis*	**0.5**	+	+	+	+	+	-	-			
C. lusitaniae	**0.6**	+	+	+	+	+	+	+	**3.0×10^9**	**1.5×10^7**	**0.5**
Candidakrusei	**0.8****	+	+	+	+	+	+/-	-	**3.0×10^9**	**3.6×10^7**	**1.2**
C. zeylanoides	**0.8**	+	+	+	+	+	+	-	**3.0×10^9**	**1.7×10^7**	**0.57**
G. capitatum	**0.8**	+	+	+	+	+	+	-	**3.0×10^9**	**5.3×10^7**	**1.76**
C. parapsilosis	**0.9**	+	+	+	+	+	-	-	**3.0×10^9**	**1.9×10^7**	**0.63**
K. ohmeri	**1.0**	+	+	+	+	+	+	-	**3.0×10^9**	**2.7×10^7**	**0.9**
Rhodo. Minuta	**1.1**	+	+	+	+	+	+/-	-	**3.0×10^9**	**4.9×10^7**	**1.63**

Keys: *** Ethanol tolerance, measured as % cell viabilityof yeast in 10% (v/v) ethanol computated according to this equation:

$$\frac{\text{count at 48hours}}{\text{Initial viable count}} \times \frac{100}{1}$$

*Control
** Values are averages of duplicate determinations.
Sedimentation rate; 0.0-0.4 = -ve
0.5-1.0 = +ve

4.5 Fermentation Activities

The abilities of the yeast strains to grow in 10-50%(w/v) glucose and ferment other sugars are shown in Tables 4.1, 4.4 and 4.5. All the isolates did not ferment lactose and raffinose. The strains of *Candida lusitaniae, Candida parapsilosis* and *Kodamaea ohmeri* fermented galactose, maltose and sucrose. Some of the strains fermented glucose with gas production, these include; *Candida krusei, Candida zeylanoides, Kodamaea ohmeri, Rhodotorula minuta*,other strains such as *Candida lusitianiae, Candida norvegensis* and *Candida parapsilosis* also fermented glucose but without gas production. (Table 4.4). The industrial strain *Saccharomyces carlbergensis* did not grow at 42^0C, but grew at 37^0C and fermented 10-40% (w/v) glucose (Table 4.4). *Rhodotorulaminuta* fermented sucrose in addition to glucose, while *Candida krusei* fermented only glucose (Table 4.5). Majority of the isolated strains fermented 10-40 % (w/v) glucose and showed growth at elevated temperatures of 32, 35, 37 and 42^oC respectively.

Table 4. 4:Fermentative Performance of Yeast Strains with Glucose at various Concentrations and Growth at Elevated Temperatures

	Fermentative performance with glucose %(w/v)								Growth at elevated temperatures (^oC)			
Yeast Strains	**37^oC***				**42^oC***				**30****	**35**s**	**37****	**42****
	10	**20**	**30**	**40**	**10**	**20**	**30**	**40**				
Candida krusei/inconspicua	++	++	++	++	++	++	++	++	+	+	+	+
C. lusitaniae	+	+	+	+	+	+	+	+	+	+	+	+
C. norvegensis	+	+	+	+	+	+	+	+	+	+	+	+
C. parapsilosis	+	+	+	+	+	+	+	+	+	+	+	+
C. zeylanoides	++	++	++	++	++	++	++	++	+	+	+	+
G. capitatum	-	-	-	-	-	-	-	-	+	+	+	+
K. ohmeri	++	++	++	++	++	++	++	++	+	+	+	+
Rhodo. minuta	+	+	+	+	+	+	+	+	+	+	+	+
Saccharomyces carlbergensis	++	++	++	++	-	-	-	-	+	+	+	-

Key: + **=*** Glucose fermentation within 48 hours without gas production

++ = * Glucose fermentation within 48 hours with gas production

- **= *** Nofermentation of glucose within 48 hours

+ **= **** Growth at elevated temperature

- = ** No growth at elevated temperature

Table 4. 5:Fermentative Pattern of Yeast Strains for Some Sugars

Yeast strain	Glucose	Galactose	Maltose	Sucrose	Lactose	Raffinose
Candida krusei	+	-	-	-	-	-
Candida lusitaniae	+	+	+	+	-	-
Candida norvegensis	+	-	-	-	-	-
Candida parapsilosis	+	+	+/-	+/-	-	-
Candida zeylanoides	+	-	-	-	-	-
Geotrichum capitatum	-	-	-	-	-	-
Kodamaea ohmeri	+	+	+	+	-	-
Rhodotorula minuta	+	-	-	+	-	-
Saccharomyces Carlbergensis	+	+	+	+	-	-

Keys: + = Sugar fermentation within 24 – 48 hours

\- = No fermentation after 24 – 48 hours.

+/- = Most strains fermented the sugar while some did not.

4.6 Mutagenic Treatments of the YeastIsolates

The effects of the mutagenic treatments on the yeast isolates using UV light irradiation and nitrous acid treament are shown in Figs 4.2 - 4.9, AppendicesIV a-g and V. The effects of ultraviolet light (UV) irradiation on the yeast strains at 4 minutes showed % survival rates which ranged from 49.72 to 83.72%.The yeast strain of *Candidalusitaniae* had the least% survival rate of 49.72% and *Rhodotorula minuta* had 83.72% as the highest % survival rate (Fig.4.2).At 6 minutes, % survival rate ranged from 45.24- 76.75%.Candida norvegensis was the least with a% survival rate of 45.24%, while *Rhodotorulaminuta* had 76.74% as the highest survival rate.(Fig. 4.3). The effects of ultraviolet light (UV) irradiation at 10 minutes, the irradiation on the yeast strains % survival ranged from 31 - 66.98%. *Candidaparapsilosis* with survival rate of 31.97% was the least and*Candida norvegensis*was the highest with survival rate of 64.77%(Fig. 4.4).

The percentage survival of the effects of UV irradiation at 15 minutes ranged from 24.59 - 57.84%.The yeast strain of *Candidaparapsilosis* had a % survival rate of 24.59%as the least, and*Geotrichum capitatum* had the highest survival rate of 57.84%(Fig4.5).The effect of UV-irradiation on the % survival at20 minutes ranged from 21.7- 45.45%.*Candidaparapsilosis* yeast strain had the least % survival rate of 21.72%,and *Kodamaeaohmeri* had the highest % survival rate of 45.45 % (F ig. 4.6).The effect of UV- irradiation on the % survival at 25 minutes ranged frorm 4.09- 37.73%.The yeast of *Candidaparapsilosis* with % survival of 4.09% was the least,while *Candidazeylanoides* with the % survival rate of 37.73% was the highest (Fig. 4.7).The percentage survival of the effect of UV-irradiation on the yeast strains at30 minutes ranged from 12.15 - 31.82%(Fig.4.8).The yeast strain of *Candidalusitaniae* with a % survival rate of 12.15% was the least and *Kodamaea ohmeri* with a% survival rate of 31.82 % was the highest (Fig4.8).The effect of nitrous acid treatment on the yeast strains at 20 minutes showed that thepercentage survival ranged from 0.81 - 3.87%.*Candidakrusei* yeast strain had the least % survival rate of 0.81%,and *Candidakrusei* had the highest % survival rate of 3.87%(Fig. 4.9).

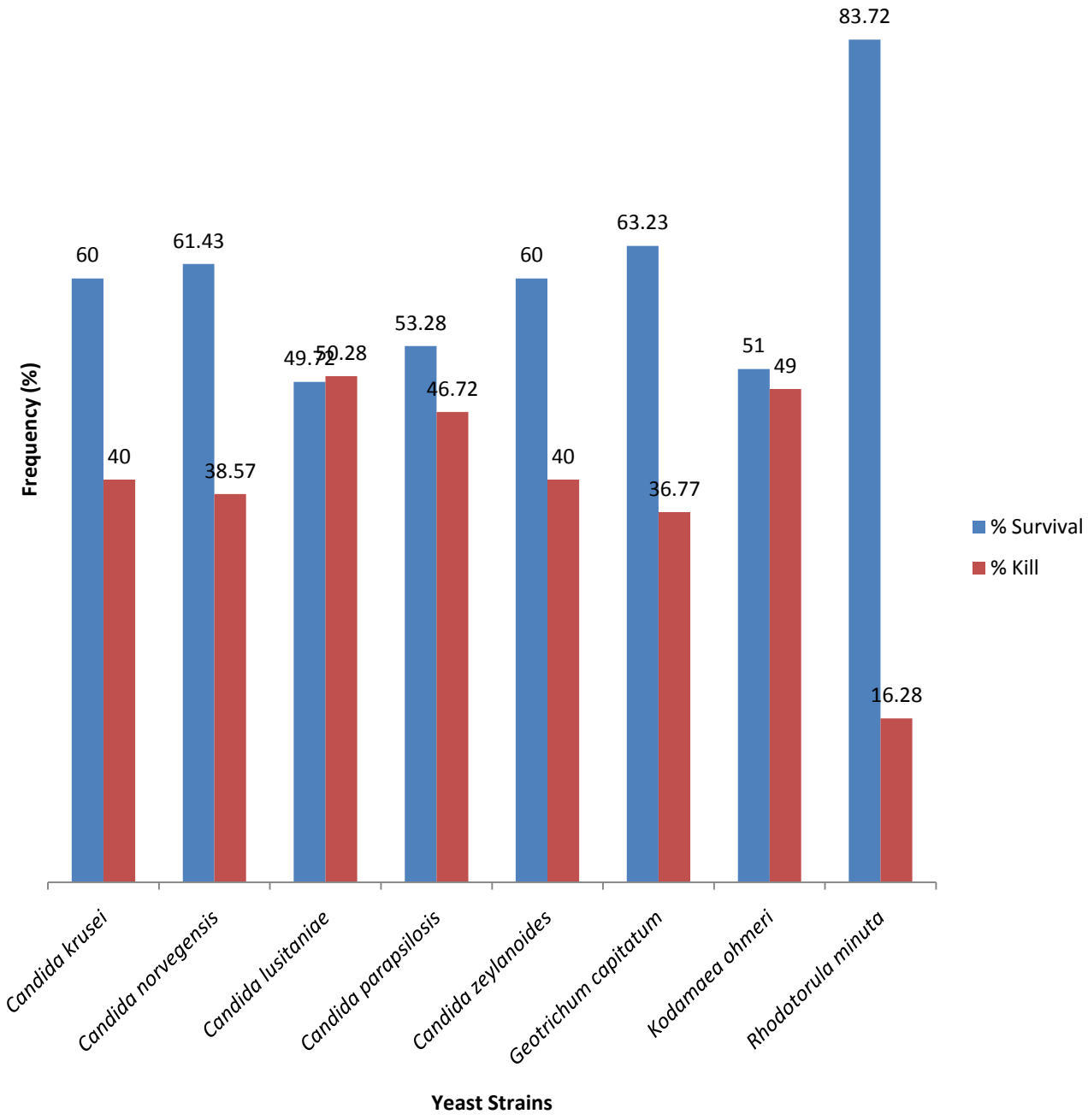


Fig 4.2: Percentage Survival and Kill Ratesof UV Irradiated Yeast Strains at 4 minutes.

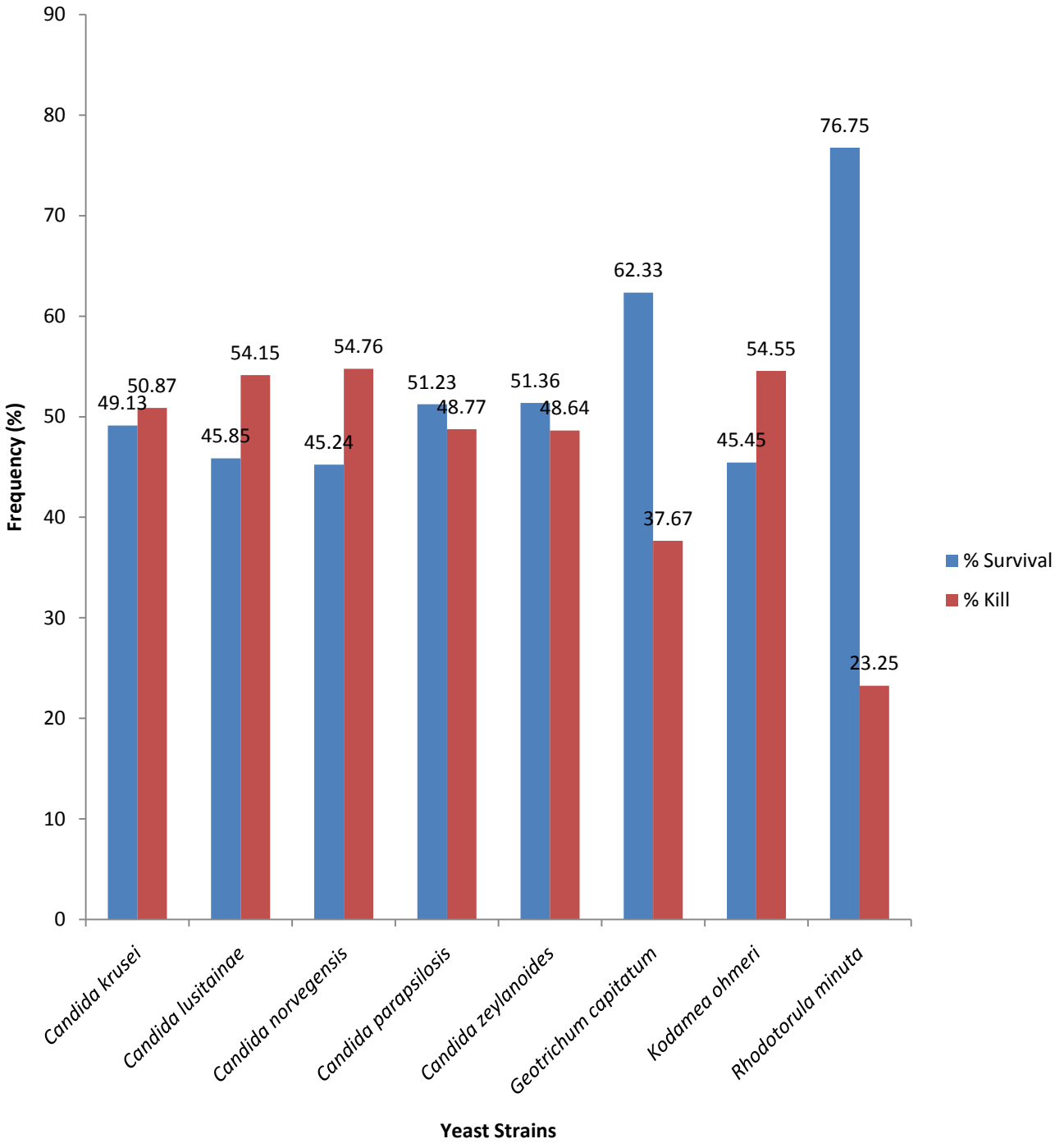


Fig 4.3: Percentage Survival and Kill Ratesof UV Irradiated Yeast Strains at 6 minutes.

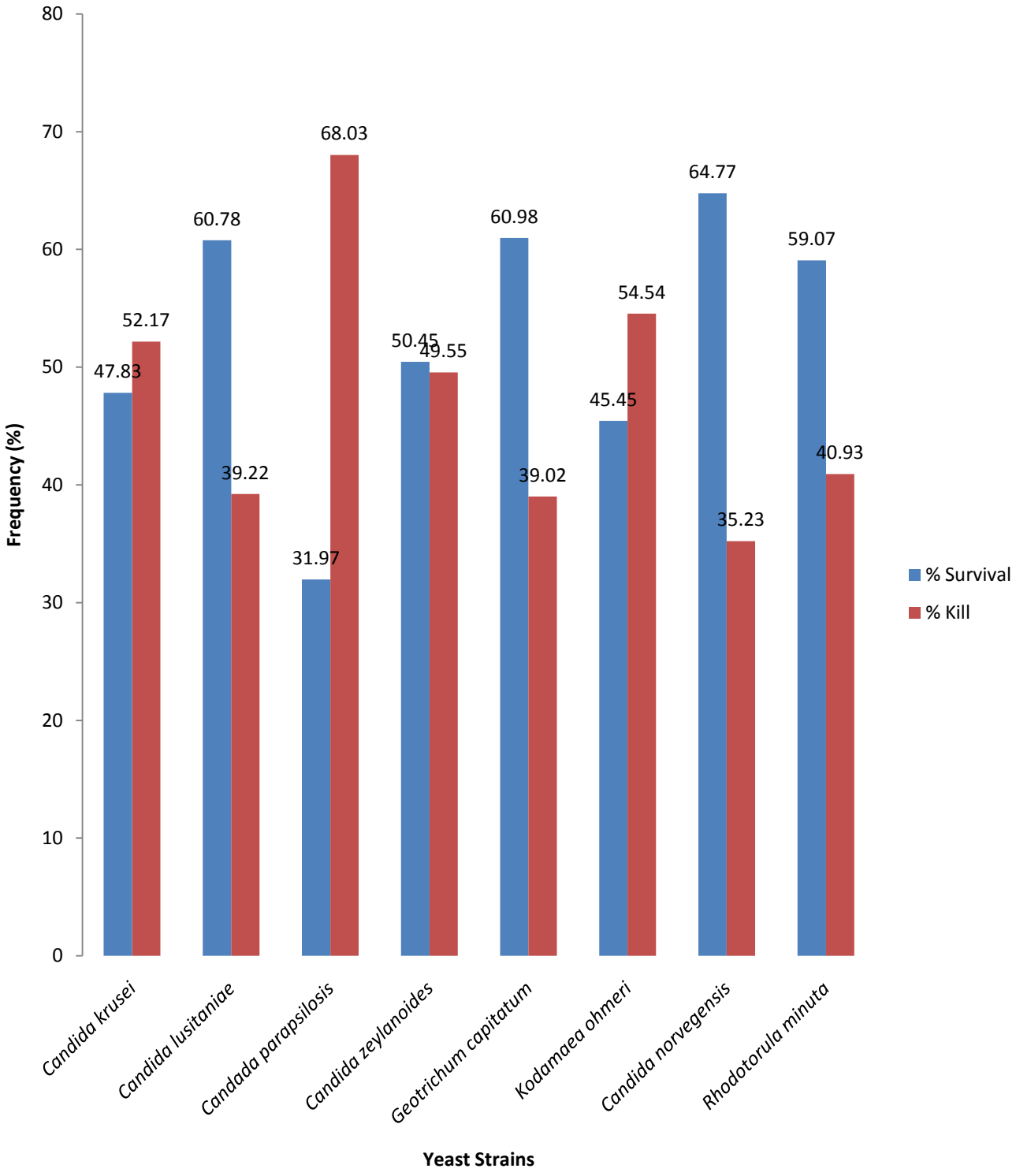


Fig 4.4: Percentage Survival and Kill Ratesof UV Irradiated Yeast Strains at 10 minutes.

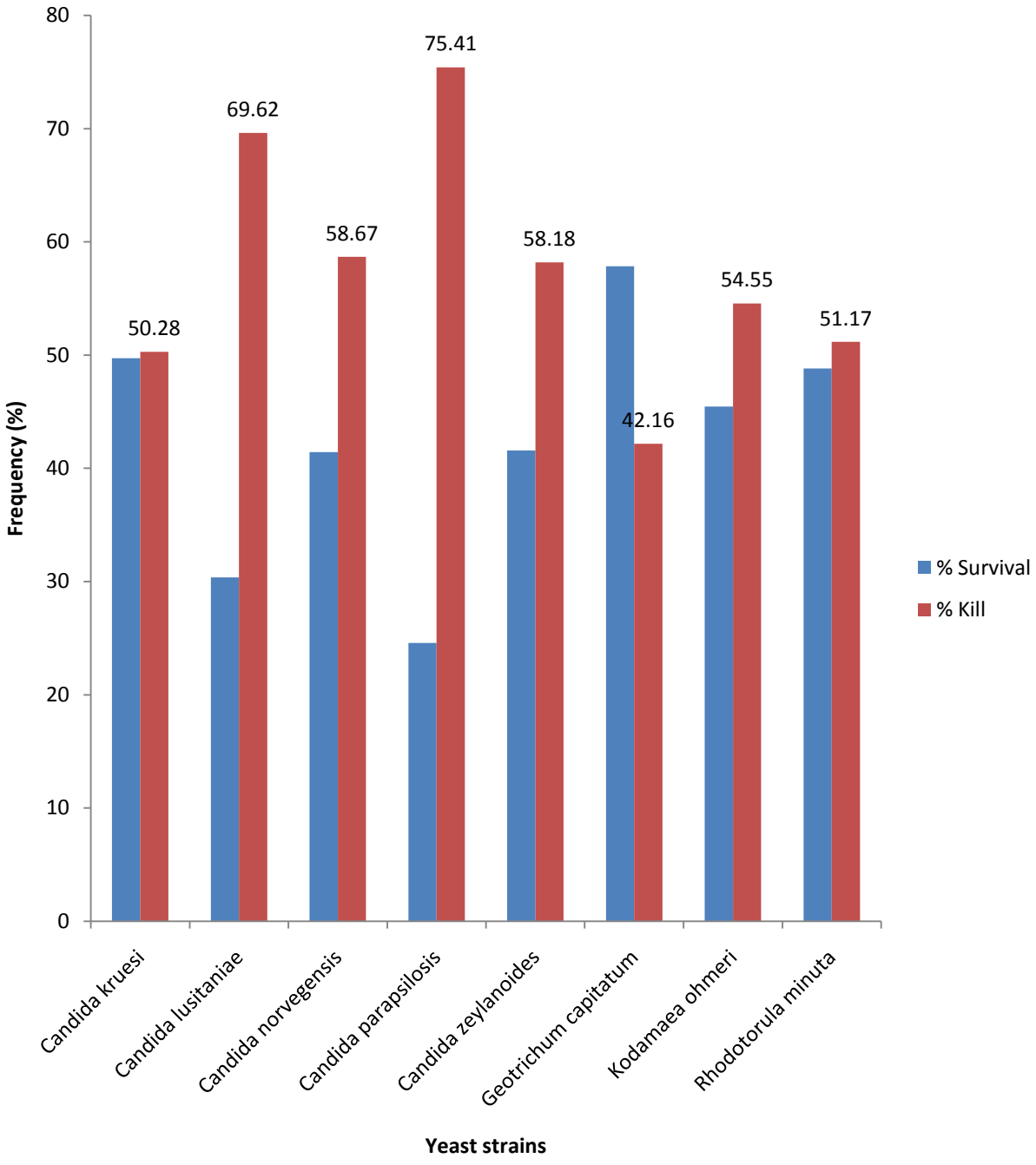


Fig 4.5: Percentage Survival and Kill Rates of UV Irradiated Yeast Strains at 15 minutes.

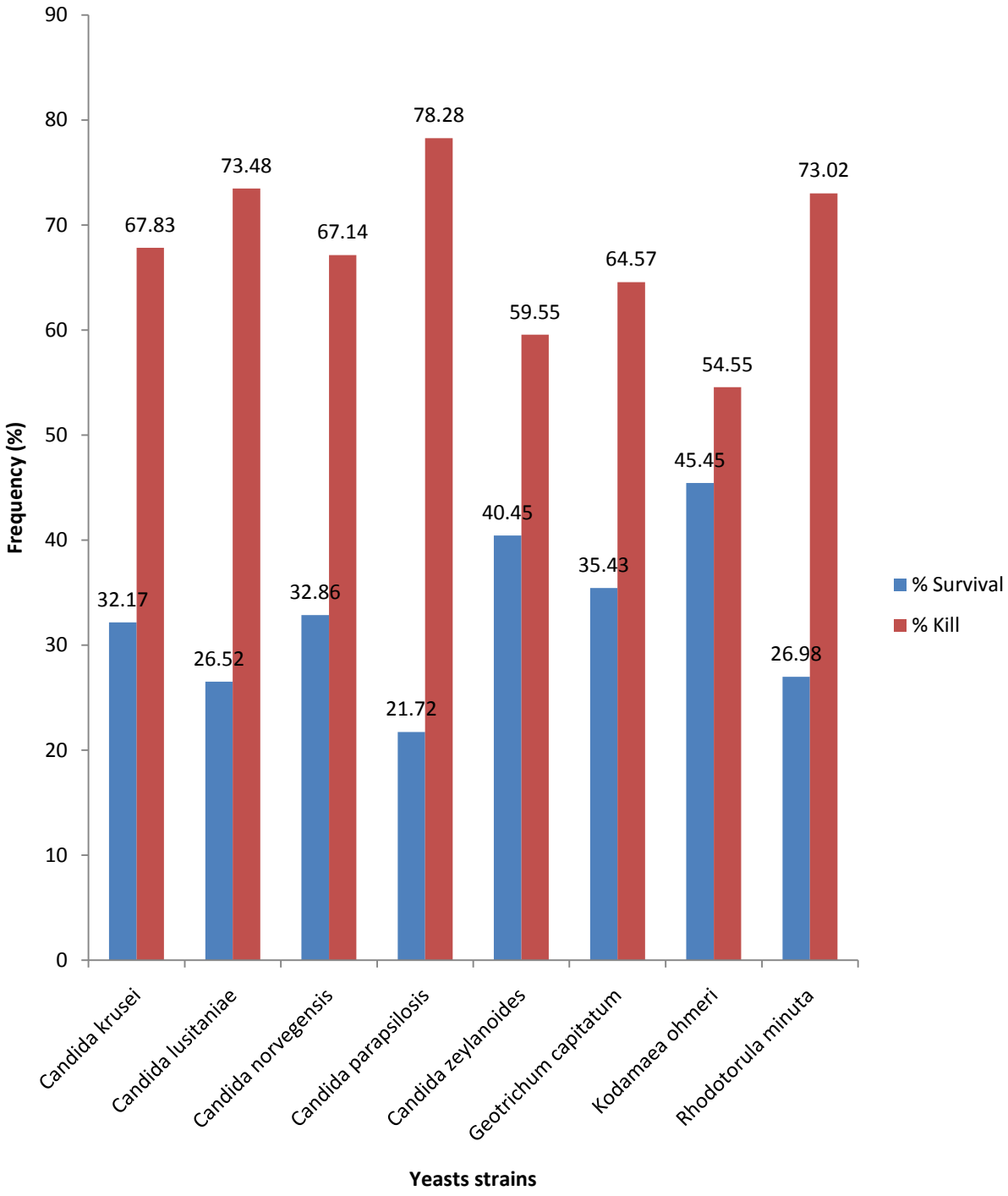


Fig 4.6:Percentage survival and kill rates of UV irradiated yeast strains at 20 minutes.

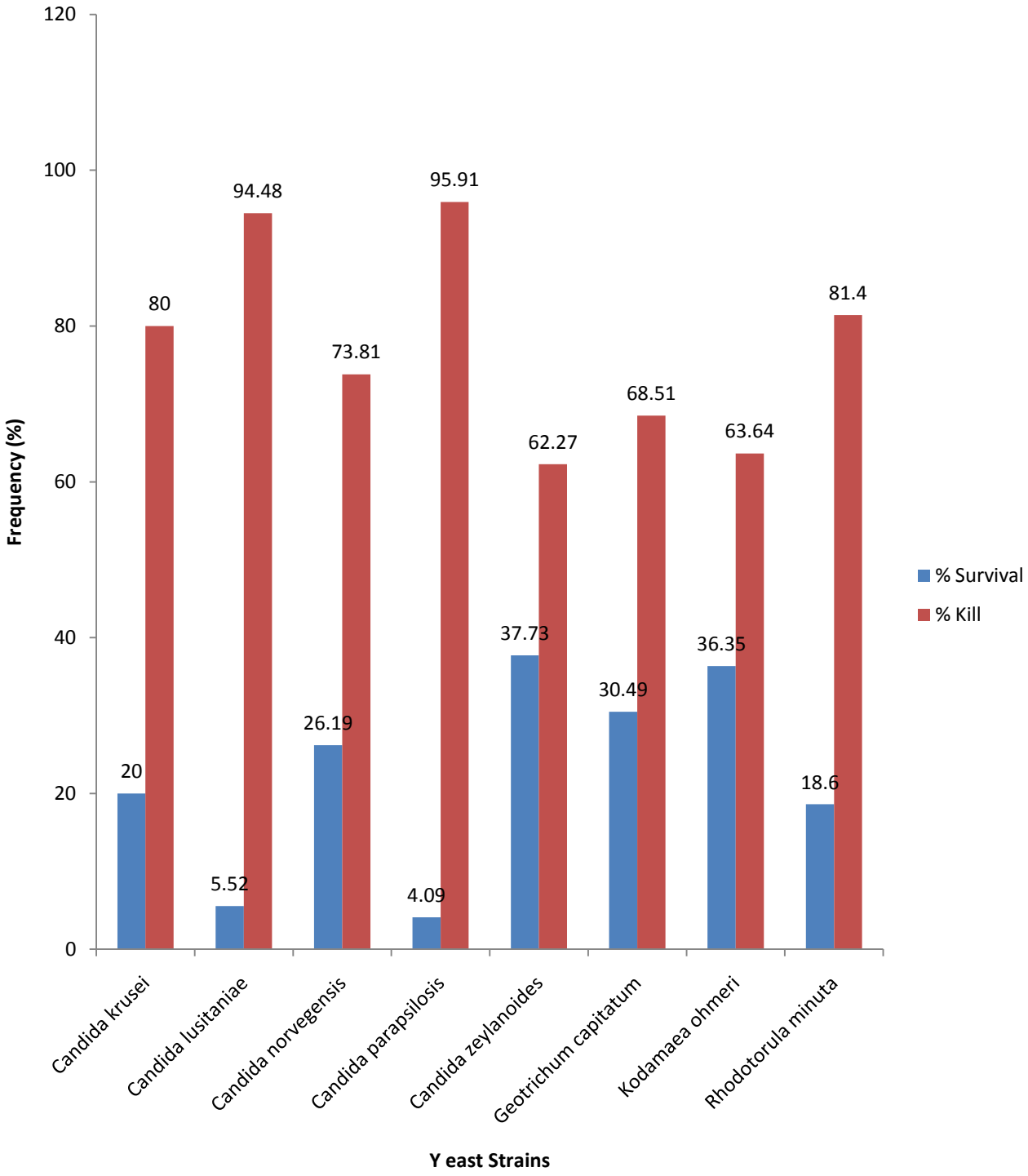


Fig 4.7: Percentage Survival and Kill Ratesof UV Irradiated Yeast Strains at 25 minutes.

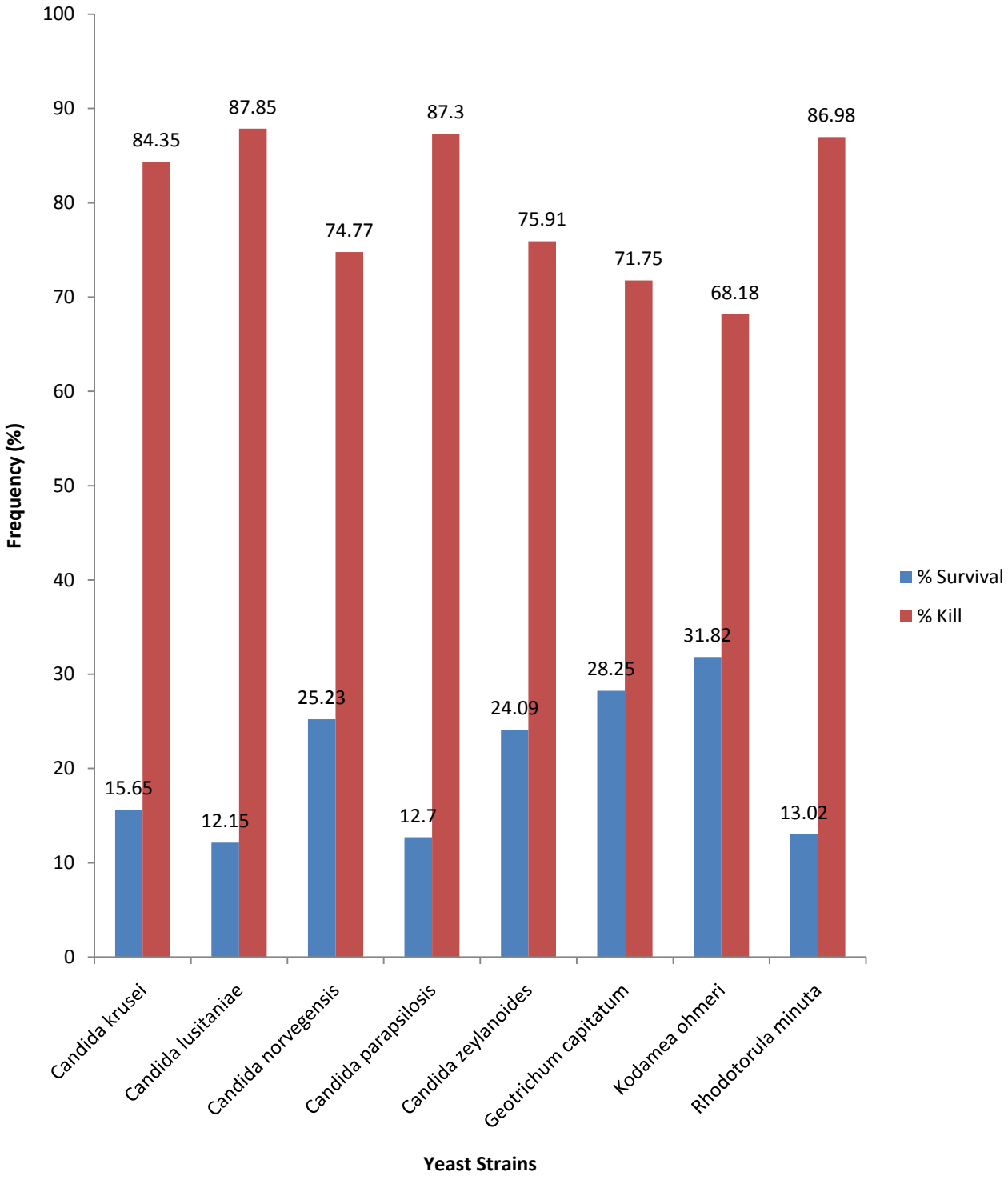


Fig 4.8: Percentage Survival and Kill Ratesof UV Irradiated Yeast Strains at 30 minutes

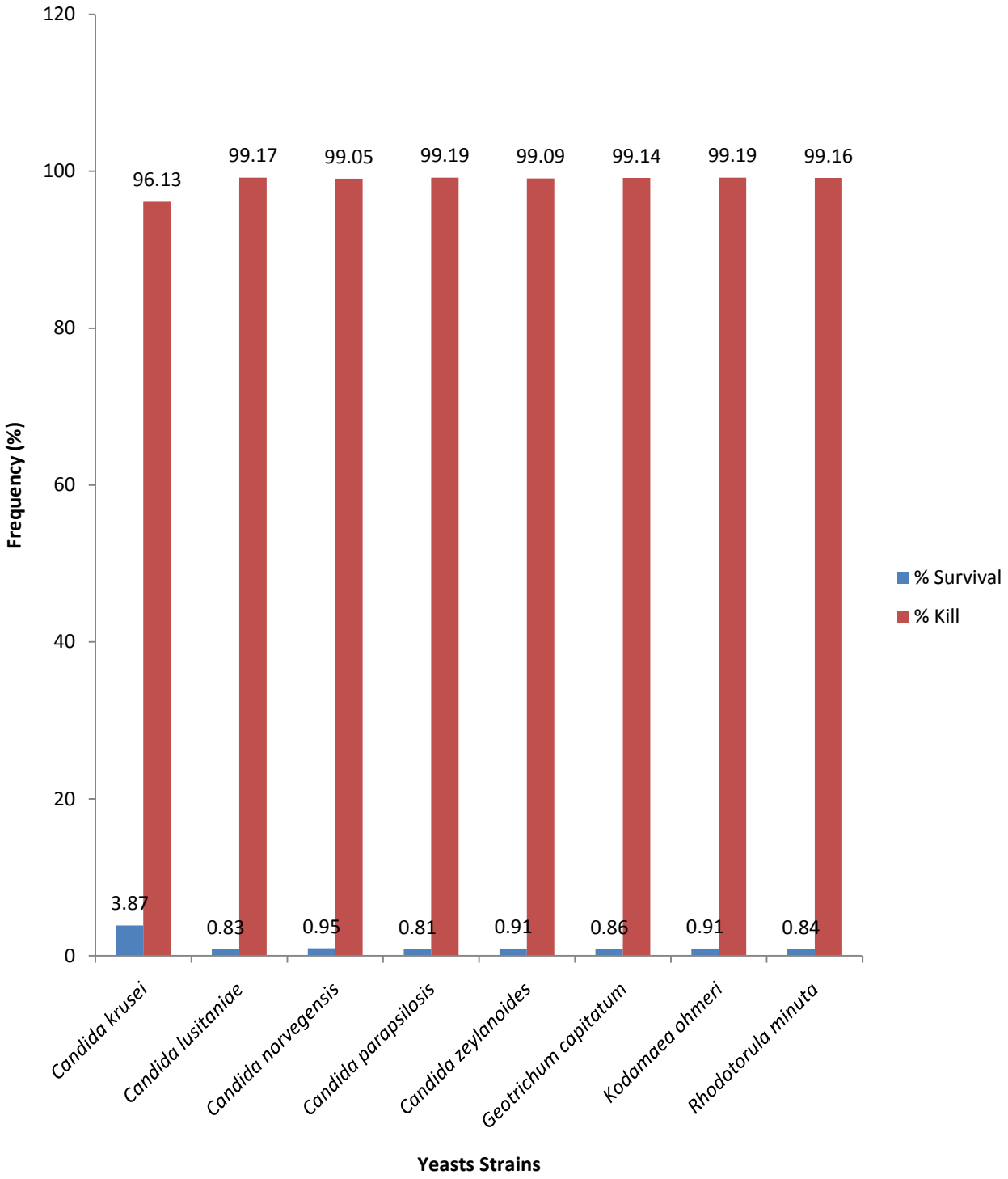


Fig 4.9: Percentage Survival and Kill Rates of Nitrous Acid at 20 Minutes

4.7 Characterisationof the Nutritional Requirements of the Identified Mutant Yeast Strains for both UV- Irradiation and Nitrous Acid Treatments.

The characteristics of the mutants from both UV light irradiation and nitrous acid reaction is shown in Table 4.6. Both UV irradiated and nitrous acid treated isolatesutilised all the eight amino acids for their growth. A stability test was conducted for four weeks to confirm the stability of the mutants, thegrowthmedium stillremained turbid(i.e.showing growth).

Table 4. 6:Characterisationof the Nutritional Requirements of the Wild and Identified Mutant Strains using Amino Acids

	Yeast strains (UV irradiated and nitrous acid treated)*								
Amino acids	**Wild yeast**	***Candida krusei***	***Candida lusitaniae***	***Candida norvegensis***	***Candida parapsilosis***	***Candida zeylanoides***	***Geotrichum capitatum***	***Kodamaea ohmeri***	***Rhodotorula minuta***
Histadine	+	+	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+
Methionine	+	+	+	+	+	+	+	+	+
Isoleucine	+	+	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	+	+	+	+	+
Valine	+	+	+	+	+	+	+	+	+
Cysteine	+	+	+	+	+	+	+	+	+

NB: The pH of minimal basal medium 4.5-5.5.
Key: * = Growth on minimal basal medium.+ = Growth (turbid), - = no growth

4.8 Fermentation of 5% Sorghum Starch with Wild and Brewing Yeast Strains for Ethanol Production.

The results in Fig 4.10 – 4.15 and Appendix VIshowed ethanol yield, cell number determination, reducing sugar and cell dry weight obtained during the fermentation of 5% sorghum starch with wild and brewing yeast strains.From the resultsthere was a gradual increase in the yield of ethanol from the first day to the fifth day when a maximum yield was obtained. These are stated as follows for isolates; INS18.18% (v/v), 2F36 17.05% (v/v), and DS6 11.36 % (v/v) (Fig. 4.10 – 4.12) respectively. Isolate 7F8 (14.78 % (v/v)) showed a maximum yield on the sixth day 14.78% (v/v) (Fig. 4.13).Isolates7F9 and DS20 had increase in ethanol yield from the first day and maximum on the seventh day10.93 % (v/v) (Fig. 4.14 – 4.15). The peak for each strain(five, six and seventh days) were selected as the optimum length of fermentation for the wild and controlled yeast strains.

The cell number, as evidence of growth, increased gradually from the first day to the third day and declined for both wild and control yeast Strains.(Fig.4.10-4.15).The population growth for isolates 2F36 and 7F8 on the first day was 0.17cells/100ml and 0.47cells/100ml and 0.26cells/100ml for isolates 2F36 and 7F8 on the third day respectively. The cell growth for isolate 7F9 was 0.21cells/100ml and 0.37cells/100ml for the first and the third day respectively.The isolate of DS6 also showed a population growth of 0.20cells/100ml on the first day and 0.40cells on the second day. The isolate of DS20 also showed a population growth of 0.16cells/100ml on the first day and 0.50cells/100ml on the third day(AppendixVI).

The cell dry weight (growth), also increasedthroughout the length of fermentation. The reducing sugar concentration also reduced gradually throughout the period of fermentation for the various isolates.(Figs.4.10-4.15).

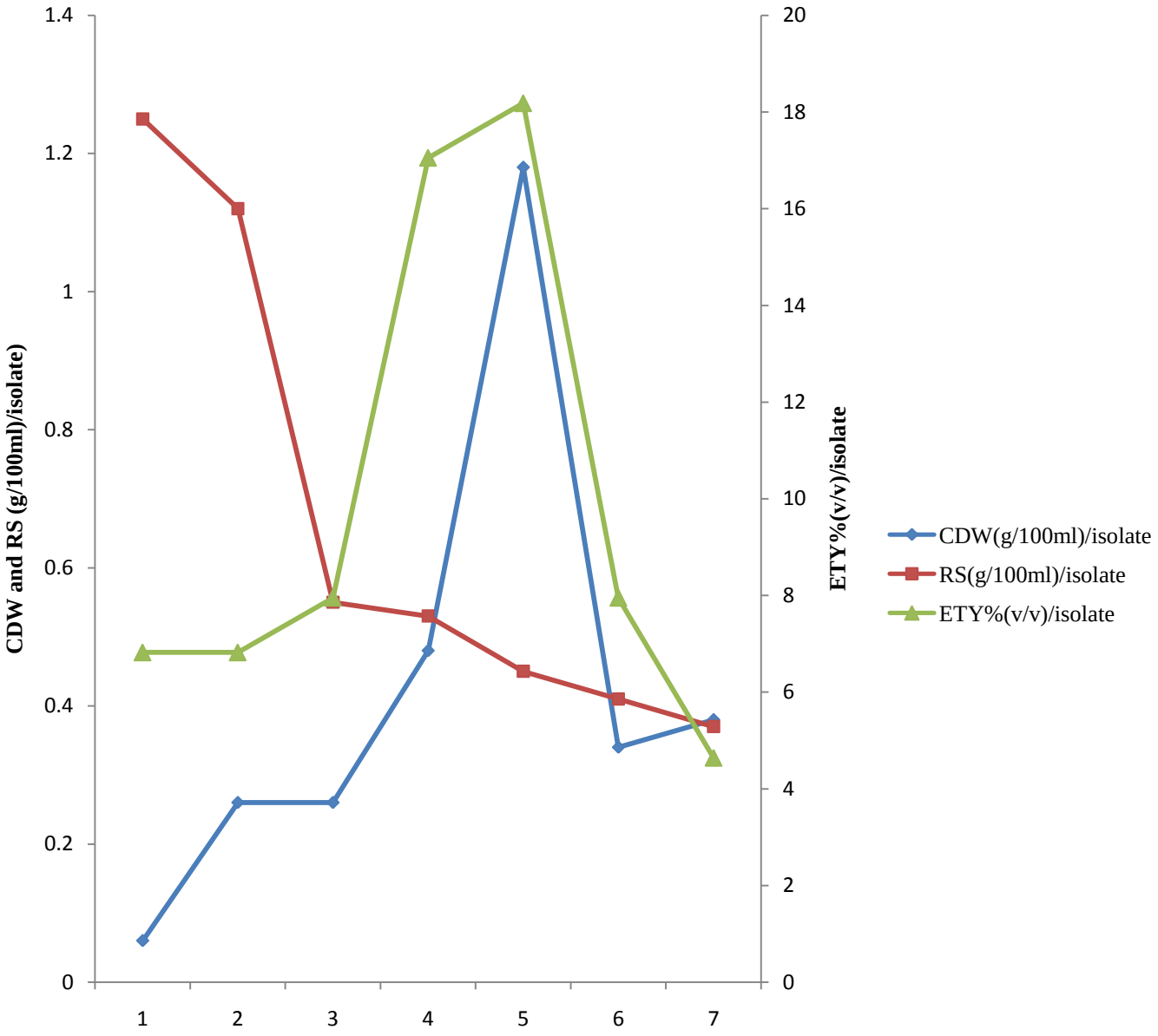


Fig 4.10: Fermentation of 5% Sorghum Starch with Brewing Yeast (INS) for Ethanol Production (pH 6.5)

Key:

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

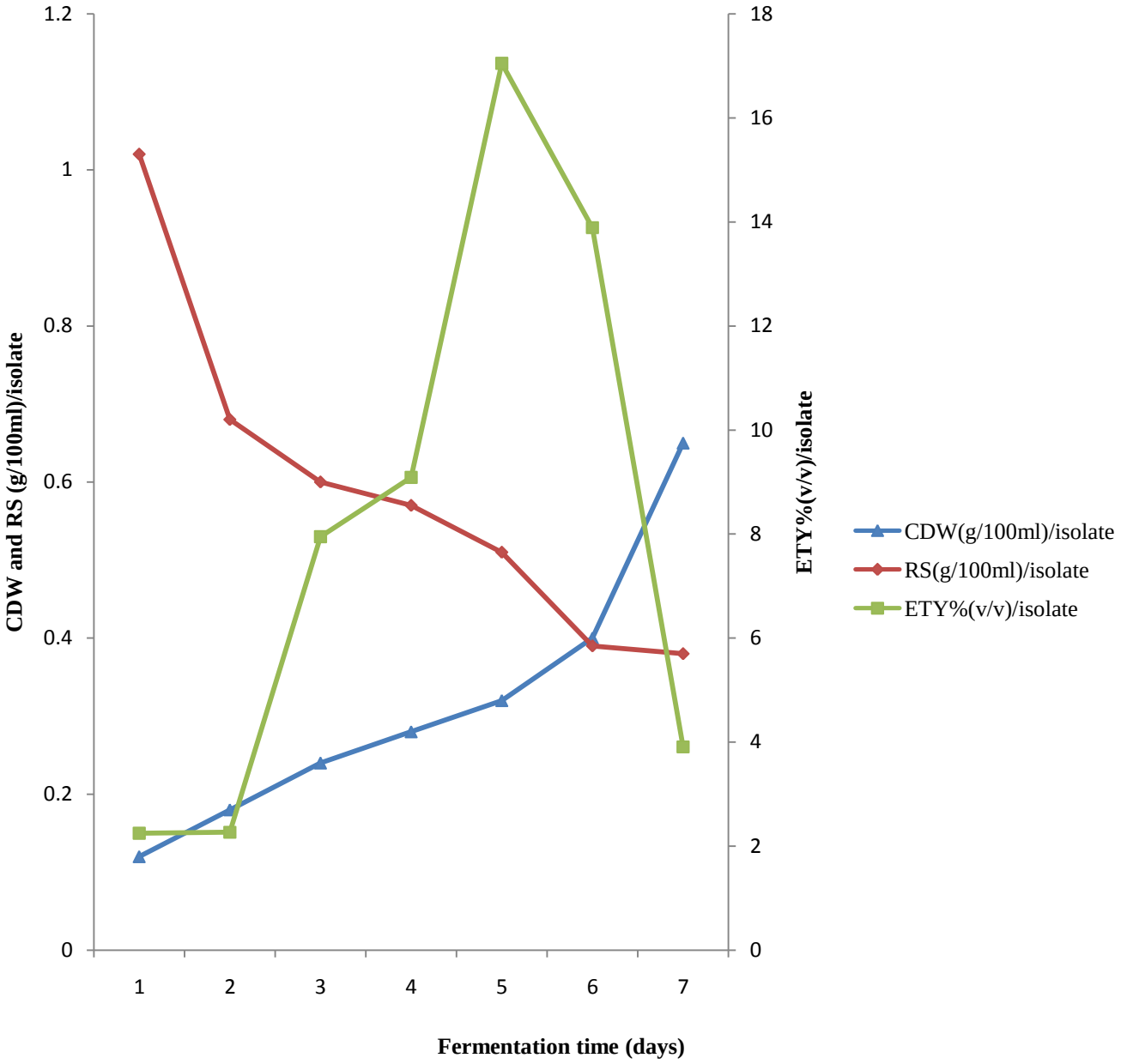


Fig: 4.11 Fermentation of 5% Sorghum Starch with Wild Yeast Strain (2F36) for Ethanol Production (pH 6.5).

Key:

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

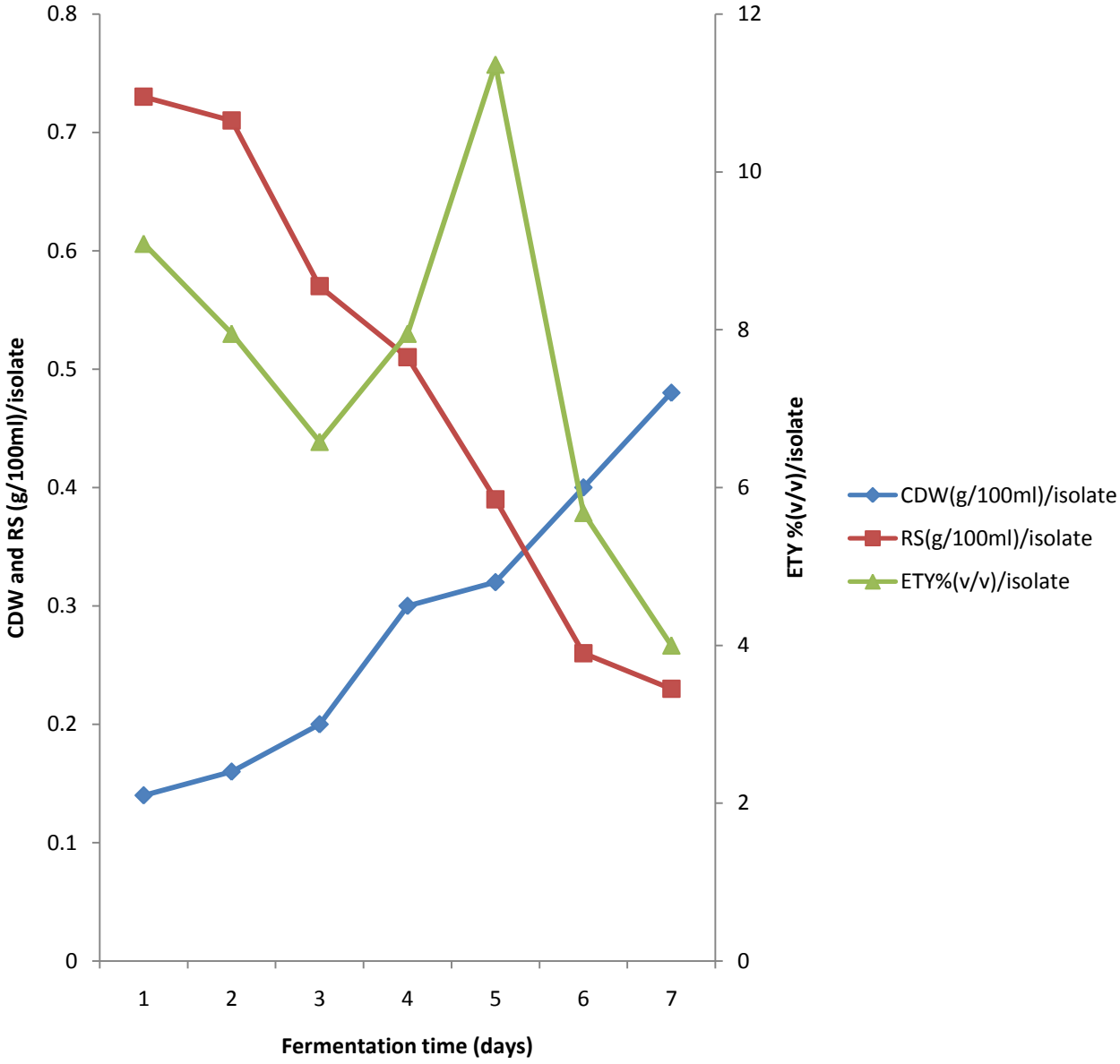


Fig 4.12: Fermentation of 5% Sorghum Starch with Wild Yeast Strain (DS6) for Ethanol production (pH 6.5)

Key:

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

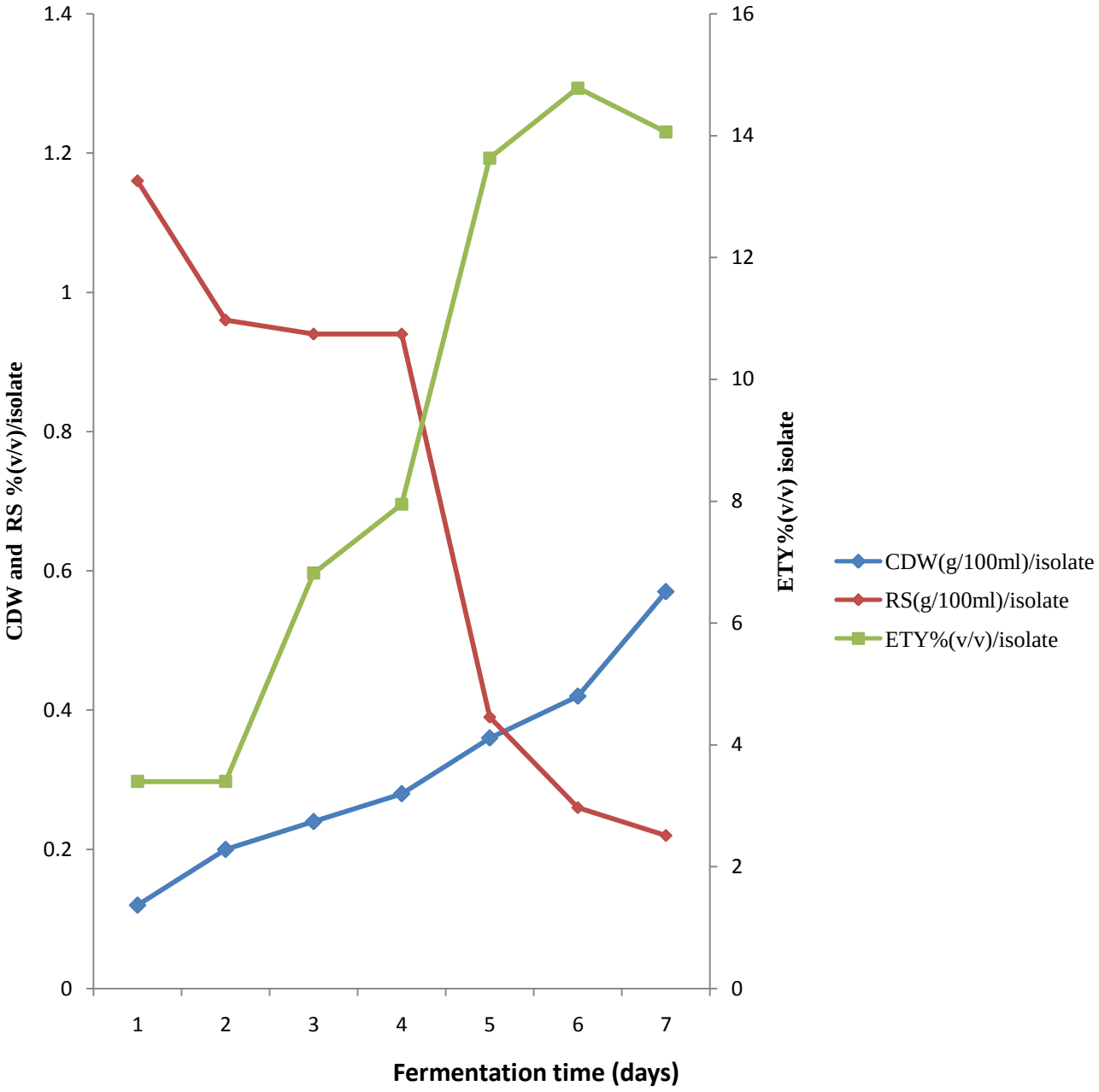


Fig 4.13: Fermentation of 5% Sorghum Starch with Wild Yeast Strain (7F8) for Ethanol Production (pH 6.5)

Key:

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

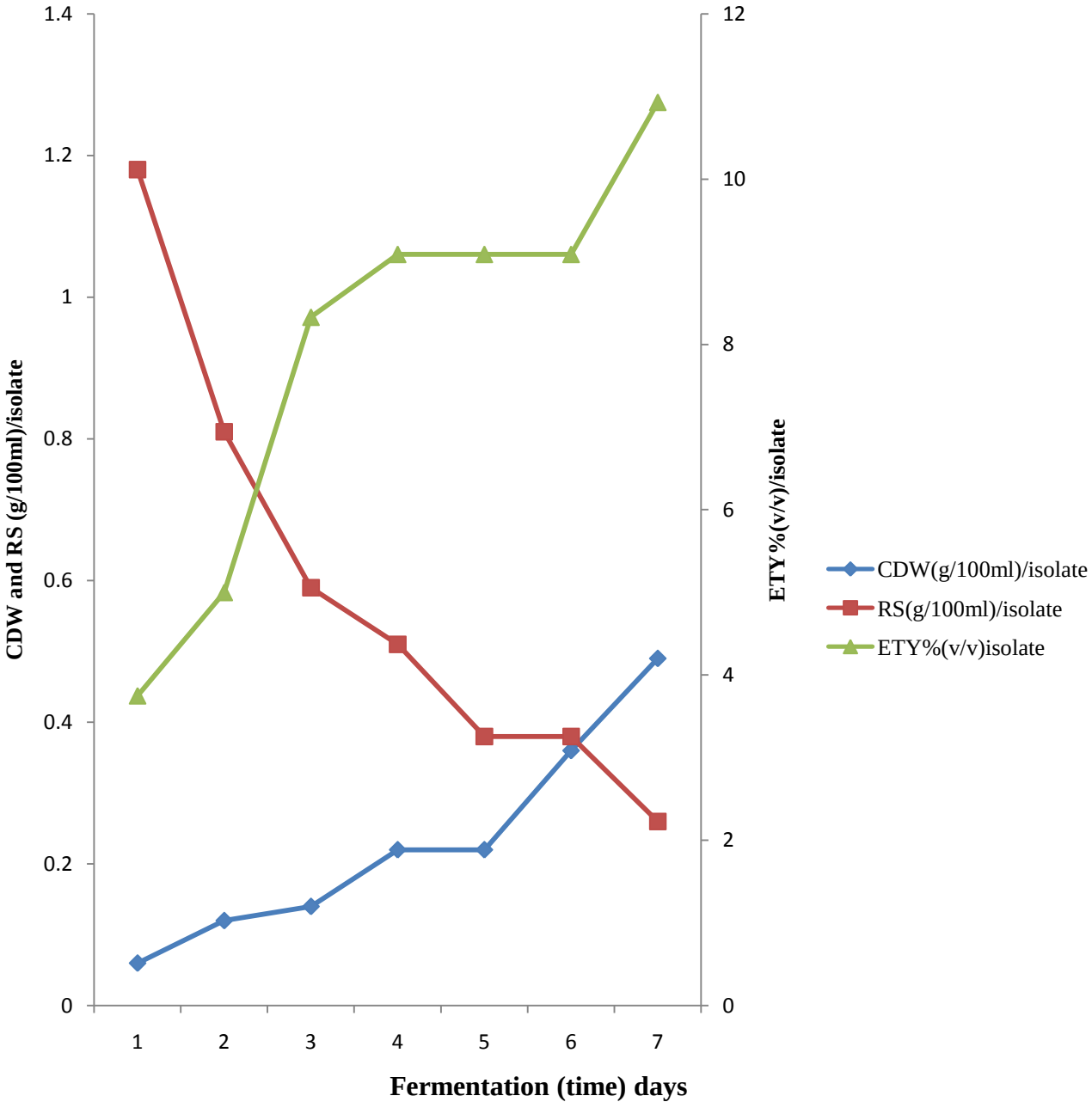


Fig 4.14: Fermentation of 5% Sorghum Starch with Wild Yeast Strain (7F9) for Ethanol Production (pH6.5)

Key:

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

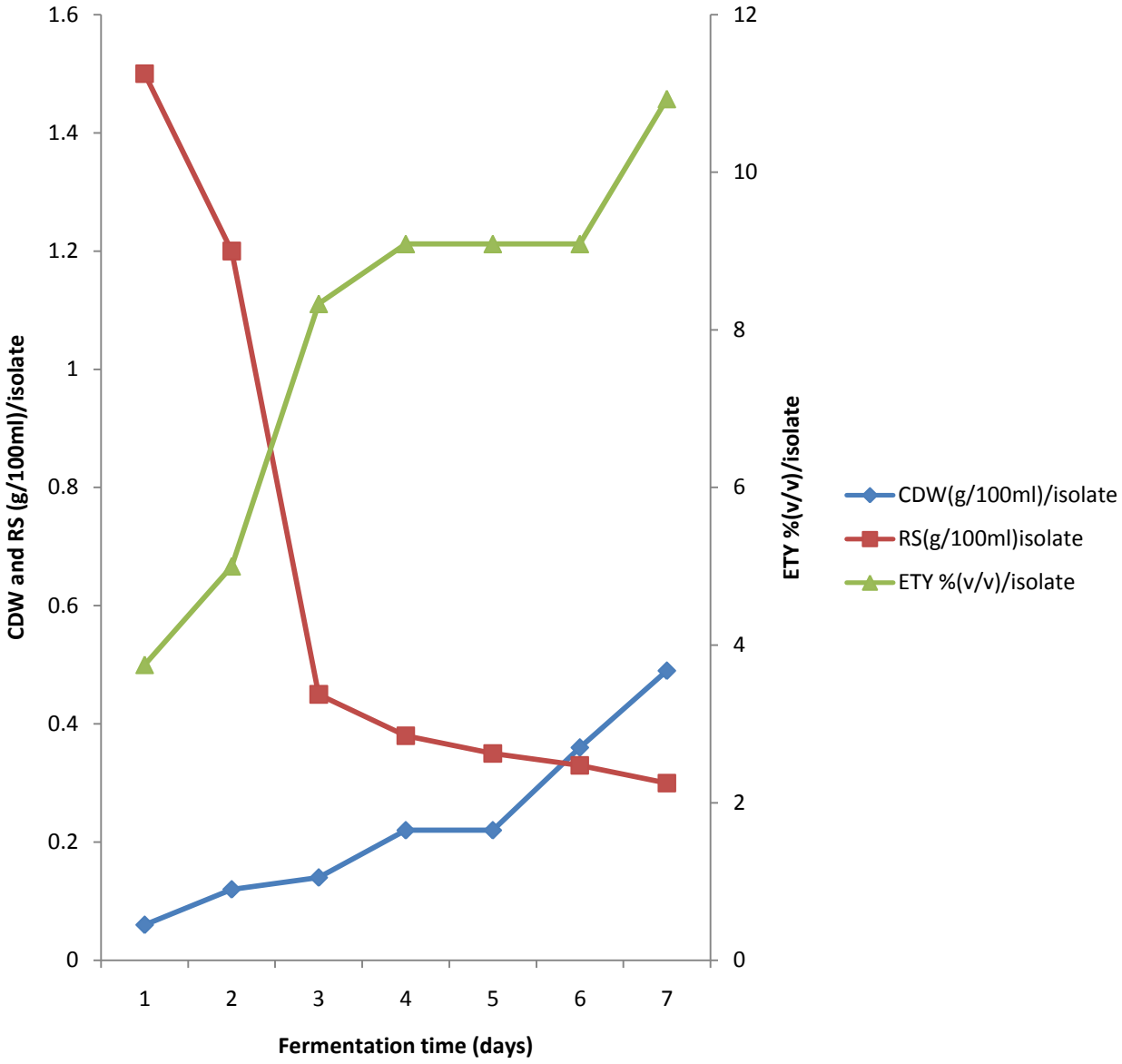


Fig 4.15: Fermentation of 5% Sorghum Starch with Wild Yeast Strain (DS20) for Ethanol Production (pH 6.5).

Key:

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

4.9 Fermentation of 5% sorghum Starch withMutants (nitrous acid) Treatment and Brewing Yeast Strains for Ethanol Production.

Figs.4.16 –4.20 and AppendixVIIshow the results obtained from fermentation of mutants (nitrous acid) treatment after 20 minutes with sorghum starch for ethanol yield, reducing sugar concentration, cell number and cell dry weight. The result showed a gradual increase in ethanol yield from the first day with a maximum yield on the fifth day and some on the seventh day. The isolates that showed increase in ethanol yield from the first to the fifth day had values that ranged from INS (6.82 - 18.18%(v/v)), 2F36 (1.14 - 17.05%(v/v)), 7F9 (6.82 - 17.05%(v/v)), DS20 (4.55 - 16.16%(v/v)), but isolates 7F8 (4.55 - 16.16%(v/v)) and DS6 (1.14 - 16.40%(v/v)), had gradual increase in ethanol yield from the first to the seventh day respectively.

The reducing sugar concentration fell gradually from the first day to the seventh day of fermentation. Thefall ranged from (0.68g/100 - 1.48g/100ml)on the firstday and (0.17g/100-0.29g/100ml)on the seventh day for isolates; 2F36, 7F9, DS20,7F8 and DS6 respectively. (Figs.4.16-4.20 and Appendix VII) The cell number (as evidence of growth) also increased gradually from the first day to the fourth and then declined. The result obtained on the first days ranged from (0.16cells/100-1.25cells/100ml and 0.18cells/100ml -0.14cells/100ml) on the fourth day for isolates of 2F36,7F9,DS20,7F8 and DS6 respectively.(Figs.4.16-4.20 and AppendixVII). The cell dry weight (growth) increased throughout the length of fermentation for all the mutant yeast strains, while for the control, the brewing yeast (INS) showed maximum growth on the fifth day and declined(Figs.4.16-4.20). The result obtained are presented thus: INS (0.06g/100 - 1.18g/100ml), 2F36 (0.12g/100 - 0.50g/100ml), 7F8 (0.12g/100 - 0.44g/100ml), 7F9 (0.20g/100 - 0.81g/100ml), DS6 (0.10g/100- 0.57g/100ml), DS20 (0.10g/100- 0.62g/100ml)(AppendixVII).

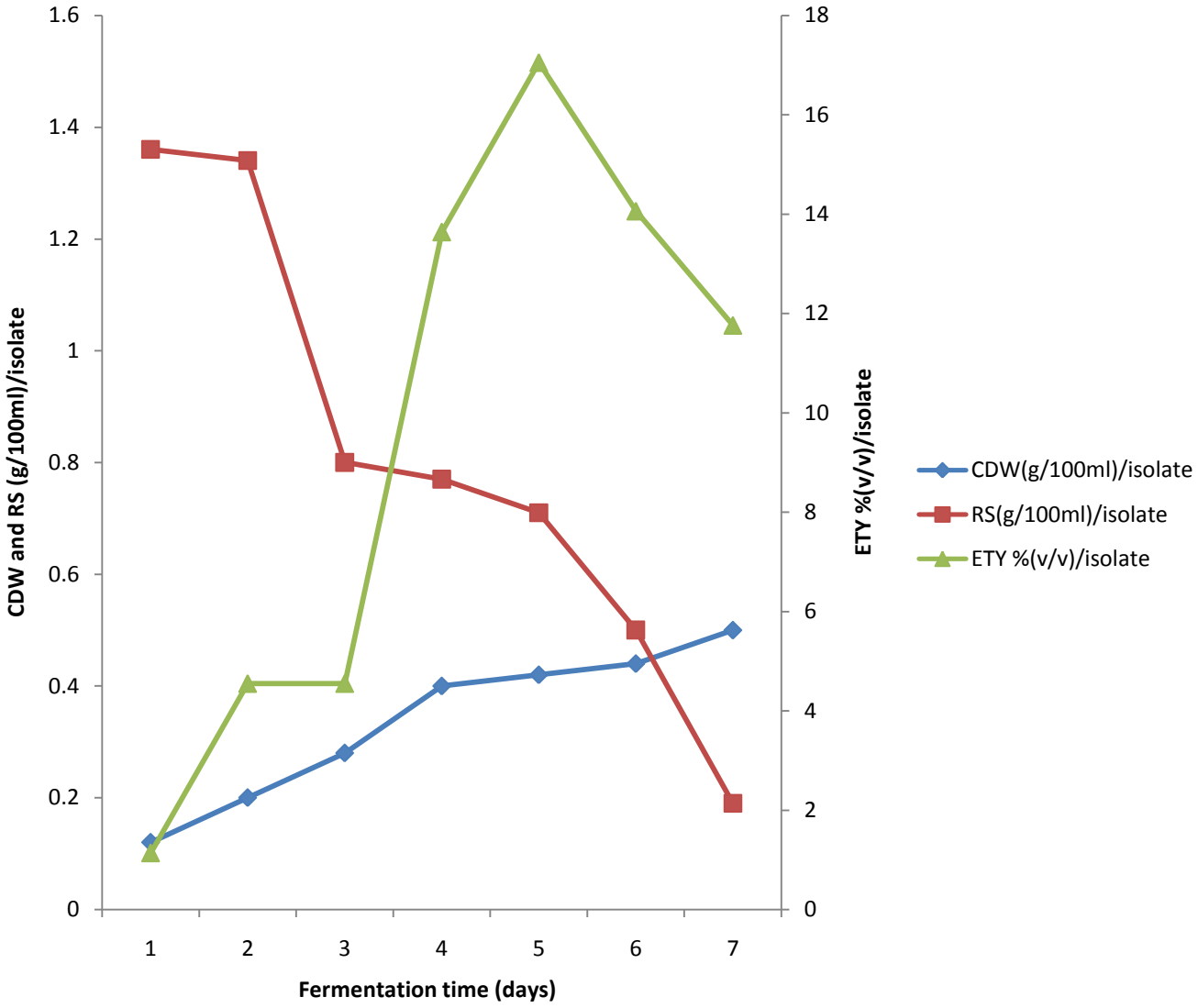


Fig 4.16: Fermentation of 5% Sorghum Starch with Mutant (nitrous acid treated) Strain (2F36) forEthanol Production at 20 minutes (pH 6.5)

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

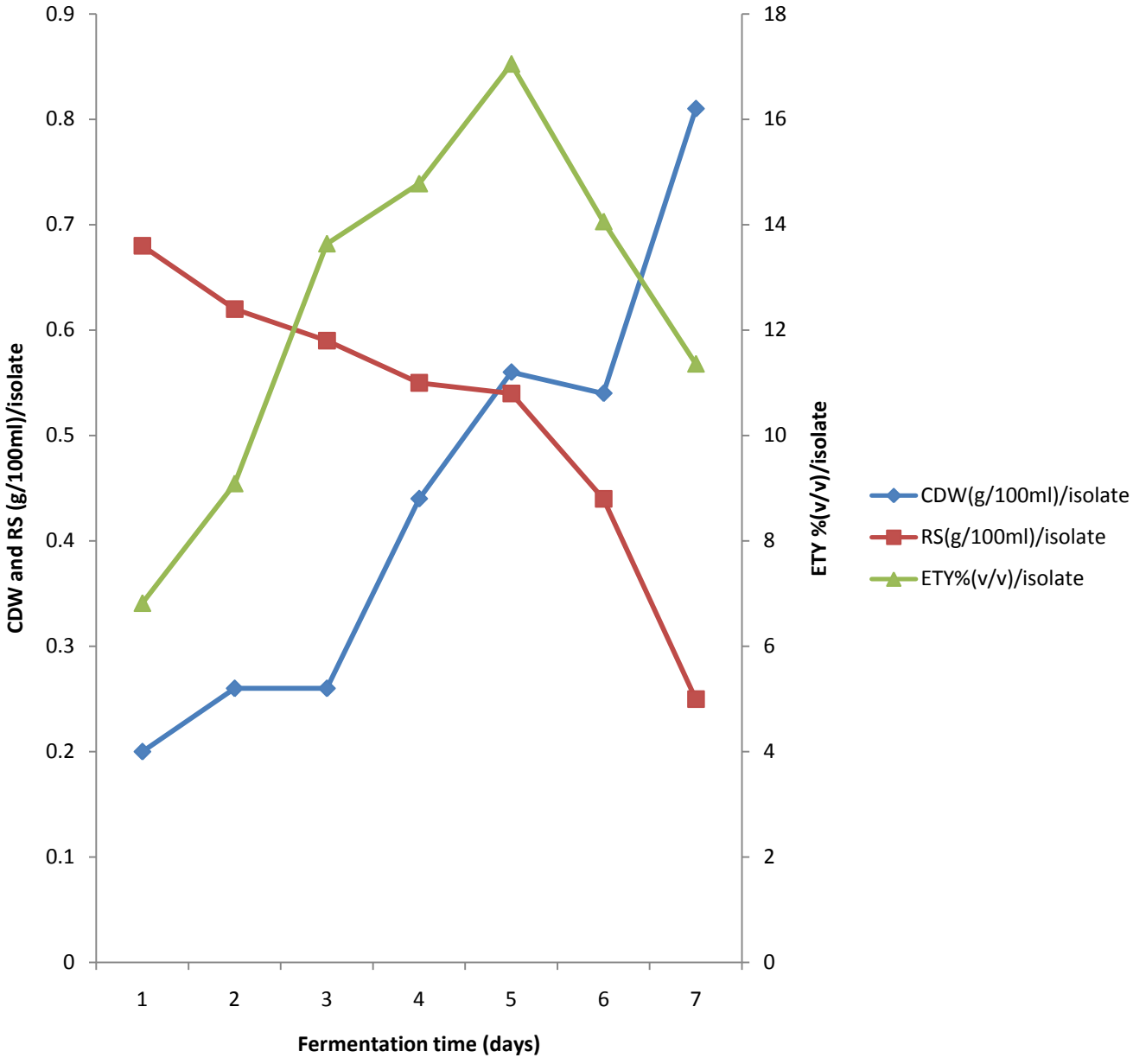


Fig 4.17: Fermentation of 5% Sorghum Starch with Mutant (nitrous acid treated) Yeast Strain (7F9) for Ethanol Production at 20 minutes (pH 6.5).

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

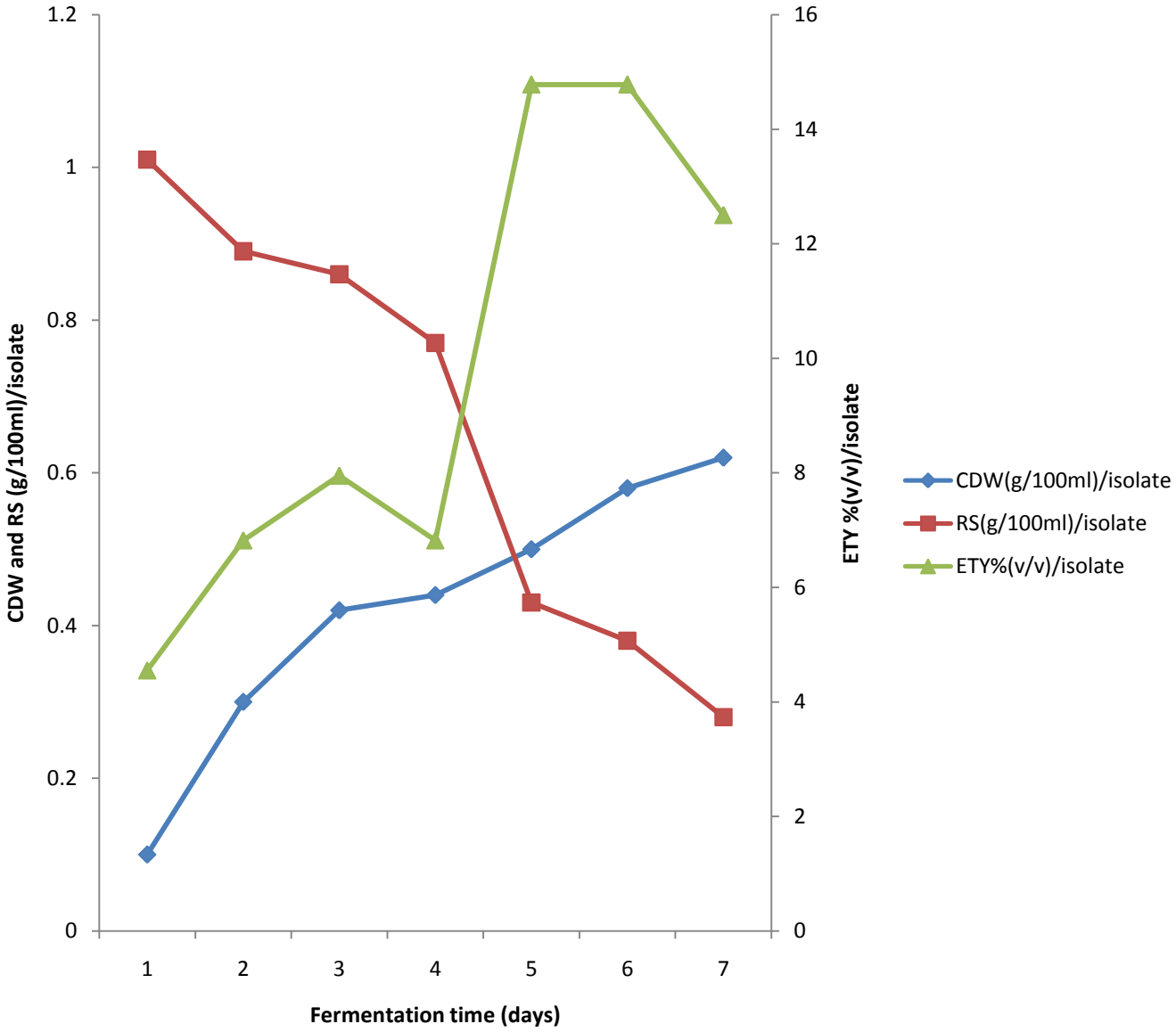


Fig 4.18: Fermenatation of Sorghum Starch with Mutant (nitrous acid treated) Yeast Strain (DS20) for Ethanol Production at 20 minutes (pH 6.5)

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

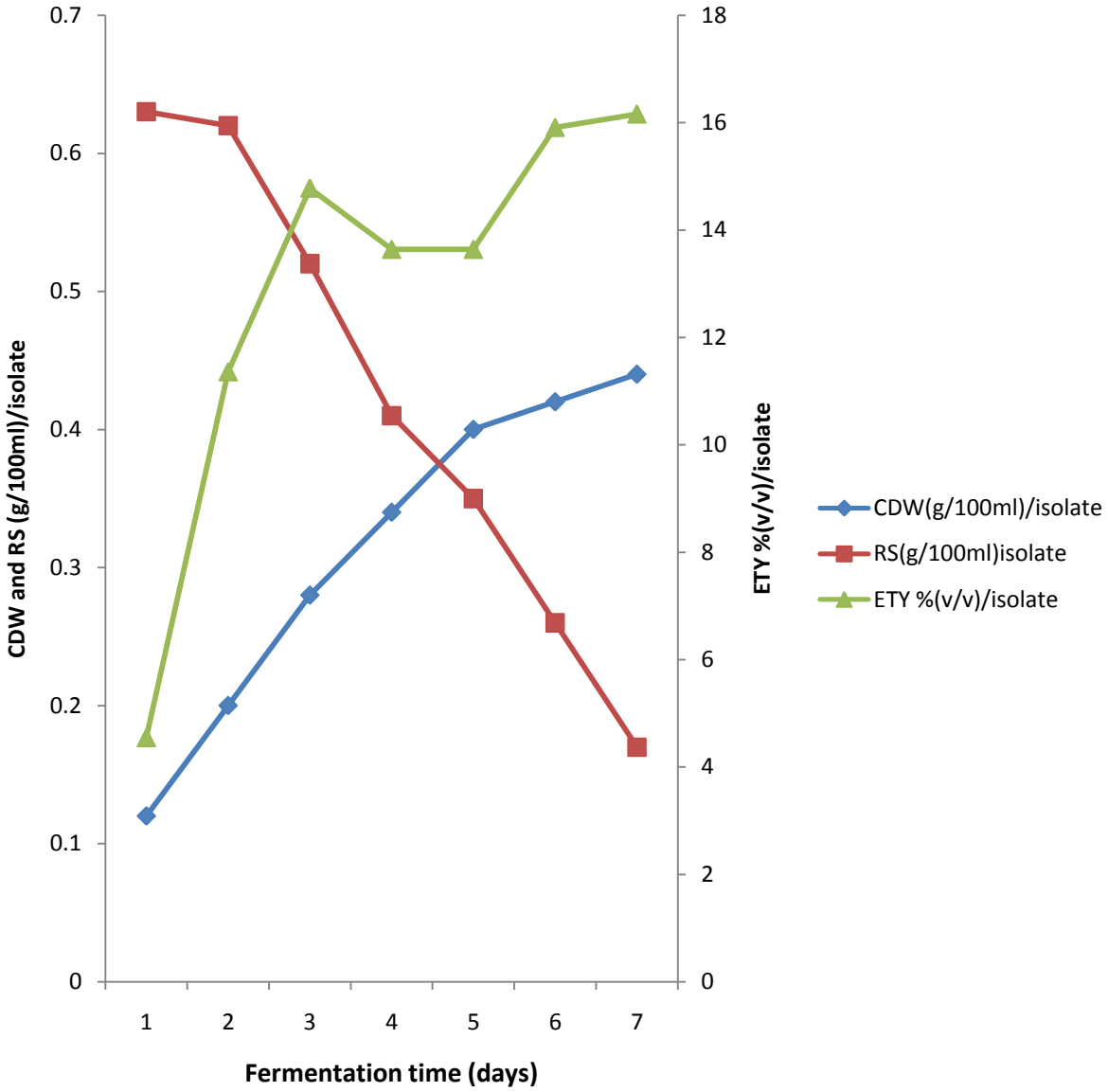


Fig 4.19: Fermentation of Sorghum Starch with Mutant (nitrous acid treated) Yeast Strain (7F8) forEthanol Production at 20 minutes (pH 6.5)

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

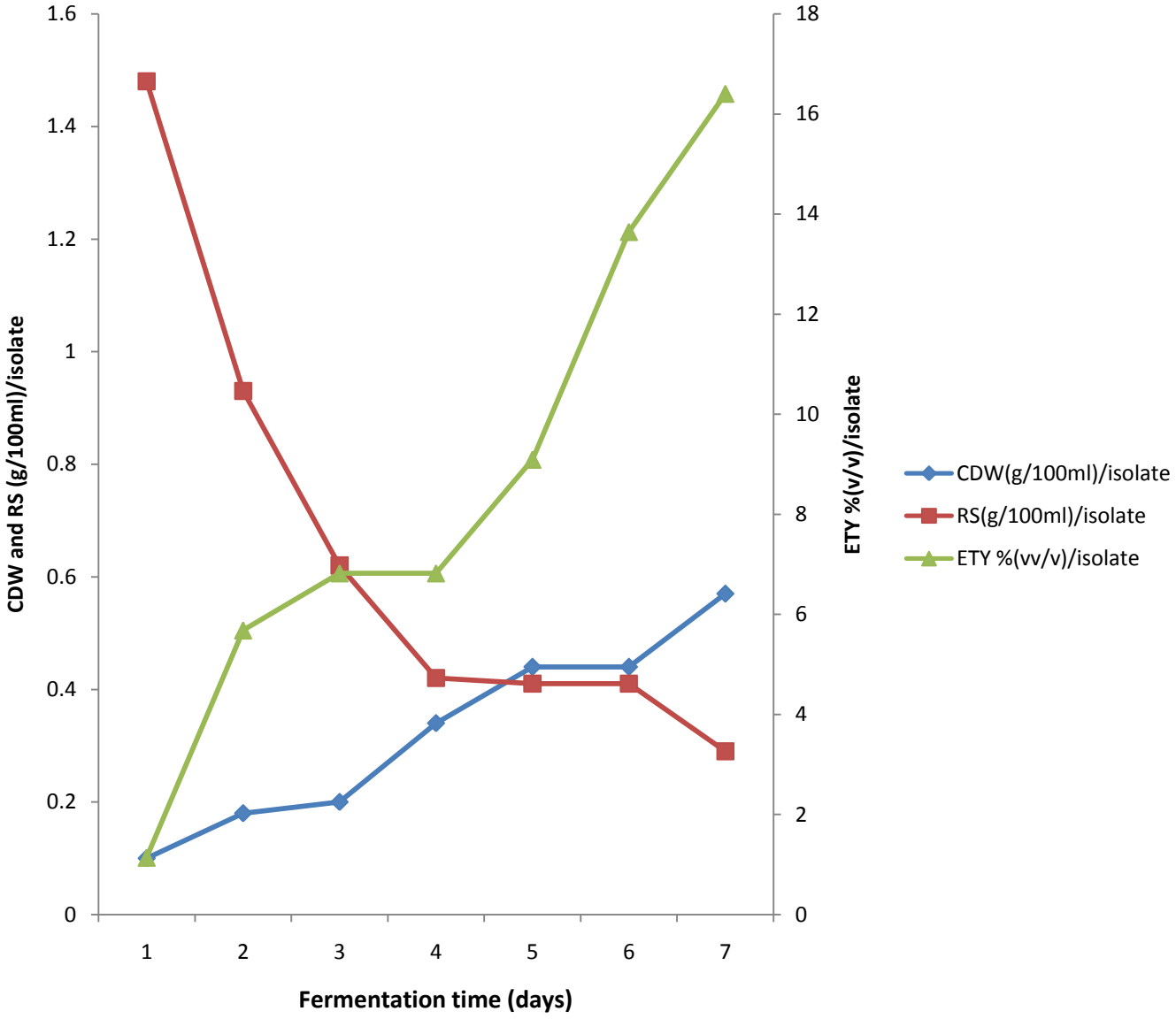


fig 4.20: Fermentation of 5% Sorghum Starch with Mutant (nitrous acid treated) Yeast Strain (DS6) for Ethanol Production at 20 minutes (pH 6.5)

CDW = Cell dry weight

RS = Reducing suger

ETY = Ethanol yield

4.10 Fermentation of 5% Sorghum Starch with Mutant (UV light-Irradiated) Yeast Strains forEthanol Production.

The results in (Tables 4.7a - 4.7g) show the effects of fermentation of 5% sorghum starch with mutants of ultraviolet (UV) light irradiated yeasts strains for ethanol production at various time intervals of 4, 6, 10, 15, 20, 25 and 30 minutes. The Tables show the results of ethanol yield (ETY), residual sugars (RS), cell number (CND) and cell dry weight (CDW) at various time intervals of mutation and the length of fermentation in days. From these results there was a gradual increase in ethanol yield from the first day through to the fifth day and then declines. The maximum yield and the optimum length of fermentation varied with time of mutation and the different mutants. The cell biomass yield,representing cell growth (cell dry weight and cell number), increased throughout the length of fermentation. The reducing sugar concentrations and amounts of sugar consumed by the cell reduced from the first day of fermentation to the seventh day.

At mutation time of 4 minutes, the ethanol yield for some of the isolates of DS20, 7F9, DS6, 2F36and 7F8, ranged from 6.68 – 14.78%(v/v), 2.25 – 11.36%(v/v), 3.41 –10.23%(v/v), 7.95 – 13.64%(v/v), and 5.68 – 15.91%(v/v) respectively. The maximum ethanol yield for each of the isolate above was obtained on the second, third, fourth and fifth day respectively. The residual sugar ranged from 0.75 – 0.26g/100ml, 0.97 – 0.33g/100ml, 0.87 – 0.20g/100ml, 0.88 – 0.25g/100ml, and 0.83 – 0.35g/100ml respectively. The cell number ranged from,0.06 – 0.38 cells/100ml, 0.18 – 0.48cells/100ml, 0.09 – 0.41cells/100ml, 0.10 – 0.37cells/100ml and 0.07 – 0.19/100ml. The cell dry weight ranged from 0.10 – 0.48g/100ml, 0.16 – 0.65g/100ml, 0.14 – 0.64g/100ml, 0.16 – 0.35g/100ml, 0.12 – 0.71g/100ml repectively (Table 4.7a).

Table 4. 7a:Fermentationof 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast for Ethanol Production at 4 minutes.

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
Days	MINS	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	4	0.16	0.12	0.16	0.14	0.10	0.10	0.07	0.18	0.09	0.06	0.88	0.83	0.97	0.87	0.75	7.95	12.5	2.25	3.41	6.82
2	4	0.24	0.14	0.24	0.16	0.22	0.16	0.08	0.20	0.12	0.20	0.86	0.57	0.88	0.75	0.70	10.23	12.5	2.25	6.82	14.78
3	4	0.32	0.14	0.24	0.24	0.28	0.16	0.10	0.26	0.12	0.21	0.72	0.55	0.88	0.68	0.64	8.33	15.91	10.23	10.23	14.78
4	4	0.42	0.18	0.28	0.32	0.30	0.17	0.10	0.26	0.12	0.22	0.66	0.52	0.79	0.64	0.52	13.64	14.78	4.55	4.55	5.55
5	4	0.46	0.20	0.34	0.34	0.32	0.19	0.14	0.26	0.13	0.24	0.42	0.49	0.51	0.55	0.50	9.09	5.68	11.36	6.82	14.78
6	4	0.48	0.20	0.48	0.48	0.34	0.28	0.15	0.43	0.18	0.30	0.25	0.35	0.33	0.33	0.43	11.36	14.78	11.36	10.23	8.82
7	4	0.35	0.71	0.65	0.64	0.48	0.37	0.19	0.48	0.41	0.38	0.25	0.35	0.33	0.20	0.26	10.55	12.5	3.13	2.25	6.82

Key: Results are means of triplicate determination

FMT = fermentation time (Days)

UVI = Ultraviolet irradiation (minutes)

CDW = cell dry weight

CND = cell number determination

RS = residual sugar

ETY = ethanol yield

2F36 = *Kodamaea ohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichum capitatum*

DS6 = *Rhodotorula minuta*

DS20 = *Candida zeylanoides*

At 6 minutes mutation, the ethanol yield for isolates 2F36, 7F8, 7F9, DS6 and DS20 ranged from 3.52 – 14.78%(v/v), 5.68 – 17.05%(v/v), 2.25 – 14.78%(v/v), 2.25 – 10.23%(v/v) and 6.82 – 14.78%(v/v)(Table 4.7b). The maximum ethanol yield for isolates 2F36, 7F8 and DS6 was obtained on day one: 14.78%(v/v), 17.05%(v/v), 10.23%(v/v), while for isolates 7F9 and DS20, the maximum yield was obtained on the third and fifth day with 14.78%(v/v). The residual sugars ranged from 0.90 – 0.39g/100ml, 0.86 – 0.40g/100ml, 1.64 – 0.24g/100ml, 1.25 – 0.25g/100ml, and 1.30 – 0.25g/100ml.The cell number ranged from 0.14 – 0.36cells/100ml, 0.12 – 0.21g/100ml, 0.18 – 0.49cells/100ml, 0.06 – 0.58cells/100ml and 0.15 – 0.33cells/100ml. The cell dry weight ranged from 0.18 – 0.42g/100ml, 0.20 – 0.62g/100ml, 0.10 – 0.43g/100ml, 0.20 – 0.51g/100ml and 0.22 – 0.44g/100ml (Table 4.7b).

At 10 minutes, the ethanol yield for isolates 2F36, 7F8, 7F9, DS6 and DS20 were as follows: 3.75 – 14.78%(v/v), 5.68 – 14.78%(v/v), 9.09 – 14.78%(v/v), 2.27 – 14.78%(v/v), and 5.68 – 18.18%(v/v). The maximum ethanol yield was obtained on the second day for isolate 2F36, 7F8 and DS6; 14.78% (v/v), and for isolate 7F9 and DS20, the maximum yield was obtained on the third day third 14.78% (v/v) and fifth day 18.18% (v/v). The residual sugar ranged from 1.24 – 0.28g/100ml, 0.66 – 0.31g/100ml, 1.78 – 0.24g/100ml, 1.14 – 0.25g/100ml and 0.86 – 0.24g/100ml.The cell number ranged from 0.16 – 0.30cells/100ml, 0.09 – 0.14cells/100ml, 0.12 – 0.62cells/100ml, 0.16 – 0.56cells/100ml, and 0.11 – 0.26cells/100ml. Cell dry weight also ranged from 0.12 – 0.55g/100ml, 0.22 – 0.71g/100ml, 0.20 – 0.48g/100ml, 0.12 – 0.64g/100ml and 0.14 – 0.45g/100ml (Table 4.7c).

Table 4.7b:Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeasts Strains for Ethanol Production at 6 minutes.

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
(Days)	(Mins)	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	6	0.18	0.20	0.10	0.20	0.22	0.14	0.12	0.18	0.06	0.15	0.90	0.86	1.64	1.25	1.30	14.78	17.05	2.25	10.23	6.82
2	6	0.18	0.28	0.16	0.22	0.22	0.16	0.12	0.25	0.13	0.18	0.82	0.84	1.03	1.23	1.21	10.23	14.78	1.14	6.82	9.09
3	6	0.18	0.34	0.18	0.28	0.24	0.17	0.16	0.25	0.14	0.20	0.68	0.79	0.88	0.95	0.84	13.64	15.91	14.78	6.82	3.75
4	6	0.24	0.39	0.24	0.30	0.24	0.19	0.17	0.30	0.14	0.25	0.66	0.73	0.75	0.78	0.77	13.64	10.23	11.36	3.75	6.82
5	6	0.24	0.40	0.34	0.34	0.24	0.21	0.18	0.30	0.21	0.30	0.49	0.68	0.70	0.73	0.73	9.09	13.64	5.68	2.27	14.78
6	6	0.31	0.50	0.42	0.46	0.39	0.36	0.19	0.37	0.26	0.32	0.41	0.48	0.33	0.28	0.23	5.68	7.81	9.76	2.25	14.78
7	6	0.42	0.62	0.43	0.51	0.44	0.36	0.21	0.49	0.58	0.33	0.39	0.40	0.24	0.25	0.25	3.52	5.68	2.27	2.25	14.78

Key:

Result are means of triplicate determination
FMT = fermentation time
UVI = ultraviolet irradiation
CDW = cell dry weight
CND = cell number determination
RS = residual sugar
ETY = ethanol yield

2F36 = *Kodamaeaohmeri*
7F8 = *Candida norvegensis*
7F9 = *Geotrichumcapitatum*
DS6 = *Rhodotorulaminuta*
DS20 = *Candida zeylanoides*

Table 4.7c:Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast Strains for Ethanol Production at10minutes.

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates)					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
(Days)	(Mins)	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	10	0.12	0.22	0.20	0.12	0.14	0.16	0.09	0.12	0.16	0.11	1.24	0.66	1.78	1.14	0.86	12.5	12.5	9.09	10.23	6.82
2	10	0.24	0.24	0.20	0.16	0.16	0.18	0.11	0.13	0.16	0.12	1.08	0.61	1.17	1.05	0.64	14.78	14.78	10.23	14.78	9.09
3	10	0.26	0.28	0.26	0.16	0.16	0.19	0.11	0.20	0.18	0.13	0.76	0.60	0.94	0.96	0.55	14.78	13.64	14.78	13.64	12.5
4	10	0.38	0.30	0.28	0.30	0.16	0.19	0.12	0.32	0.18	0.15	0.72	0.57	0.71	0.95	0.55	11.36	12.5	13.63	5.46	14.78
5	10	0.42	0.30	0.34	0.34	0.20	0.19	0.12	0.39	0.25	0.20	0.47	0.56	0.66	0.90	0.55	6.25	11.36	12.5	3.75	18.18
6	10	0.46	0.34	0.35	0.58	0.38	0.24	0.14	0.52	0.32	0.23	0.45	0.36	0.62	0.33	0.52	5.68	8.59	11.36	3.41	13.64
7	10	0.55	0.71	0.48	0.64	0.45	0.30	0.14	0.62	0.56	0.26	0.28	0.31	0.24	0.25	0.24	3.75	5.68	13.64	2.27	5.68

Key:

Result are means of triplicate determination

FMT = fermentation time

UVI = ultraviolet irradiation

CDW = cell dry weight

CND = cell number determination

RS = residual sugar

ETY = ethanol yield

2F36 = *Kodamaeaohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichumcapitatum*

DS6 = *Rhodotorulaminuta*

DS20 = *Candida zeylanoides*

At 15 minutes mutation time, the ethanol yield ranged from 12.5 – 17.05%(v/v), 5.68 – 13.64%(v/v), 2.25 – 14.78%(v/v), 3.41 – 10.23%(v/v), and 6.82 –14.78%(v/v) for isolates 2F36, 7F8, 7F9, DS6 and DS20 respectively. The maximum ethanol yield was obtained on the second day for isolates 2F36, 7F9 and DS6, while isolates 7F8 and DS20 was day one and third respectively.The residual sugar for isolates 2F36, 7F8, 7F9, DS6 and DS20 ranged from 1.10 – 0.43g/100ml, 0.77 – 0.47g/100ml, 0.79 – 0.40g/100ml, 1.07 – 0.29g/100ml, and 1.07 – 0.17g/100ml.The cell number ranged from 0.14 – 0.40cells/100ml, 0.13 – 0.31cells/100ml, 0.14 – 0.25cells/100ml, 0.11 – 0.34cells/100ml and 0.10 – 0.28cells/100ml. The cell dry weight ranged from 0.16 – 0.46g/100ml, 0.14 – 0.63g/100ml, 0.12 – 0.44g/100ml, 0.12 – 0.30g/100ml, and 0.14 – 0.38g/100ml for isolates 2F36, 7F8, 7F9, DS6 and DS20 respectively (Table 4.7d).

At 20 minutes mutation, the ethanol yield, the residual sugar, cell number and cell dry weight for isolates 2F36, 7F8, 7F9, DS6 and DS20 are presented in Table 4.7e as follows: The ethanol yield ranged from 4.95 – 18.18%(v/v), 9.38 – 18.18%(v/v), 9.55 – 18.18%(v/v), 2.27 – 15.91%(v/v) and 6.82 – 17.05%(v/v). The maximum ethanol yield was obtained for the various isolates above thus: fourth, third, fourth and second day respectively. The residual sugar ranged from 1.14 – 0.51g/100ml, 1.29 – 0.33g/100ml, 0.87 – 0.28g/100ml, 1.20 – 0.29g/100ml and 1.14 – 0.38g/100ml.The cell number ranged from 0.10 – 0.38cells/100ml, 0.08 – 0.15cells/100ml, 0.07 – 0.45g/100ml, 0.13 – 0.42cells/100ml and 0.11 – 0.25cells/100ml. The cell dry weight ranged from 0.10 - 0.32g/100ml, 0.10 – 0.58g/100ml, 0.14 – 0.44g/100ml, 0.16 – 0.58g/100ml and0.12 – 0.45g/100ml (Table 4.7e).

Table 4.7d:Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast Strains for Ethanol Production at 15minutes.

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
(DAYS)	(MINS)	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	15	0.16	0.14	0.12	0.12	0.14	0.14	0.13	0.14	0.11	0.10	1.10	0.77	0.79	1.07	1.07	12.5	13.64	11.36	3.41	9.72
2	15	0.20	0.16	0.18	0.12	0.16	0.15	0.14	0.16	0.12	0.12	1.01	0.77	0.77	1.05	0.63	14.78	11.36	14.78	10.23	13.64
3	15	0.24	0.20	0.20	0.24	0.20	0.15	0.15	0.16	0.15	0.14	0.81	0.71	0.71	1.02	0.59	11.36	11.36	12.5	2.25	13.64
4	15	0.28	0.34	0.28	0.24	0.24	0.16	0.16	0.21	0.18	0.23	0.75	0.64	0.68	0.76	0.58	11.36	13.64	9.09	2.27	14.78
5	15	0.32	0.34	0.34	0.24	0.26	0.20	0.17	0.23	0.21	0.27	0.72	0.57	0.62	0.68	0.53	13.64	8.59	9.09	2.25	13.64
6	15	0.38	0.46	0.38	0.26	0.28	0.21	0.21	0.24	0.22	0.27	0.43	0.57	0.54	0.35	0.26	17.05	7.95	12.5	9.09	7.95
7	15	0.46	0.63	0.44	0.30	0.38	0.40	0.31	0.25	0.34	0.28	0.43	0.47	0.40	0.29	0.17	10.16	5.68	2.25	16.40	6.82

Key: Result are means of triplicate determination

FMT = fermentation time

UVI = ultraviolet irradiation

CDW = cell dry weight

CND = cell number determination

RS = residual sugar

ETY = ethanol yield

2F36 = *Kodamaeaohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichumcapitatum*

DS6 = *Rhodotorulaminuta*

DS20 = *Candida zeylanoides*

At 25 minutes mutation, the ethanol yield, residual sugar, cell number and cell dry weight for isolates 7F8, DS20, DS6, 7F9 and 2F36 are shown in (Table 4.7f). The ethanol yield ranged from 6.82 – 14.78%(v/v), 2.25 – 17.05%(v/v), 2.25 – 18.18%(v/v), 4.55 – 18.18%(v/v) and 5.68 – 15.91%(v/v). The maximum ethanol yield was obtained on the second, third, fourth and sixth day for the different isolates as stated in (Table 4.7f). The residual sugars ranged from 0.61 – 0.29g/100ml, 0.65 – 0.25g/100ml, 1.24 – 0.29g/100ml, 1.30 – 0.41g/100ml, and 1.14– 0.33g/100ml.The cell number ranged from 0.08 – 0.15cells/100ml, 0.11 – 0.25cells/100ml, 0.13 – 0.42cells/100ml, 0.07 – 0.45cells/100ml, and 0.10 – 0.38cells/100ml. The cell dry weight ranges from 0.10 – 0.58g/100ml, 0.12 – 0.45g/100ml, 0.16 – 0.58g/100ml, 0.14 – 0.44g/100ml, 0.10 – 0.32g/100ml. (Tables 4.7f)

At 30 minutes mutation, the ethanol yield, residual sugar, cell number and cell dry weight for isolates 2F36, 7F8, 7F9, DS6 and DS20 are presented in (Table 4.7g.)The ethanol yield ranged from 5.64 – 17.05%(v/v), 1.14 – 14.78%(v/v), 1.14 – 15.91%(v/v), 2.27 – 11.36%(v/v), and 1.14 – 17.05%(v/v). The maximum ethanol yield was obtained on the second day for isolate DS20 17.05%(v/v), 7F8 on the third day 14.78%(v/v), 2F36 and 7F9, fourth 17.05%(v/v), 15.91%(v/v), respectively.The residual sugar ranged from 1.73 – 0.23g/100ml, 1.21 – 0.28g/100ml, 1.43 – 0.40g/100ml, 1.07 – 0.29g/100ml, and 1.01 – 0.42g/100ml.The cell number ranged from 0.11 – 0.29cells/100ml, 0.16 – 0.37cells/100ml, 0.21 – 0.28cells/100ml, 0.11 – 0.34cells/100ml and 0.09 – 0.33cells/100ml. The cell dry weight 0.08 – 0.30g/100ml, 0.14 – 0.51g/100ml, 0.28 – 0.53g/100ml, 0.10 – 0.44g/100ml, 0.12 – 0.88g/100ml. (Table4.7g).

Table 4.7e:Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast Strains for Ethanol production at 20minutes.

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
(Days)	(Mins)	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	20	0.14	0.18	0.14	0.10	0.16	0.18	0.08	0.17	0.15	0.13	1.14	1.29	0.87	1.20	1.14	5.68	13.64	13.64	14.78	17.05
2	20	0.26	0.28	0.24	0.12	0.18	0.20	0.10	0.17	0.18	0.13	1.12	0.73	0.63	1.10	0.99	13.64	15.91	14.78	15.91	17.05
3	20	0.40	0.34	0.28	0.20	0.24	0.20	0.10	0.19	0.20	0.15	1.00	0.64	0.49	1.02	0.87	15.91	18.18	12.5	7.95	13.64
4	20	0.44	0.38	0.30	0.26	0.28	0.32	0.11	0.21	0.25	0.18	0.98	0.62	0.43	0.94	0.70	18.18	17.05	18.18	3.41	15.91
5	20	0.58	0.40	0.35	0.28	0.30	0.32	0.14	0.21	0.30	0.24	0.66	0.57	0.43	0.87	0.53	11.36	13.64	9.55	3.41	9.09
6	20	0.65	0.50	0.44	0.48	0.32	0.36	0.15	0.24	0.32	0.27	0.54	0.51	0.38	0.49	0.38	11.36	13.64	12.5	2.27	7.95
7	20	0.78	0.64	0.65	0.61	0.40	0.37	0.16	0.47	0.33	0.34	0.51	0.33	0.28	0.29	0.38	4.95	9.38	15.27	2.27	6.82

Key: Result are means of triplicate determination

FMT = fermentation time

UVI = ultraviolet irradiation

CDW = cell dry weight

CND = cell number determination

RS = residual sugar

ETY = ethanol yield

2F36 = *Kodamaea ohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichum capitatum*

DS6 = *Rhodotorula minuta*

DS20 = *Candida zeylanoides*

Table 4.7f:Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast Strains for Ethanol Production at 25 minutes.

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
(Days)	(Mins)	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	25	0.10	0.10	0.14	0.16	0.12	0.10	0.08	0.07	0.13	0.11	1.14	0.61	1.30	1.24	0.65	14.78	14.78	14.78	10.23	13.64
2	25	0.10	0.12	0.16	0.32	0.16	0.12	0.10	0.13	0.14	0.11	0.97	0.57	0.99	1.02	0.62	11.36	14.78	14.78	12.5	17.05
3	25	0.26	0.18	0.22	0.48	0.22	0.18	0.11	0.21	0.20	0.12	0.85	0.57	0.89	1.01	0.55	9.09	12.5	10.23	18.18	11.36
4	25	0.26	0.20	0.24	0.48	0.24	0.20	0.12	0.23	0.21	0.20	0.76	0.52	0.75	0.83	0.51	5.68	6.82	18.18	5.68	11.36
5	25	0.26	0.20	0.26	0.48	0.28	0.35	0.15	0.24	0.22	0.22	0.65	0.51	0.58	0.48	0.49	9.09	14.78	13.63	4.55	2.27
6	25	0.28	0.30	0.30	0.56	0.32	0.35	0.15	0.44	0.28	0.24	0.71	0.44	0.46	0.47	0.42	15.91	14.78	4.68	4.55	2.25
7	25	0.32	0.58	0.44	0.58	0.45	0.38	0.15	0.45	0.42	0.25	0.33	0.29	0.41	0.29	0.25	13.64	13.38	4.55	2.25	2.25

Key: Result are means of triplicate determination

FMT = fermentation time

UVI = ultraviolet irradiation

CDW = cell dry weight

CND = cell number determination

RS = residual sugar

ETY = ethanol yield

2F36 = *Kodamaea ohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichum capitatum*

DS6 = *Rhodotorula minuta*

DS20= *Candida zeylanoides*

Table 4.7g:Fermentation of 5% Sorghum Starch with Mutant (UV light) Irradiation Yeast Strains for Ethanol Production at 30 minutes. .

FMT	UVI	CDW(g/100ml/isolates)					CND(cells/100ml)/isolates					RS(g/100ml)/isolates					ETY%(v/v)/isolates				
(Days)	(Mins)	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20	2F36	7F8	7F9	DS6	DS20
1	30	0.08	0.14	0.28	0.10	0.12	0.11	0.16	0.21	0.11	0.09	1.73	1.21	1.43	1.07	1.01	13.64	1.14	7.95	11.36	1.14
2	30	0.12	0.16	0.30	0.10	0.16	0.12	0.18	0.21	0.18	0.11	0.74	0.74	0.92	0.92	0.88	6.82	1.14	11.36	4.55	17.05
3	30	0.12	0.22	0.39	0.12	0.22	0.13	0.20	0.25	0.18	0.14	0.54	0.73	0.90	0.74	0.73	11.36	14.78	13.64	6.82	14.78
4	30	0.14	0.26	0.40	0.16	0.24	0.15	0.21	0.28	0.22	0.21	0.53	0.55	0.84	0.70	0.70	17.05	7.95	15.91	10.23	2.27
5	30	0.20	0.39	0.42	0.28	0.46	0.16	0.28	0.30	0.22	0.23	0.42	0.48	0.83	0.43	0.64	17.05	11.15	5.47	2.27	1.14
6	30	0.28	0.44	0.44	0.35	0.58	0.18	0.32	0.31	0.31	0.31	0.29	0.38	0.62	0.29	0.47	10.23	11.36	2.27	7.95	4.55
7	30	0.30	0.51	0.53	0.44	0.88	0.29	0.37	0.38	0.34	0.33	0.23	0.28	0.40	0.29	0.42	5.64	11.15	1.14	11.72	13.64

Key: Result are means of triplicate determination

FMT = fermentation time

UVI = ultraviolet irradiation

CDW = cell dry weight

CND = cell number determination

RS = residual sugar

ETY = ethanol yield

2F36 = *Kodamaea ohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichum capitatum*

DS6 = *Rhodotorula minuta*

DS20 = *Candida zeylanoides*

The result in the (Table 4.8) showed the maximum yield of ethanol obtained from the brewing, wild strains and nitrous acid-treated mutants. The brewing yeast gave the highest yield of18.18%(v/v), while the wild yeast also gave a maximum yield of 17.05%(v/v) for isolate 2F36 and 11.36%(v/v) for isolate DS6. All these were obtained on the fifth day of fermentation. Other wild yeast isolate 7F8 gave a maximum yield of 14.78% on the 6th day, while isolates 7F9 and DS20 gave maximum yields of 10.93%v/v on the 7th day of fermentation.

The nitrous acid-treated mutant of isolates 2F36 and 7F9 gave a maximum yield of 17.05%(v/v) each while DS20 gave 14.78%(v/v) on the 5th day of fermentation. Isolate DS6 and 7F8 showed maximum yield of 16.40%(v/v) and 16.16%(v/v) on the 7th day respectively.

Table 4.9 shows the maximum yield of ethanol fromUV light mutant yeast strains from fermentation of 5% sorghum starch. The maximum yield for the mutants was obtained at mutation time of 20minutes with a maximum yield of 18.18%(v/v) for isolates 2F36, 7F8 and 7F9 on the third and fourth day of fermentation. For isolates DS6, the maximum ethanol yield was 18.18%(v/v) at 25 minutes mutation on the third day of fermentation and 17.05 %(v/v) for isolate DS20 on the second day of fermentation at mutation time of 20 minutes.

Summarily, when the wild type yeast strain was compared with mutants obtained from UV light irradiation, it was observed that the length of fermentation reduced from the fifth to fourth day for isolate 2F36, sixth to third for isolate 7F8, seventh to fourth for isolate 7F9, fifth to third for DS6 and for isolate DS20 from seventh to second day (Table 4.9). The mutants of 2F36, 7F8 and 7F9 at 20 minutes and that of DS6 at 25 minutes mutation had the same maximum yield with the control strain 18.18%(v/v). However, two mutants of nitrous acid (DS6 and 7F8) gave a maximum yield on the seventh day of fermentation (16.16 and 16.40%) respectively (Table 4.8).

Table 4.8 Production Rate/Maximum Yields of Ethanol from Brewing, Wild and Mutant Yeast Strains from5% Sorghum Starch Fermentation.

Yeast strains and treatment	Isolate code	Production rates/maximum ethanol yield %(v/v)	Fermentation time (days)
Brewing	INS	18.18	5
Wild	2F36	17.05	5
Wild	DS6	11.36	5
Wild	7F8	14.78	6
Wild	7F9	10.93	7
Wild	DS20	10.93	7
Nitrous acid mutants (20 mins)	2F36	17.05	5
Nitrous acid mutants (20 mins)	7F9	17.05	5
Nitrous acid mutants (20 mins)	DS20	14.78	5
Nitrous acid mutants (20 mins)	DS6	16.40	7
Nitrous acid mutants (20 mins)	7F8	16.16	7

Key:

INS = Brewing yeast (*Saccharomyces carlbergensis*

2F36 = *Kodamaea ohmeri*

DS6 = *Rhodotorula minutas*

DS20 = *Candida zeylanoides*

7F8 = *Candida norvegensis*

7F9 = *Geotrichum capitatum*

Table 4.9:Production Rate/Maximum Yield of Ethanol %(v/v) Wild, Nitrous Acid and UV light-Irradiated (Mutants) Yeasts Strains from Fermentation of 5% Sorghum Starch.

Isolate code	UVT. (mins)									
									Mutants	
	4	6	10	15	20	25	30	Wild	NA	UV
2F36	13.64 (4)*	15.91 (4)	14.78 (3)	17.05 (6)	18.18 (4)	15.91 (6)	17.05 (4)	17.0(5)	17.05(5)	18.18(4)
7F8	15.91 (3)	17.05 (1)	14.78 (2)	13.64 (4)	18.18 (3)	14.78 (5)	14.78 (3)	14.78(6)	16.16(6)	18.18(3)
7F9	11.36 (5)	14.78 (3)	14.78 (3)	14.78 (2)	18.18 (4)	18.18 (4)	15.91 (4)	10.93(7)	17.05(5)	18.18(4)
DS6	10.23 (6)	10.23 (1)	14.78 (2)	16.40 (7)	17.05 (2)	18.18 (3)	11.72 (7)	11.36(5)	16.40(7)	18.18(3)
DS20	14.78 (3)	14.78 (5)	18.18 (5)	14.78 (4)	17.05 (2)	17.05 (2)	17.05 (2)	10.93(7)	14.78(5)	17.05(2)

Key: DS6 = *Rhodotorulaminuta*

2F36 = *Kodamaeaohmeri*

7F8 = *Candida norvegensis*

DS20 = *Candida zeylanoides*

[*] = Fermentation time (days)

7F9 = *Geotrichumcapitatum*

NA= Nitrous acid

UVT= Ultra Violet light time

4.11 Fermentative Ability

This was determined by gas production from glucose during fermentation of sugars. The gas (CO_2) produced during baking is responsible for the increase in volume and porosity of the bread. Hence, a good bakers yeast is expected to liberate gas from glucose. Majority of the yeast strains isolated from the study were found to produce gas vigorously (Table 4.10).

4.12 Growth in 3 and 10% NaCl

Since a pinch of sodium chloride formsone of the basic ingredients of bread, it is expected that bakers yeast should tolerate as much as 3% NaCl. It is however, inhibited by a large quantity as much as 10% depending on the type of yeast. All the yeasts tested had positive growth in 3% NaCl but most of the yeasts were inhibited in 10% (Table 4.10).

4.13 Growth in 50% Glucose

A considerable amount of sucrose or honey which contains glucose was usually added as sweetener to bread so baker's yeast ought to be osmophilic or osmo-tolerant in nature, that is, possess the ability to grow in high concentration of sugar. The strains were tested for growth in 50% glucose, the result obtained showed that they were osmophilic yeasts except the species of *Geotrichum* (Table4.10).

4.14 Growth at Elevated Temperatures

Fermentation is anaerobic in nature and heat is released as the cells respire.Baker's yeasts are able to grow at a temperature slightly higher than room temperature. The yeasts strains tested were able to grow at 30, 35, 37 and 42^0C (Table 4.1 and 4.10).

Table 4.10:Performance of Yeast Strains with respect to the Markers

Yeast strains	Fermentative ability	Growth in		Growth in 50% glucose	Growth at elevated temperatures				Dough leavening ability	
		3% NaCl	10% Nacl		30^0C	35^0C	37^0C	42^0C	Sweetened	Unsweetened
Instant dried yeast (1DY) (*Saccharomyces cerevisiae*)	**	+	_	+	+	+	+	_	+	+
Candida norvegensis	*	+	+	+	+	+	+	+	+	+
Candida zeylanoides	**	+	_	+	+	+	+	+	+	+
Geotrichumcapitatum	*	+	_	_	+	+	+	+	+	+
Kodamaeaohmeri	**	+	_	+	+	+	+	+	+	+
Rhodotorulaminuta	*	+	+	+	+	+	+	+	+	+

Key

**	Fermentation with gas production
*	Fermentation without gas production
+	Positive result
-	Negative result

4.15 Growth in 3% Ethanol

The strains of yeast isolated in this study showed tolerance and adapted to high concentration of ethanol (Table 4.4). The strains tested were able to grow in 3% ethanol, though evaporated during baking (ethanol).It is expected that the growth of baker's yeast is not inhibited by the presence of 3% ethanol. Since the yeast strains isolated in this study possess this important characteristic, they can be used for baking(Table 4.10).

4.16 Dough Leavening Tests

The isolated yeast strains having proved to possess markers peculiar to baker's yeast were tested for their fermentative ability in dough leavening both for sweetened and unsweetened types.

4.16.1 Sweetened dough leavening test

The results showing the leavening capacities of the yeasts strains of wild, mutant (nitrous acid) and the control commercial baker's yeast are presented in (AppendicesVIII, IXand Figs. 4.21 and 4.22). The proof height of the sweetened dough fermented with the wild yeasts ranged from 27- 193ml, nitrous acid-induced mutants, 45 – 185ml and the commercial baker's yeast 32 - 182ml (Fig 4.22 and 4.24).

The rate of fermentation for the yeast strains of both wild and nitrous acid-induced showed a uniform pattern since all of them showed lagging periods in the course of the 180 minutes and subsequent rise from 24-48 hours of incubation periods (Figs 4.21 and 4.23). The baker's yeast used as the control also showed a lagging period and subsequent rise within the 180 minutes and

declined from 24 h - 48 h of incubation periods. (Fig 4.21 and Fig 4.22). The leavening capacities of wild strains, nitrous acid-induced mutants and the control commercial baker's yeast (*Saccharomyces cerevisiae*) measured as increase in dough volume or leavening abilities ranged from 75 – 275ml, 84- 275ml at 48 h and 98 – 280ml at 180 minutes and 98 – 130ml at 48h for the control samplerespectively (Appendices VIII and IX). The commercial baker's yeast in both sweetened and unsweetened dough fermented the dough faster than the wild and the mutants from zero to 180 minutes. The wild type and mutants were slower and fermented the dough from zero - 48 h. The baker's yeast showed a better leavening activity at 180 minutes and declined from 24- 48 h in leavening activity from 280- 130ml. The wild and mutant strains also exhibited a good performance of leavening from 24- 48 h and the best result was obtained at 48 h of incubation periods (Fig 4.22 and 4.24).

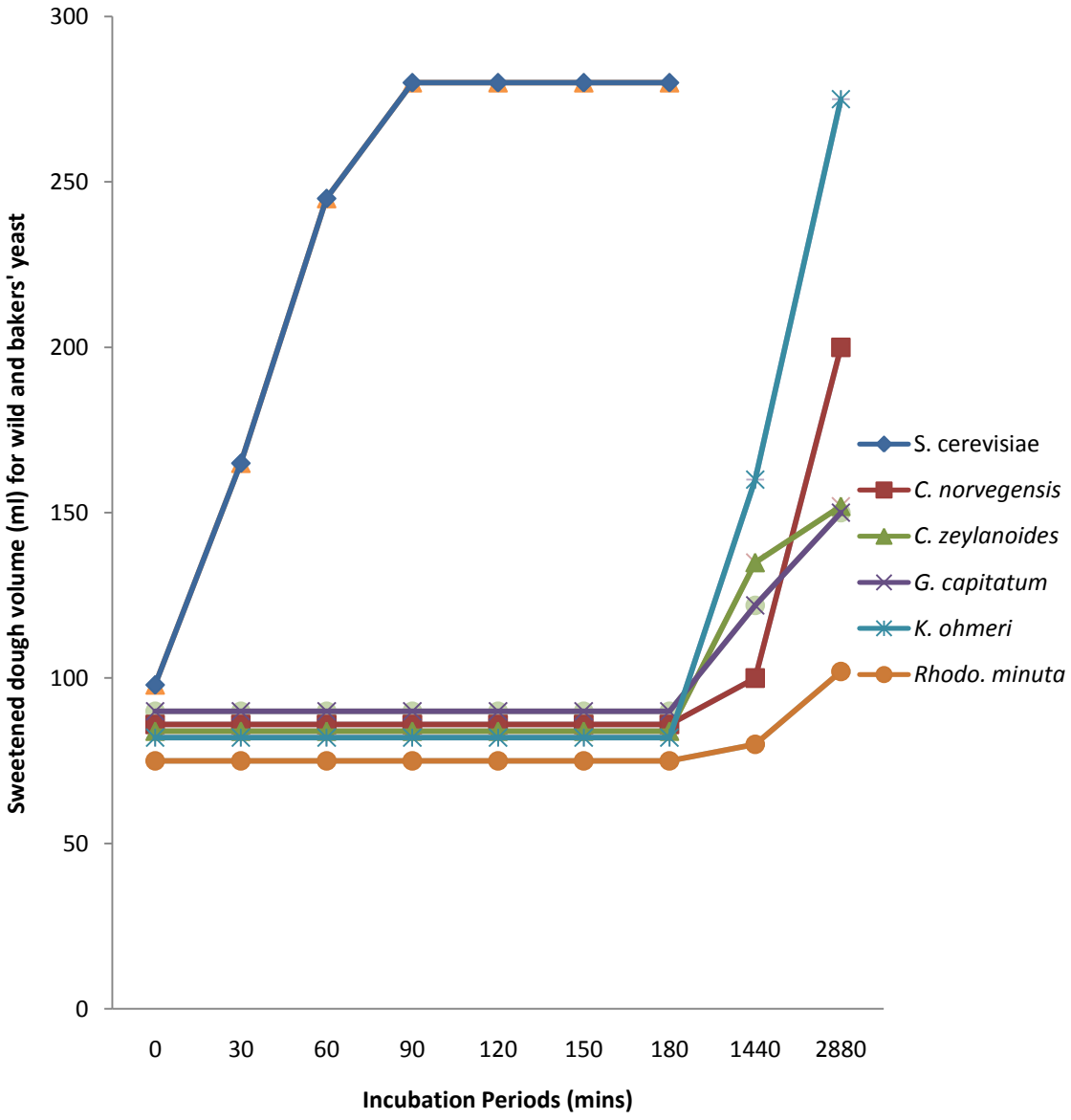


Fig 4.21: Leavening Profiles of Wild Strains and Baker's Yeast Sweetened Dough

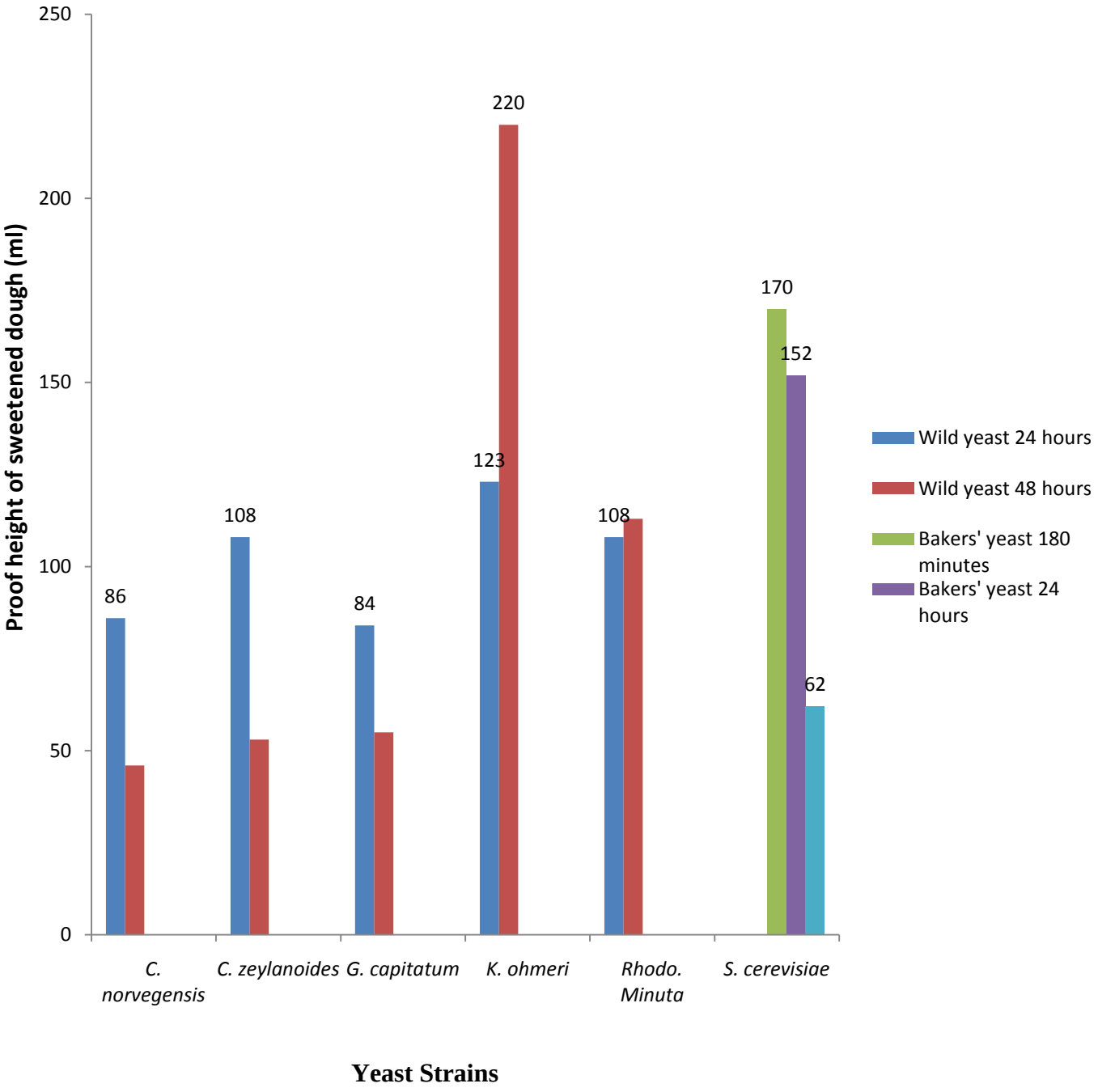


Fig 4.22: Proof Heightsof Wild Strains and Baker's Yeast Sweetened Dough

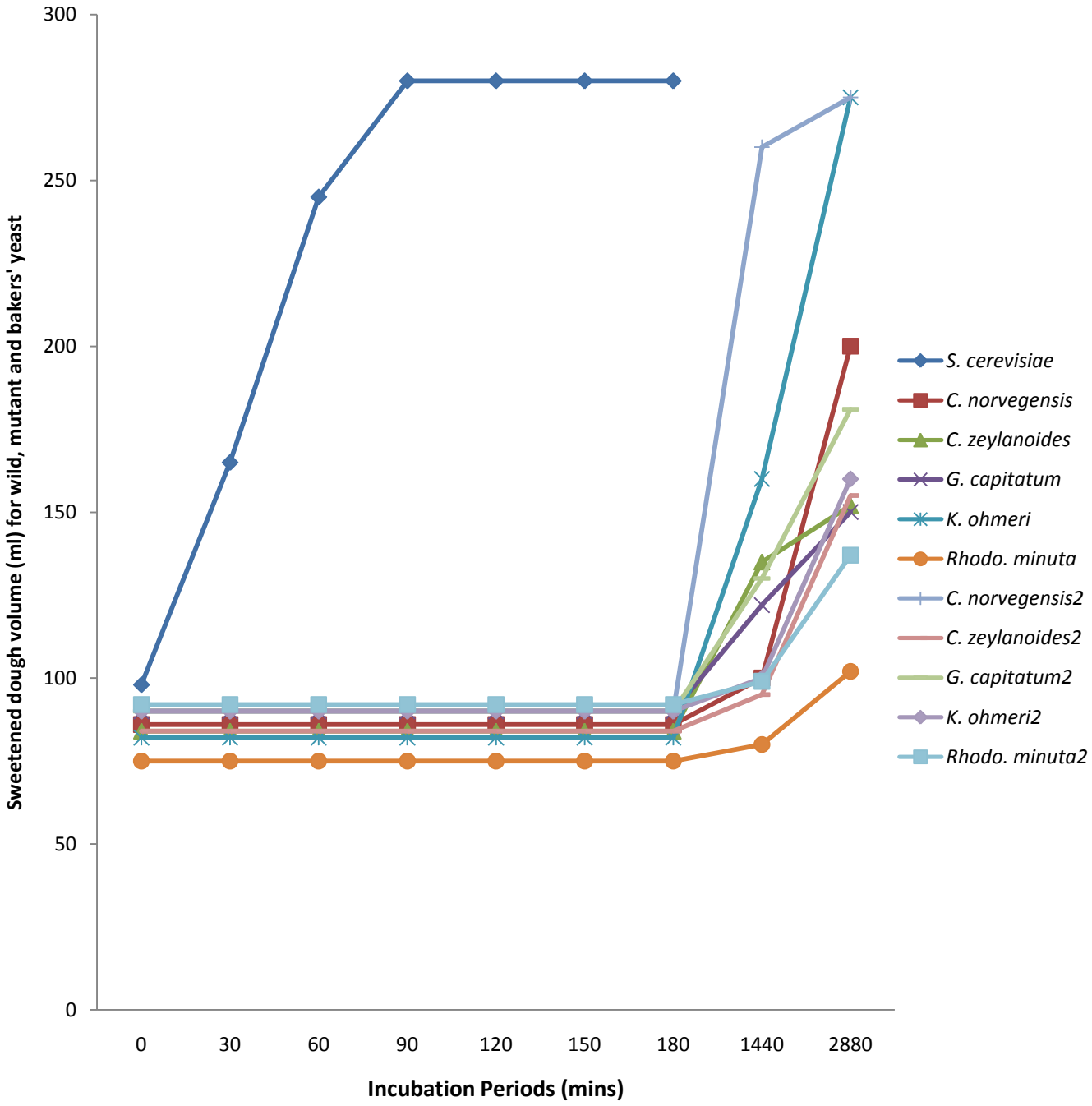


Fig 4.23: Leavening Profiles of Wild, Mutant (nitrous acid) Treatment and Baker's Yeast in Sweetened Dough

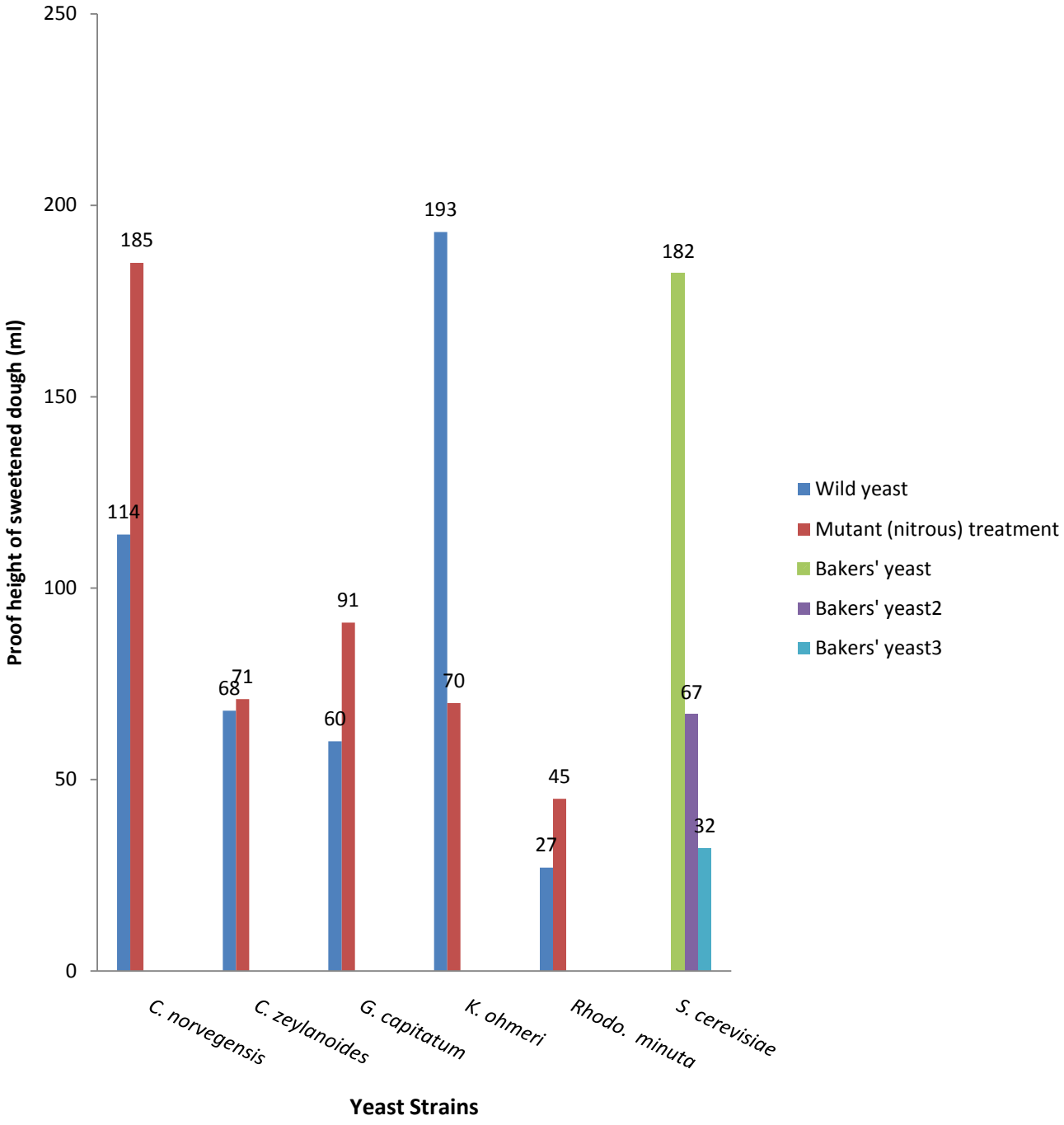


Fig 4.24: Proof Heights of Sweetened Dough Fermented Wild Strains, Mutant (nitrous acid) Treatment and Baker' s Yeast.

4.16.2 Unsweetened dough leavening test.

The test was carried out as in the sweetened dough and values were recorded for wild, mutants and commercial baker's yeast as presented in (Appendicies X and XI). The proof heights for the unsweetened dough fermented with the wild yeasts which ranged from 46 – 145ml, nitrous acid-induced mutants; 50- 80ml and the control baker's commercial yeast 62 – 170ml (Fig 4.25). The leavening abilities of the yeasts in the fermentation of the unsweetened dough for wild andnitrous acid-induced mutants showed a uniform pattern since all of them exhibited a lagging in the course of 180 minutes and subsequent rise from 24-48 h (1440 – 2880 miuntes) of incubation periods(Fig 4.26). The baker's yeast, the control strain, also showed a lagging period and subsequent rise within 180 minutes and declined from 24- 48 h of incubation periods.

The leavening abilities ranged from 71 – 220ml for unsweetened wild type of yeasts, nitrous acid-induced mutants, 70 – 150ml and the baker's yeast ranged from 78 – 248ml (Appendix X, XI and Figs4.26 and 4.27a). From the results obtained, *Kodamaea ohmeri* is taken as the best among the wild strains in terms of leavening activity in both sweetened and the unsweetened dough (Fig 4.24,4.25 and 4.27b). Most of the wild yeast strains used for sweetened dough showed a better leavening performance when mutated (nitrous acid). The strain of *Candida norvegensis* showed the best in leavening activity for the sweetened dough (Fig. 4.24). However, it was observed that the mutation caused a reduction in the leavening activity of the wild type of *Kodamaea ohmeri* for sweetened dough and an increase in*Rhodotorula minuta* for the unsweetened dough (Figs.4.24 and 4.25).

The result also revealed that for the unsweetened dough, majority of the wild yeast strains had a better leavening capacity than the mutants of nitrous acid (Appendices.X, XI and Figs 4.27b).

The wild strains and mutants from nitrous acid treated sweetened dough at incubation period of 48 hours showed a better leavening capacity than the commercial baker's yeast (Appendices.VIII, IX and Fig. 4.22). The commercial baker's yeast which was the control, showed the best leavening capacity at 180 minutes and poor leavening capacity at 48 h for both sweetened and unsweetened dough (Appendices.s VIII and X). The sweetened dough had a better leavening capacity than the unsweetened dough in wild, nitrous acid-induced mutants and baker's yeast (Fig 4.27b). However, there was a remarkable improvement in leavening capacities of the yeast strains of*Kodamaea ohmeri*and*Rhodotorula minuta*(wild strains) in the unsweetened doughwhen compared with their performance in sweetened dough (Figs. 4.24 and Fig 4.27b).

The results for the dough leavening test statistically showed that there were no significant differences in the mean performance between the bakers yeast and the wild strains in the sweetened and the unsweetened dough ($P>0.05$) appendices XII and XV.

The mutants of nitrous acid and UV light when compared with the wild type statistically, their mean performances varied between the different species. Some species showed significant differences at $P> 0.05$ at different time intervals 24 and 48 hfor sweetened and unsweetened doughrespectively (AppendicesXIV -XVII).

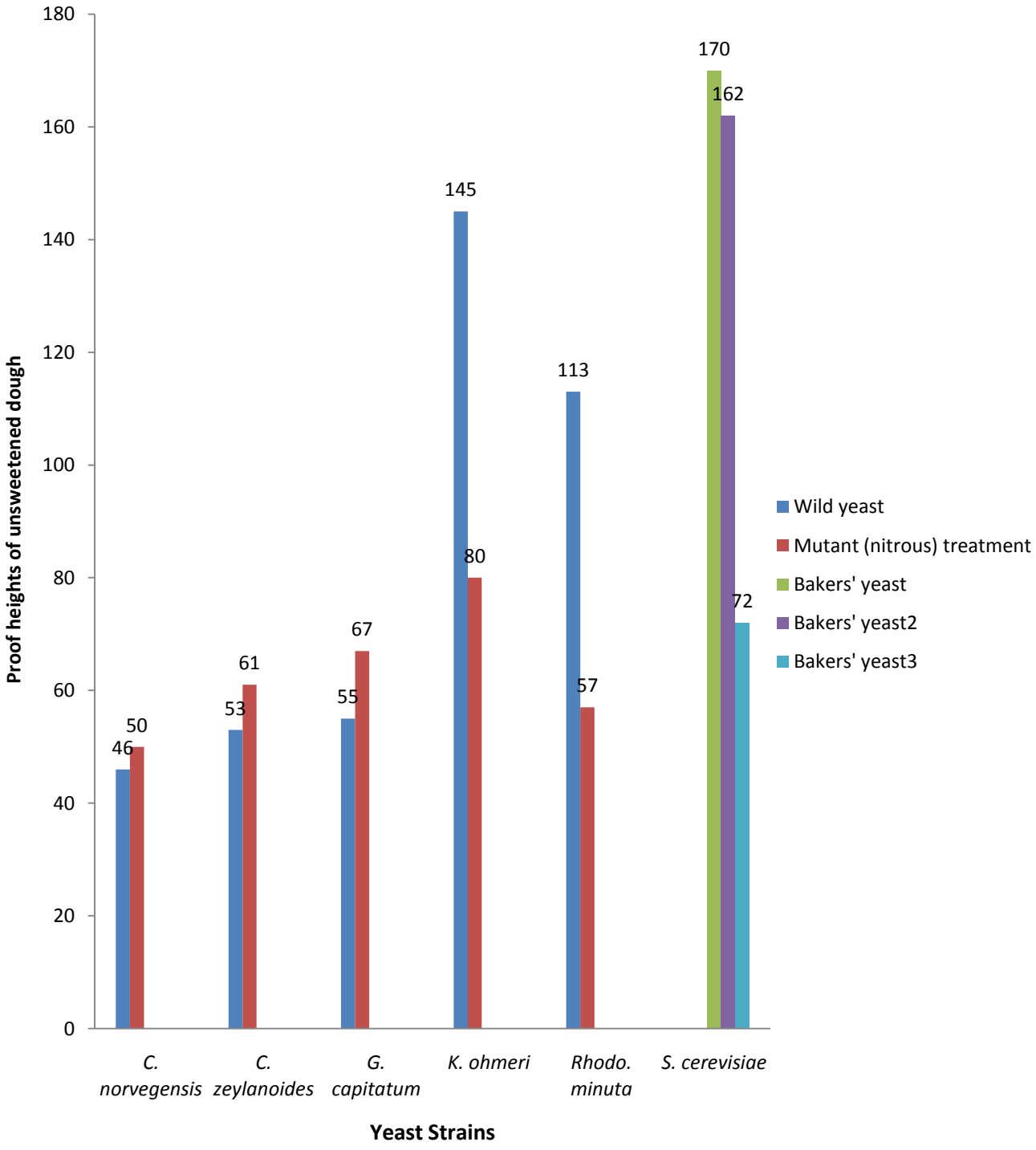


Fig 4.25: Proof Heights of Unsweetened Dough Fermented with Wild Strains,Mutant (nitrous acid) Treatment and Baker's Yeast

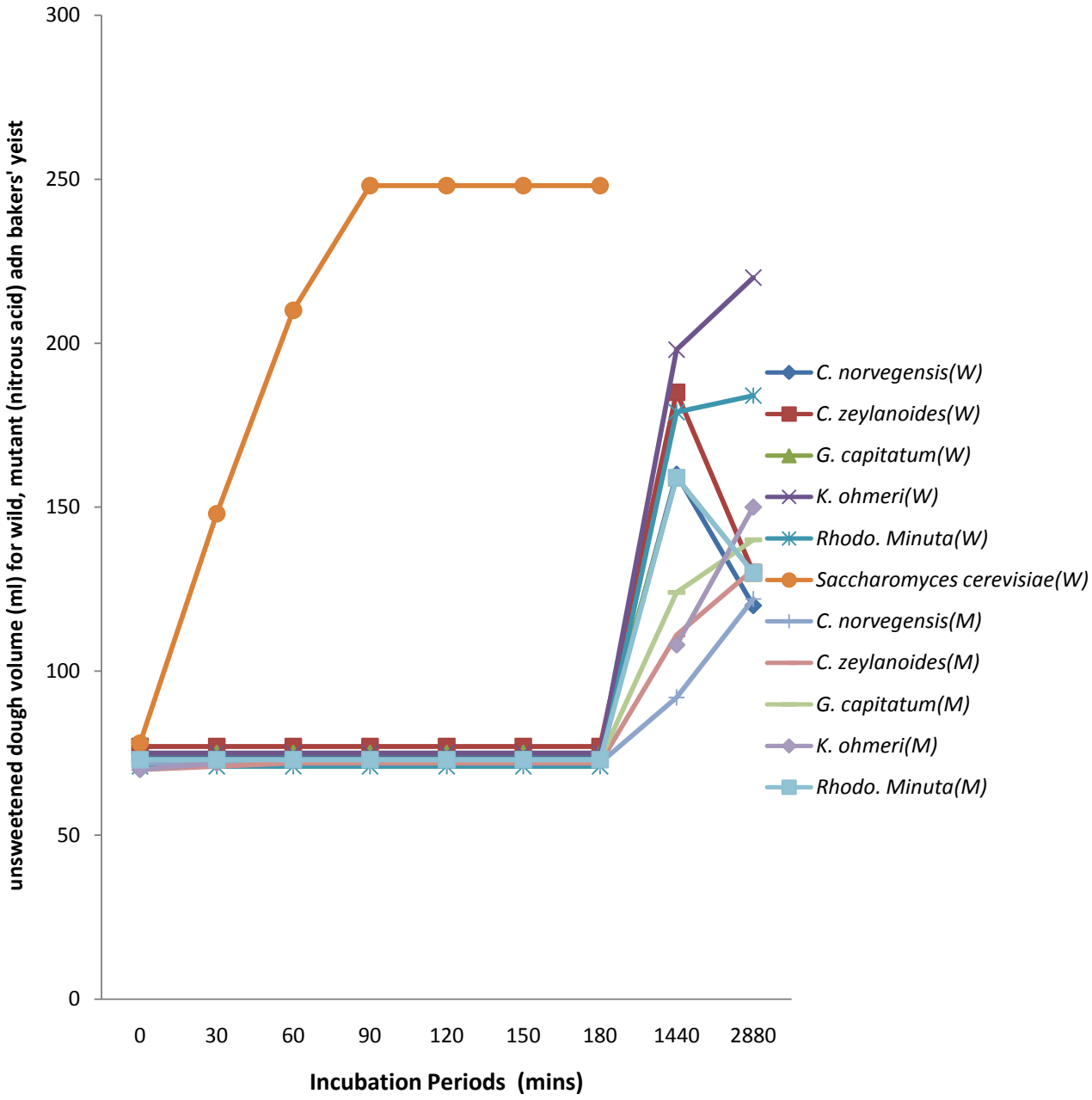


Fig 4.26: Leavening Profile of Unsweetened Dough Fermented with Wild Strains, Mutant (nitrous acid) Treatment and Baker's Yeast.

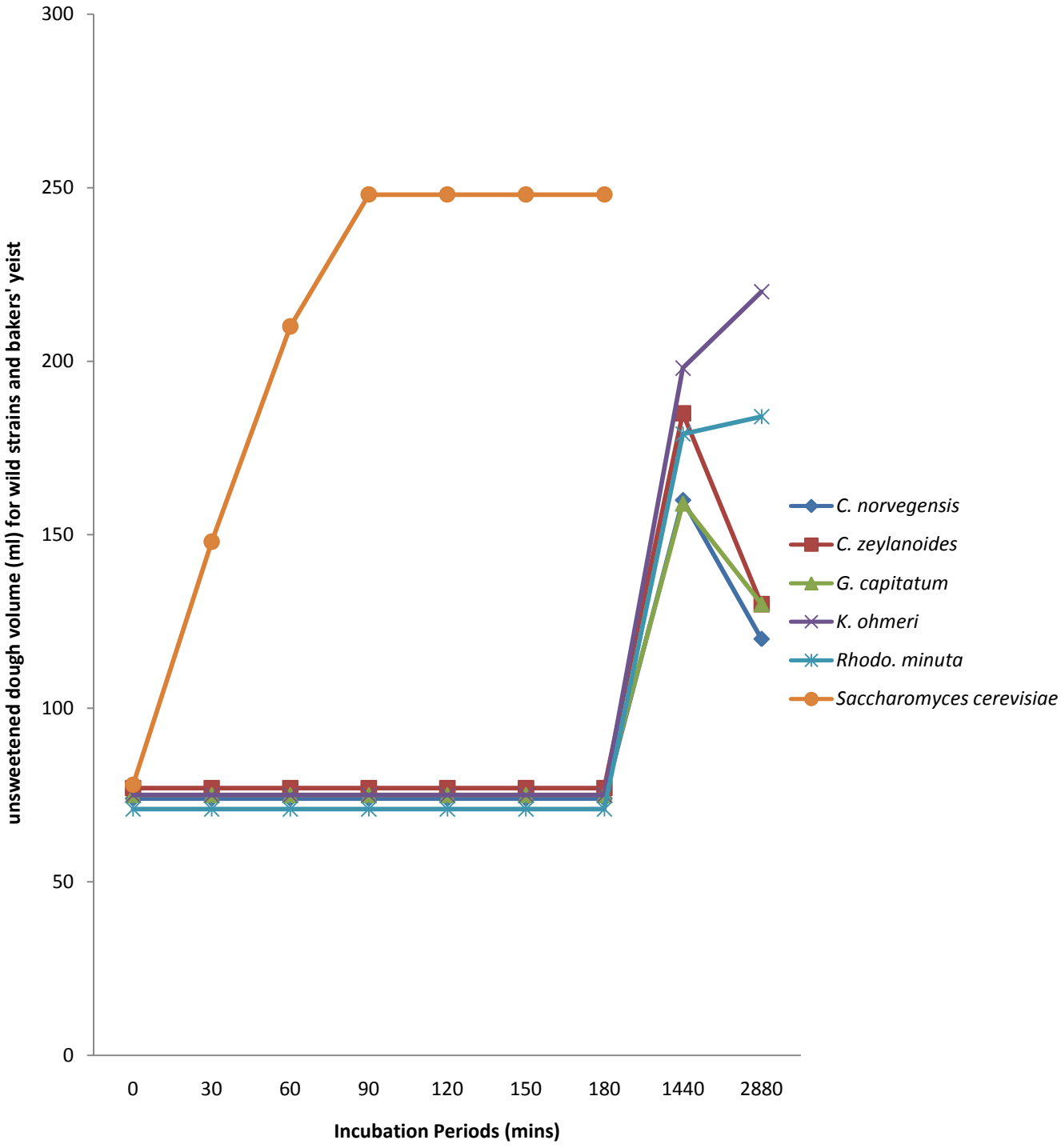


Fig 4.27a: Leavening Profile of Unsweetened Dough Fermented with Wild Yeast and Baker's Yeast.

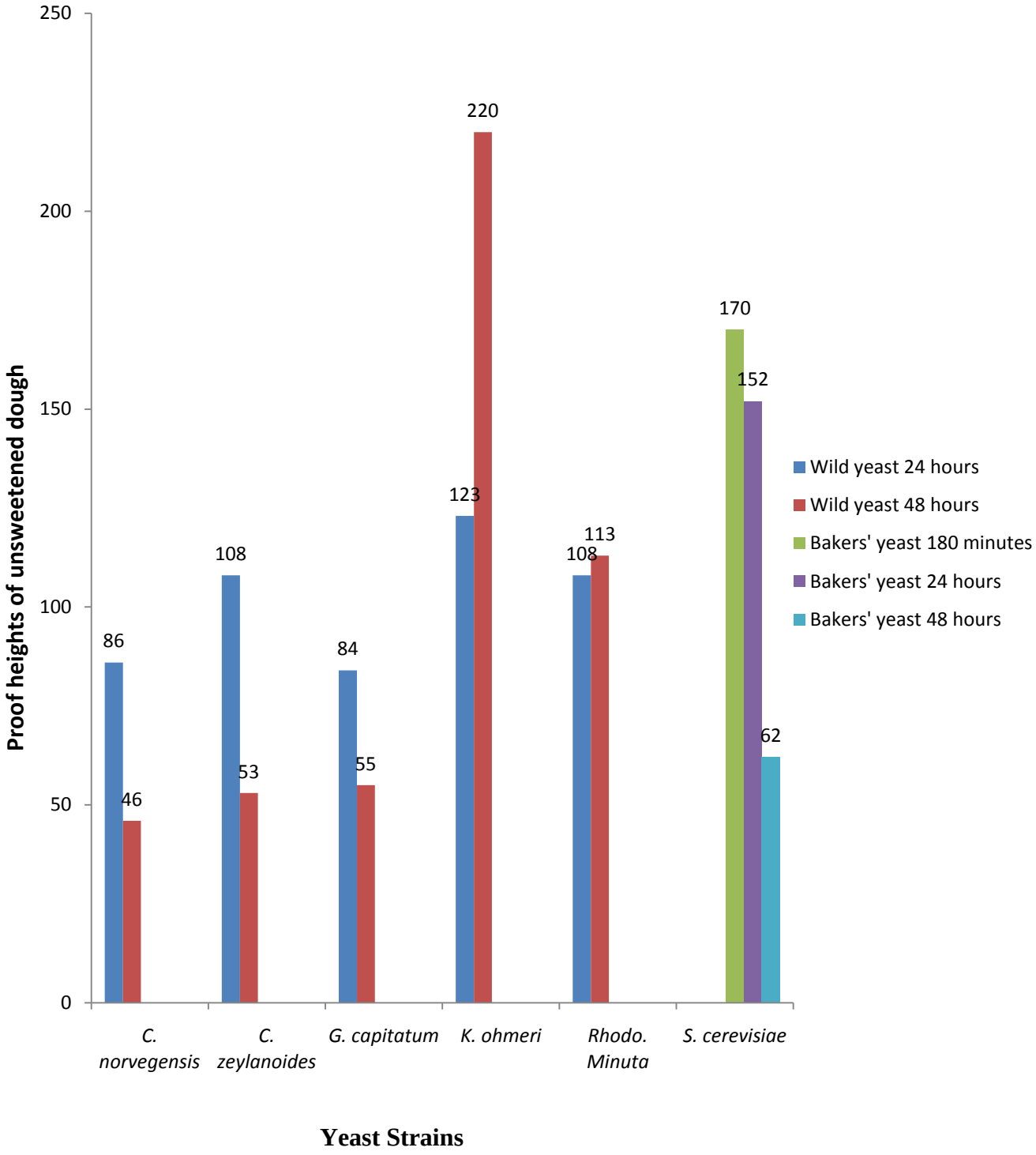


Fig 4.27b: Proof Heightsof Unsweetened Dough Fermented with Wild Yeast and Baker's Yeast.

4.16.3 Ultra Violet (UV) light-Irradiated Yeast Strains used for Sweetened and Unsweetened Dough Test

The test was carried out as described in sweetened and unsweetened dough for wild and nitrous acid mutants. They only differred in the UV light exposure time intervals of 4, 6, 10, 15, 20, 25 and 30 minutes. The results were recorded as presented in Table 4.11 and 4.12.The dough leavening capacitiesof the UV- light-irradiated yeast strains (mutants) in relation to time intervals (minutes) ranged from 4 mins 60 – 104ml, 6mins 65 – 112ml, 10mins 60 – 108ml, 15mins 66 – 86ml, 20mins 68 – 116ml, 25mins 70 – 150ml and 30mins 70 – 94ml (Table 4.12).

The results for unsweetened dough ranged from 4mins69 – 175ml, 6mins70 – 185ml, 10mins70 – 195ml, 15mins71 – 230ml, 20mins70– 203ml, 25mins70 – 170ml and 30mins70 – 186ml) (Table 4.13). The above dough leavening activities were observed with various isolates obtained from the study namely: *Candida norvegensis, Candida zeylanoides, Geotrichum capitatum, Kodamaea ohmeri* and *Rhodotorula minuta* for both sweetened and unsweetened dough.Considering the result obtained from the sweetened dough, it was observed that at 4 minutes, the mutant that gave the best leavening activity was *Candida norvegensis* (25ml) and the least was*Candida zeylaniodes* (2ml) (Fig 4.28a). At 6 and 10 minutes, *Kodamaea ohemri* and *Candida norvegensis* muntants gave the leavening activites of 46 and 20ml (Fig 4.28c and Fig 4.28e). The mutant strain of *Kodamaea ohmeri*, at 15, 20 and 25 minutes intervals gave the best leavening activities of 12, 36 and 25 ml (Figs. 4.28g, 4.281 and 4.28k). At 30 minutes, *Candida norvegensis* gave the best leavening actitity of 24ml (Fig. 4.28m).However, combining the leavening capacities of sweetened dough from UVmutation times from 4- 30 minutes intervals showed that the least mutants in terms of leavening capacity is the yeast strain *Candida zeylanoides (*Appendix XVIII).

Table 4.11:Assessment of Leavening Capacities of MutantYeast Strains with UV-Light at Different Time Intervals in Sweetened Dough

Yeast strain	Dough volume (ml) at Different Incubation Times (mins)									
	Time interval (mins)	0mins	30mins	60mins	90mins	120mins	150mins	180mins	24hrs	48hrs
Candida norvegensis	4	60	65	75	75	75	75	75	75	85
	6	65	70	70	70	70	70	70	70	80
	10	60	70	70	70	70	80	80	80	80
	15	76	76	76	76	76	76	76	78	80
	20	70	70	70	70	70	75	75	75	80
	25	70	70	70	70	70	75	75	75	75
	30	70	74	74	74	74	84	84	84	94
Rhodotorula minuta	4	73	73	73	73	73	73	73	77	80
	6	73	73	73	73	73	73	73	78	89
	10	73	73	73	73	73	73	73	76	82
	15	73	73	73	73	73	73	73	77	78
	20	73	73	73	73	73	73	73	75	76
	25	74	74	74	74	74	74	74	76	82
	30	77	77	77	77	77	77	77	86	88
Geotrichum capitatum	4	65	65	67	67	67	67	67	70	80
	6	75	75	77	77	77	77	77	84	112
	10	91	91	91	91	91	91	91	95	108
	15	66	66	66	66	68	68	68	68	74
	20	68	68	69	69	69	69	69	74	77
	25	76	76	76	76	76	76	76	82	96
	30	72	72	73	73	73	73	73	75	80
Kodamaea ohmeri	4	80	80	80	80	80	80	80	84	104
	6	84	84	84	84	84	84	84	90	130
	10	90	90	90	90	90	90	90	90	104
	15	74	74	74	74	74	74	74	76	86
	20	80	80	80	80	80	80	80	80	116
	25	90	90	90	90	90	90	90	94	150
	30	90	90	90	90	90	90	90	92	92
Candida zeylanoides	4	76	76	76	76	76	76	76	77	78
	6	75	75	75	75	75	75	75	77	78
	10	76	76	76	76	76	76	76	77	80
	15	77	77	77	77	77	77	77	77	79
	20	73	73	73	73	73	73	73	73	74
	25	82	82	82	82	82	82	82	82	83
	30	79	79	79	79	79	79	79	79	80

Values are means of duplicate readings.

Table 4.12:Assessment of Leavening Capacities of Mutant Yeast Strains with UV-Light at Different Time Intervals in Unsweetened Dough

Yeast strain	Dough volume (ml) at Different Incubation Times (mins)									
	Time interval (mins)	0mins	30mins	60mins	90mins	120mins	150mins	180mins	24hrs	48hrs
Candida norvegensis	4	70	70	70	70	70	70	70	108	138
	6	75	75	75	75	75	75	75	118	185
	10	75	75	75	75	75	75	75	85	85
	15	75	75	75	75	75	75	75	90	90
	20	75	75	75	75	75	75	75	95	95
	25	72	72	72	72	72	72	72	80	80
	30	72	72	72	72	72	72	72	118	118
Candida zeylanoides	4	72	72	72	72	72	72	72	146	166
	6	72	72	72	72	72	72	72	100	116
	10	70	70	70	70	70	70	70	140	195
	15	74	74	74	74	74	74	74	118	149
	20	74	74	74	74	74	74	74	99	114
	25	74	74	74	74	74	74	74	98	120
	30	75	75	75	75	75	75	75	100	155
	4	69	69	69	69	69	69	69	102	129
Geotrichum capitatum	6	73	73	73	73	73	73	73	102	119
	10	72	72	72	72	72	72	72	185	190
	15	71	71	71	71	71	71	71	114	168
	20	73	73	73	73	73	73	73	191	203
	25	70	70	70	70	70	70	70	135	170
	30	73	73	73	73	73	73	73	161	186
Kodamaea ohmeri	4	70	70	70	70	70	70	70	135	175
	6	76	76	76	76	76	76	76	146	182
	10	76	76	76	76	76	76	76	157	194
	15	76	76	76	76	76	76	76	186	230
	20	70	70	70	70	70	70	70	125	170
	25	70	70	70	70	70	70	70	75	100
	30	70	70	70	70	70	70	70	100	125
Rhodotorula minuta	4	70	70	70	70	70	70	70	90	120
	6	70	70	70	70	70	70	70	88	110
	10	70	70	70	70	70	70	70	200	150
	15	75	75	75	75	75	75	75	220	170
	20	75	75	75	75	75	75	75	190	180
	25	75	75	75	75	75	75	75	100	120
	30	78	78	78	78	78	78	78	180	140

Values are Means of Duplicate Readings.

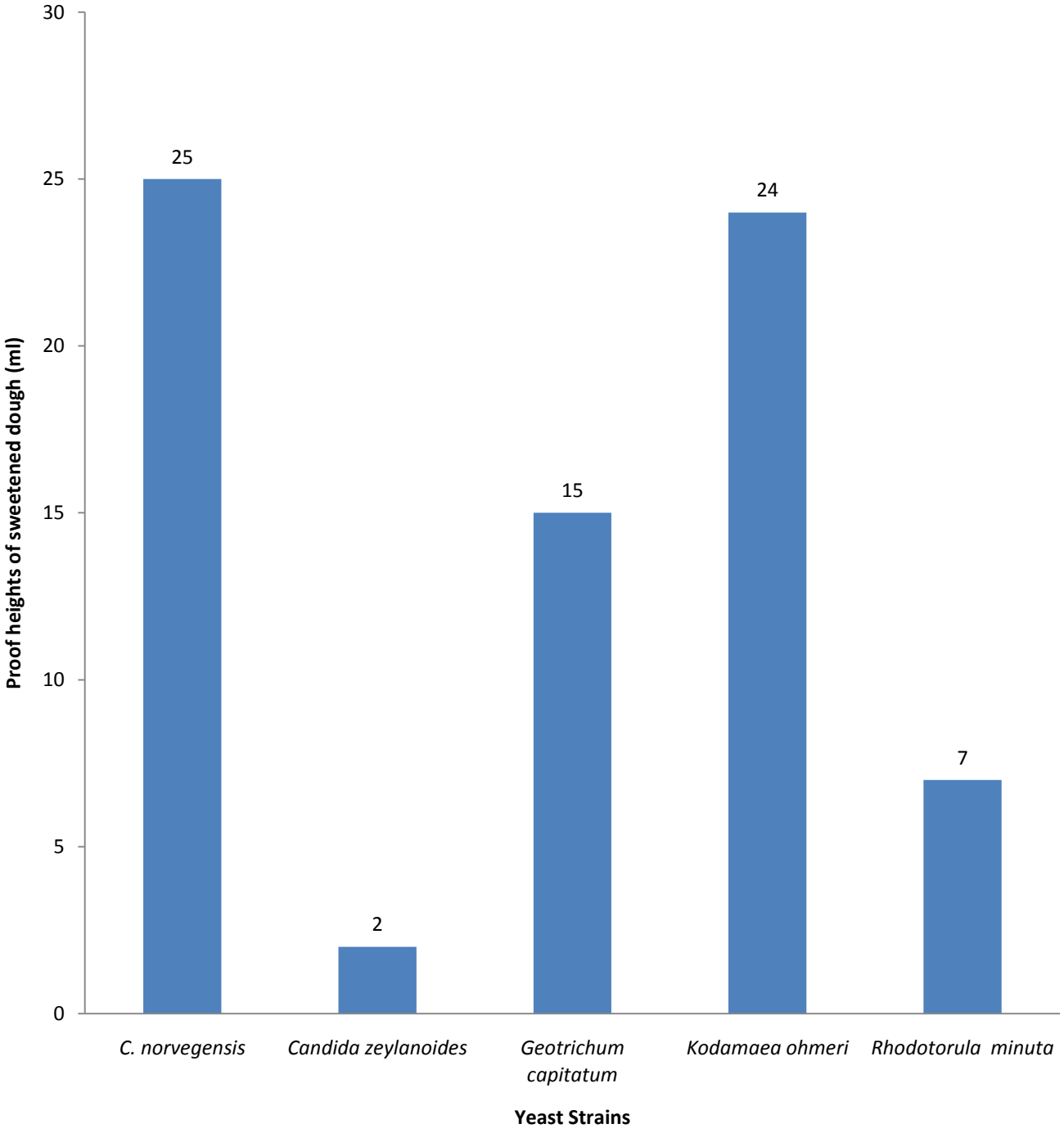


Fig 4.28a: Proof Heights of Sweetened Dough of UV Mutants at 4mins

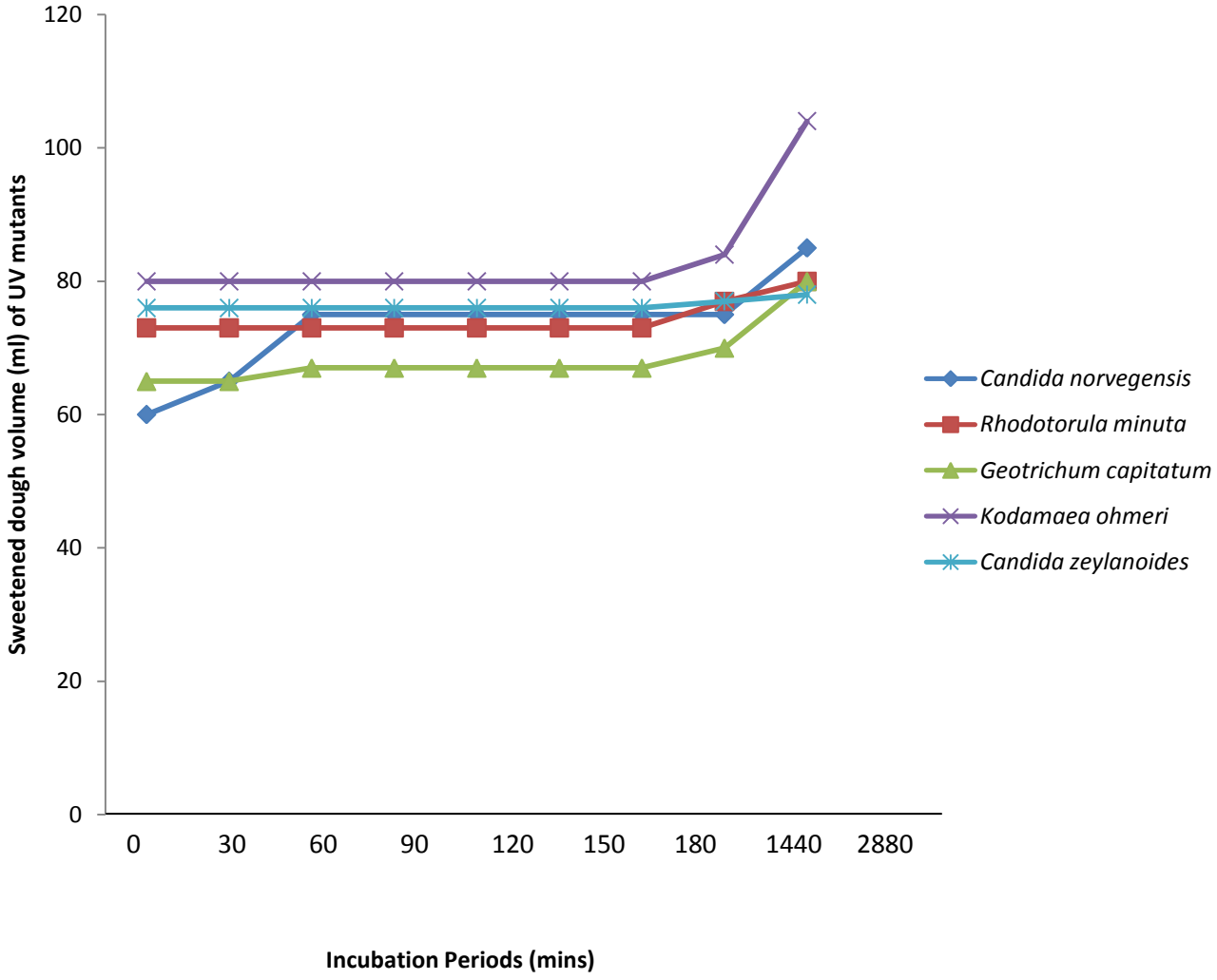


Fig 4.28b: Leavening Profile of Sweetened Dough for UV Mutants at 4 mins.

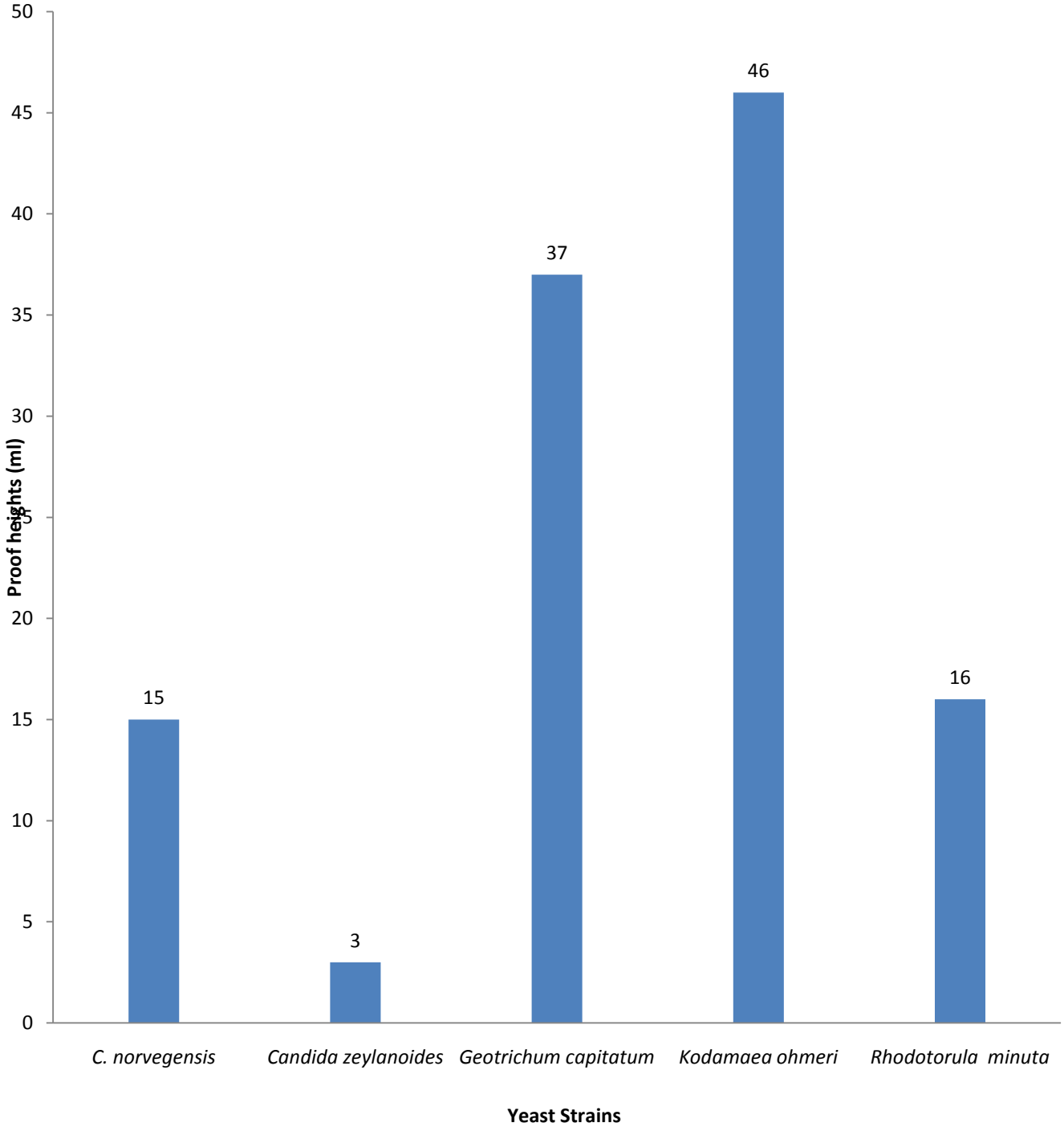


Fig 4.28c: Proof Heights of Sweetened Dough of UV Mutants at 6 mins

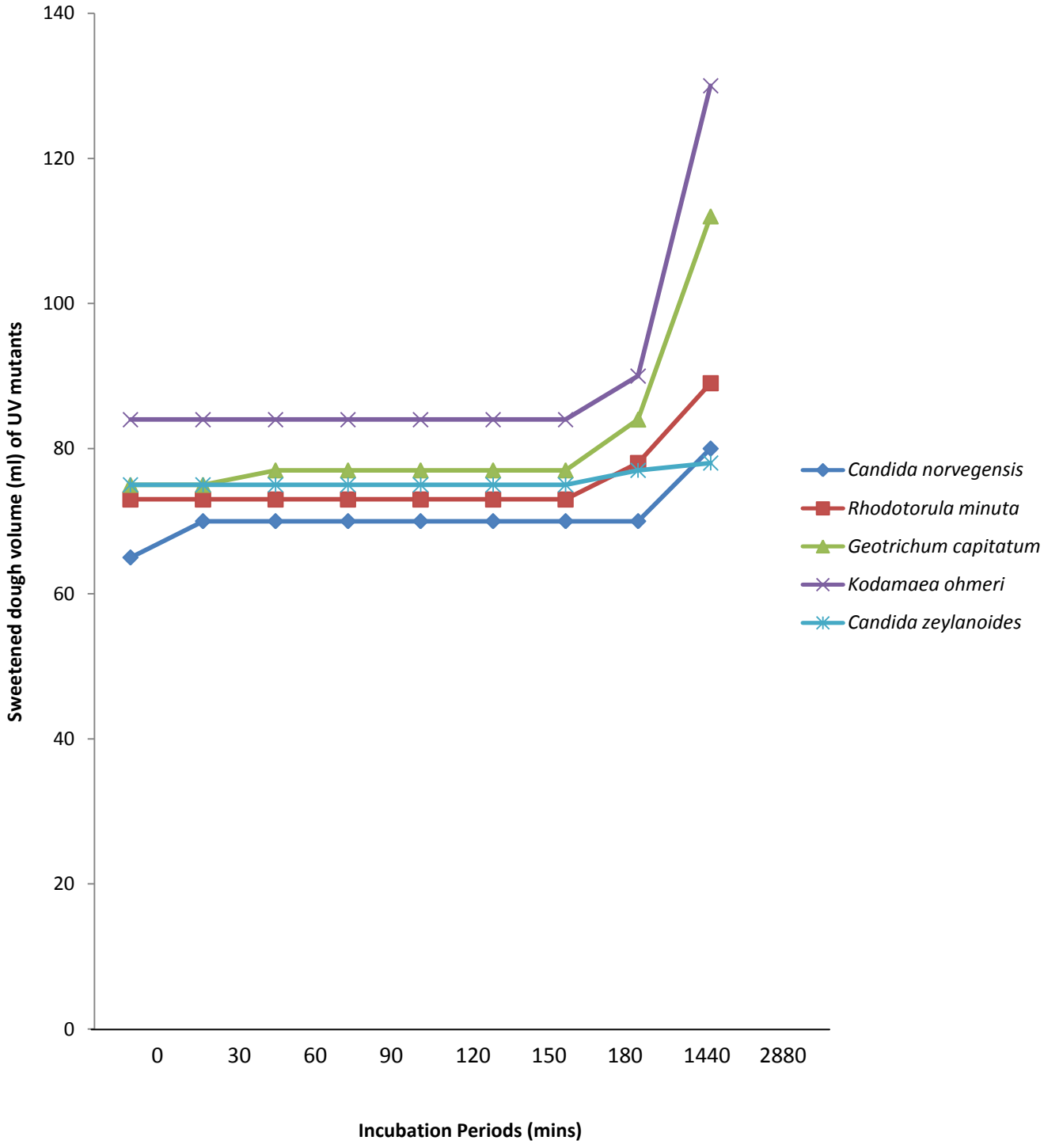


Fig4. 28d: Leavening Profile of Sweetened Dough for UV Mutants at 6 mins.

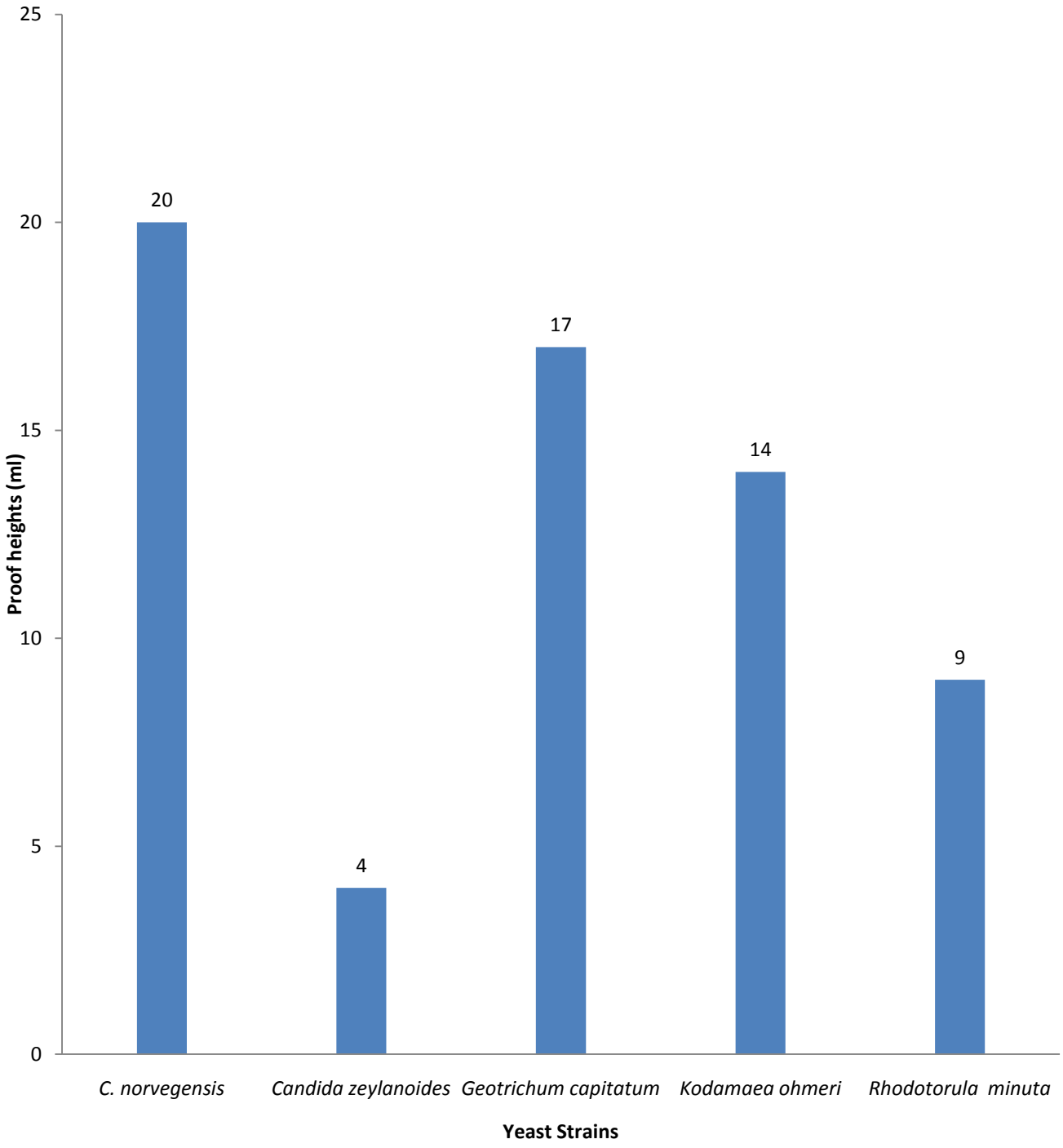


Fig 4.28e: Proof Heights of Sweetened Dough of UV Mutants at 10 mins

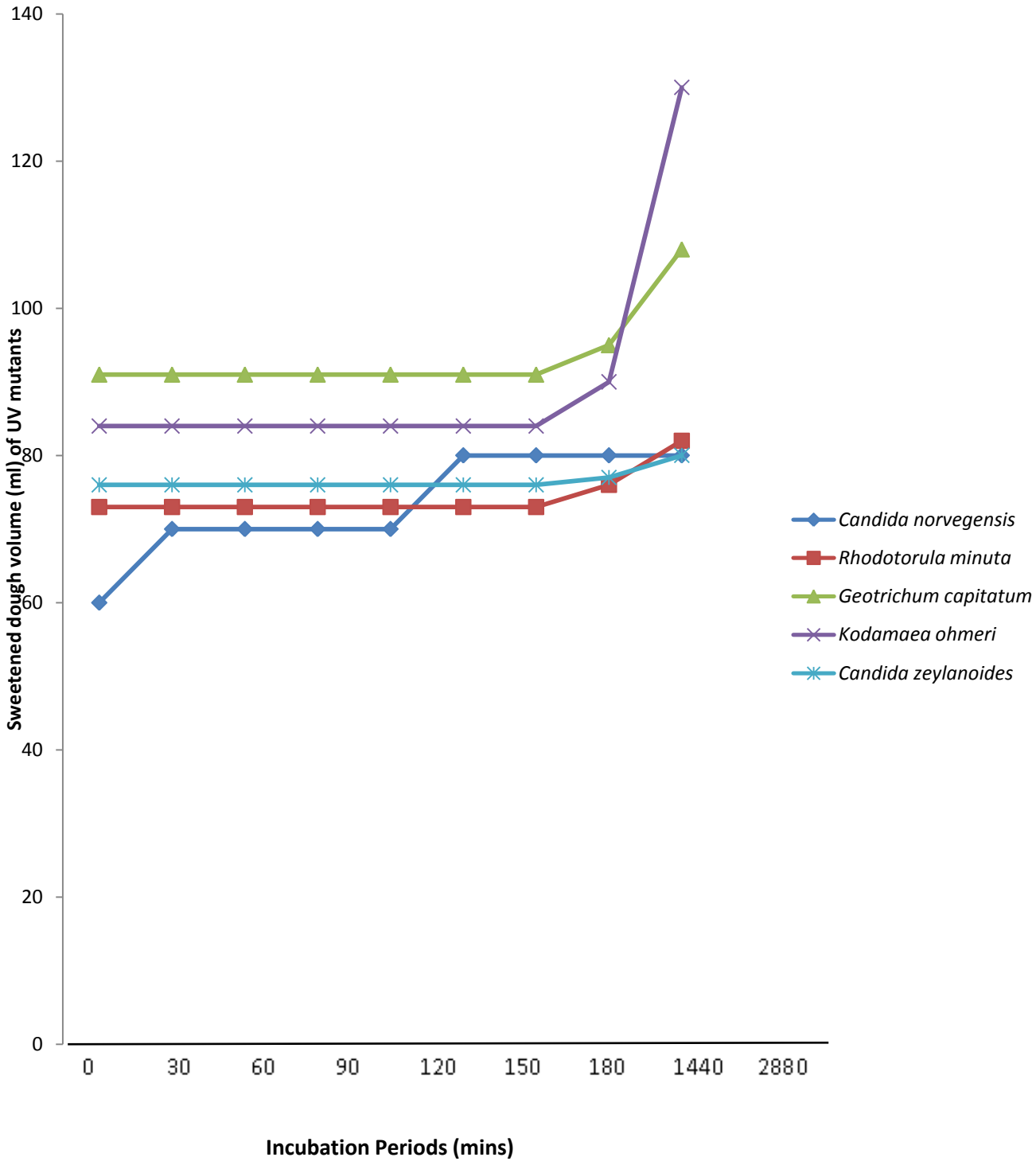


Fig 4.28f: Leavening Profile of Sweetened Dough for UV Mutants at 10 mins

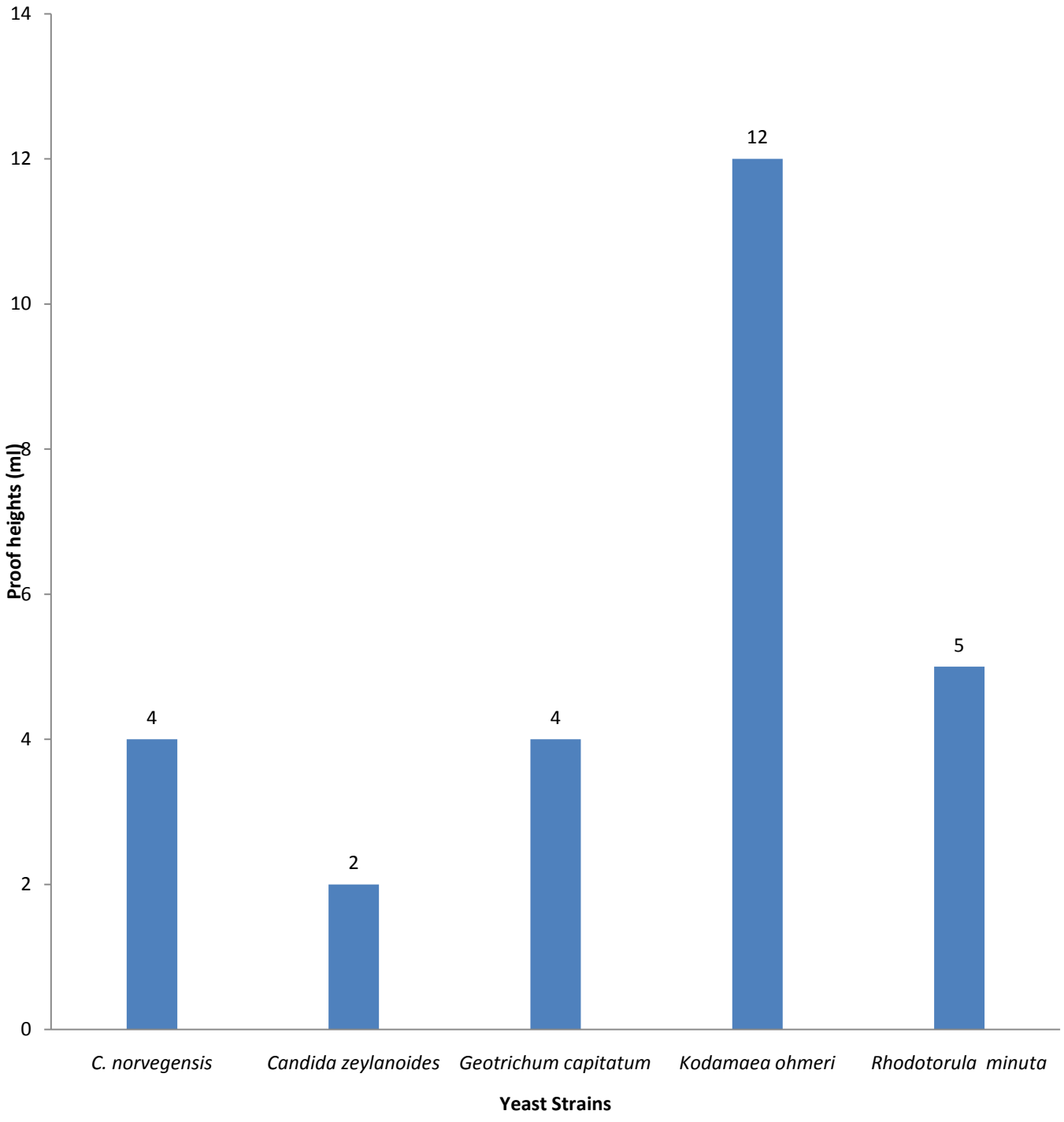


Fig 4.28g: Proof Heights of Sweetened Dough of UV Mutants at 15 mins

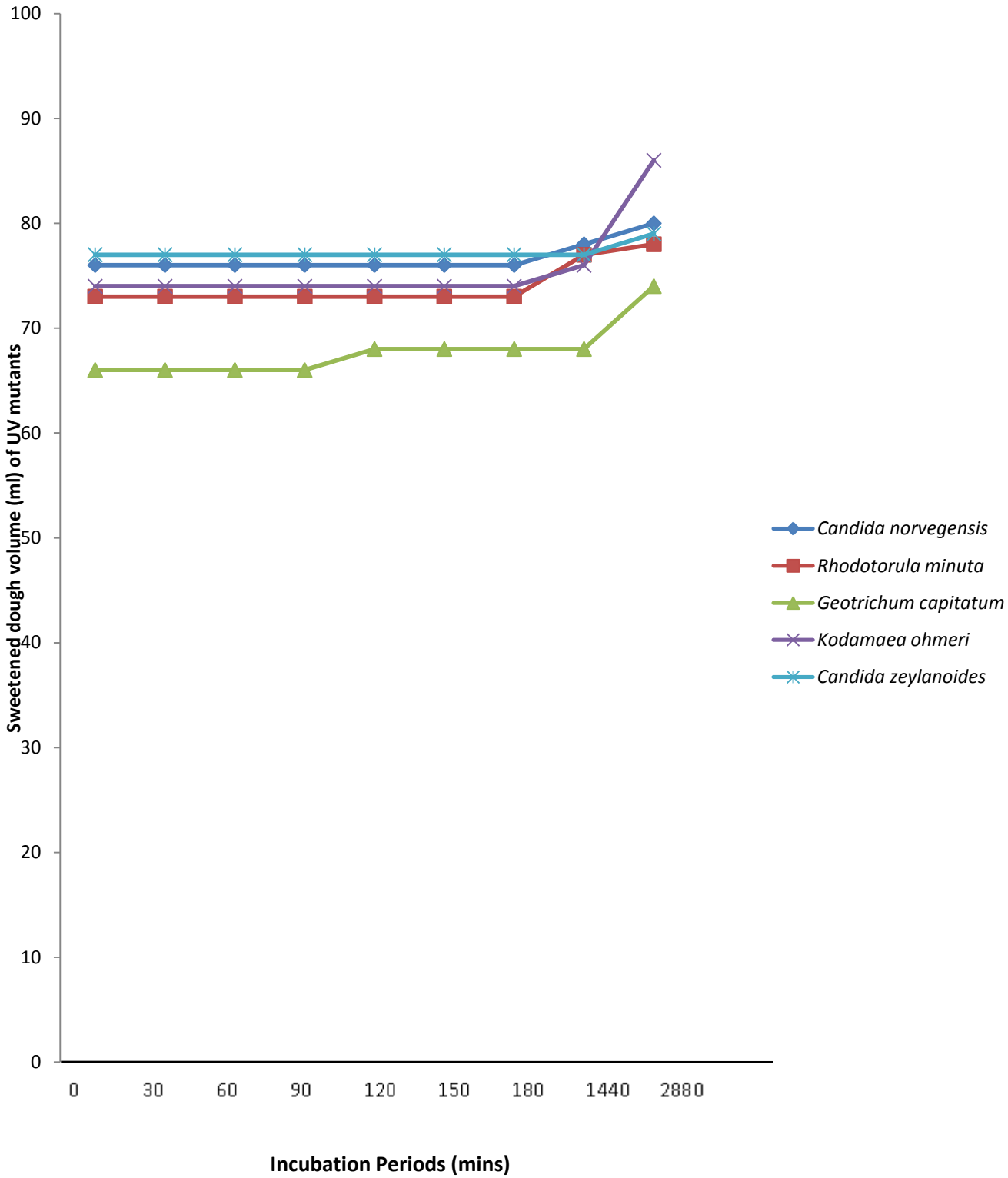


Fig 4.28h: Leavening Profile of Sweetened Dough for UV Mutants at 15 mins

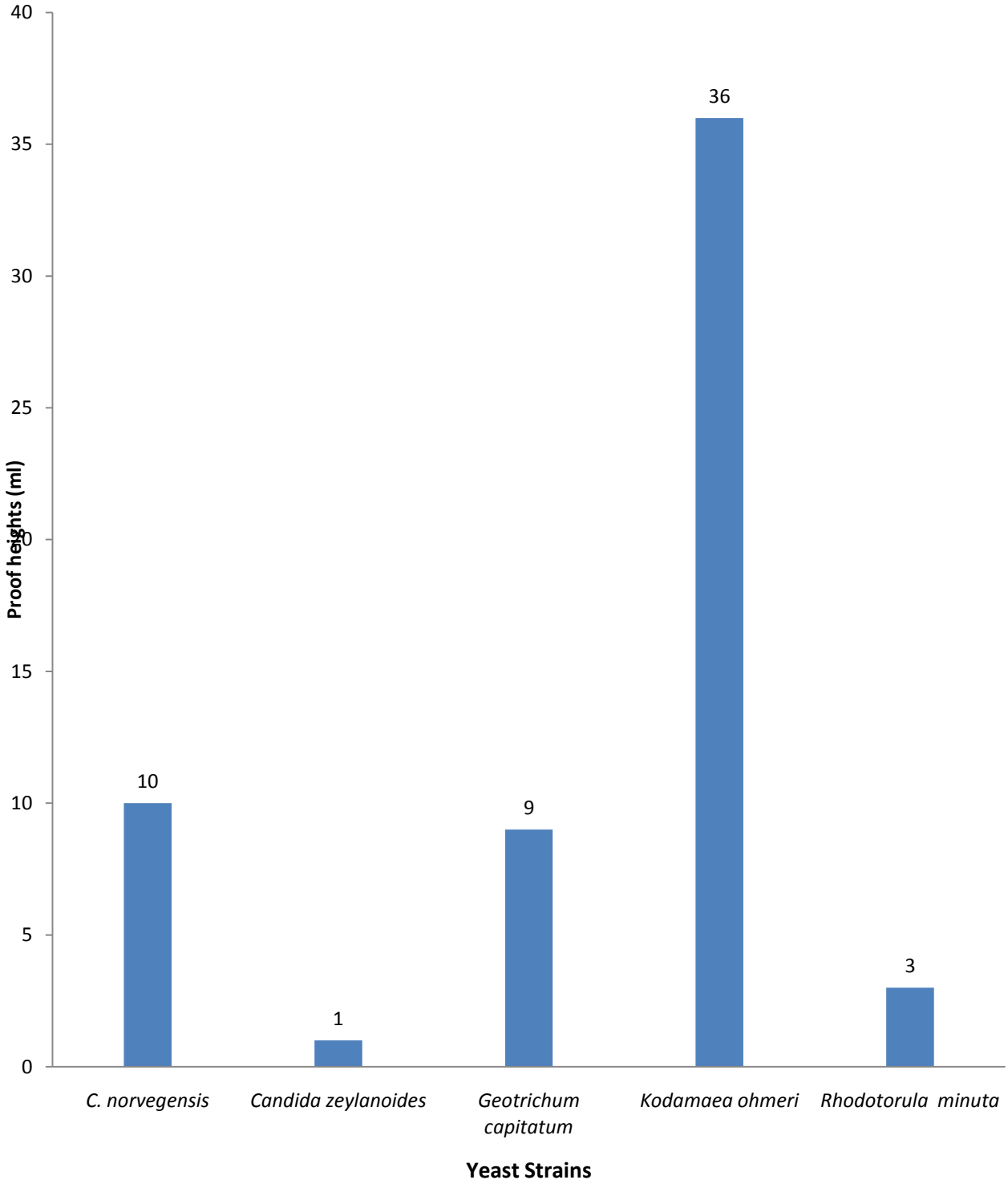


Fig 4.28i: Proof Heights of Sweetened Dough of UV Mutants at 20 mins

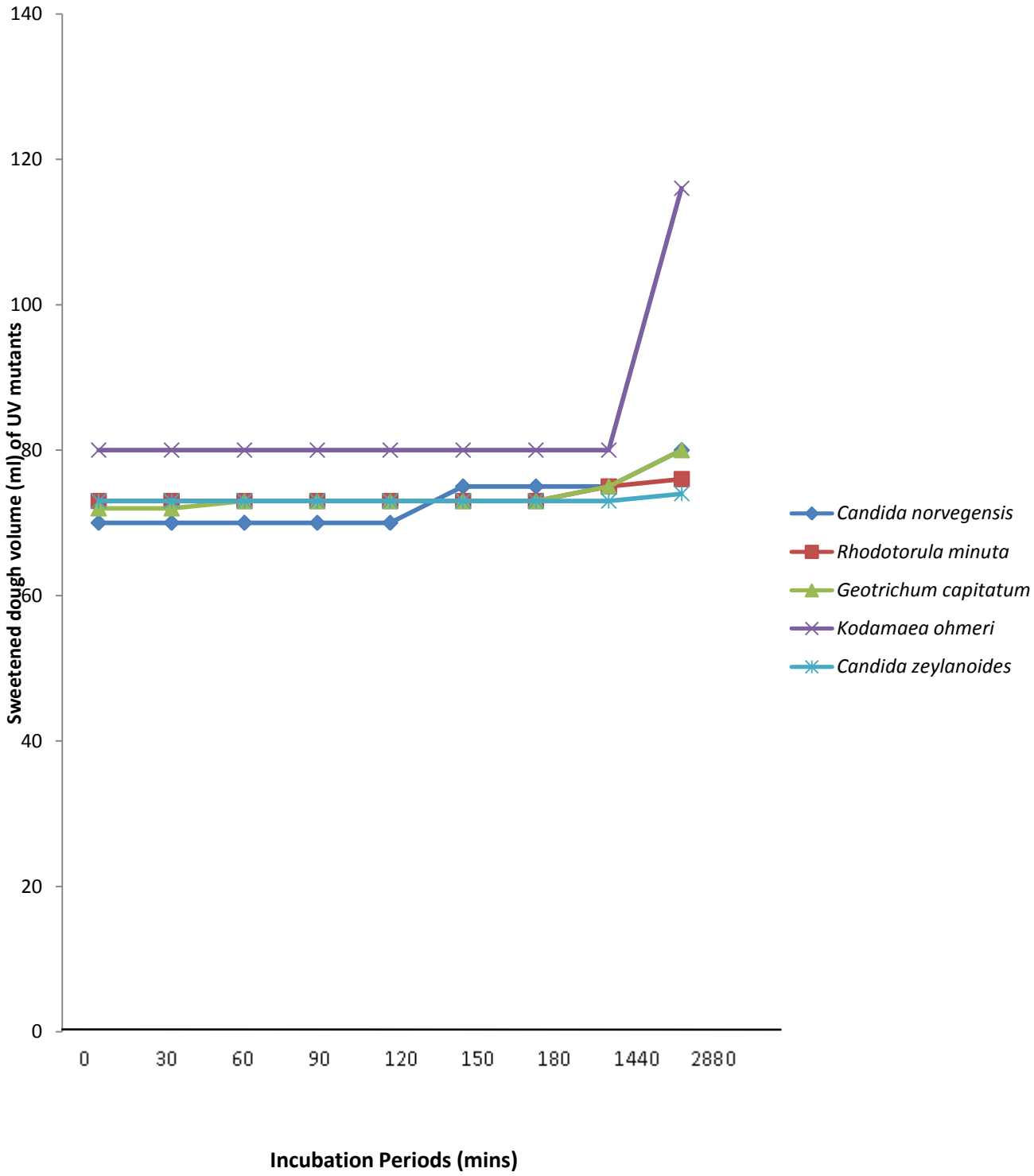


Fig 4.28j: Leavening Profile of Sweetened Dough for UV Mutants at 20 mins

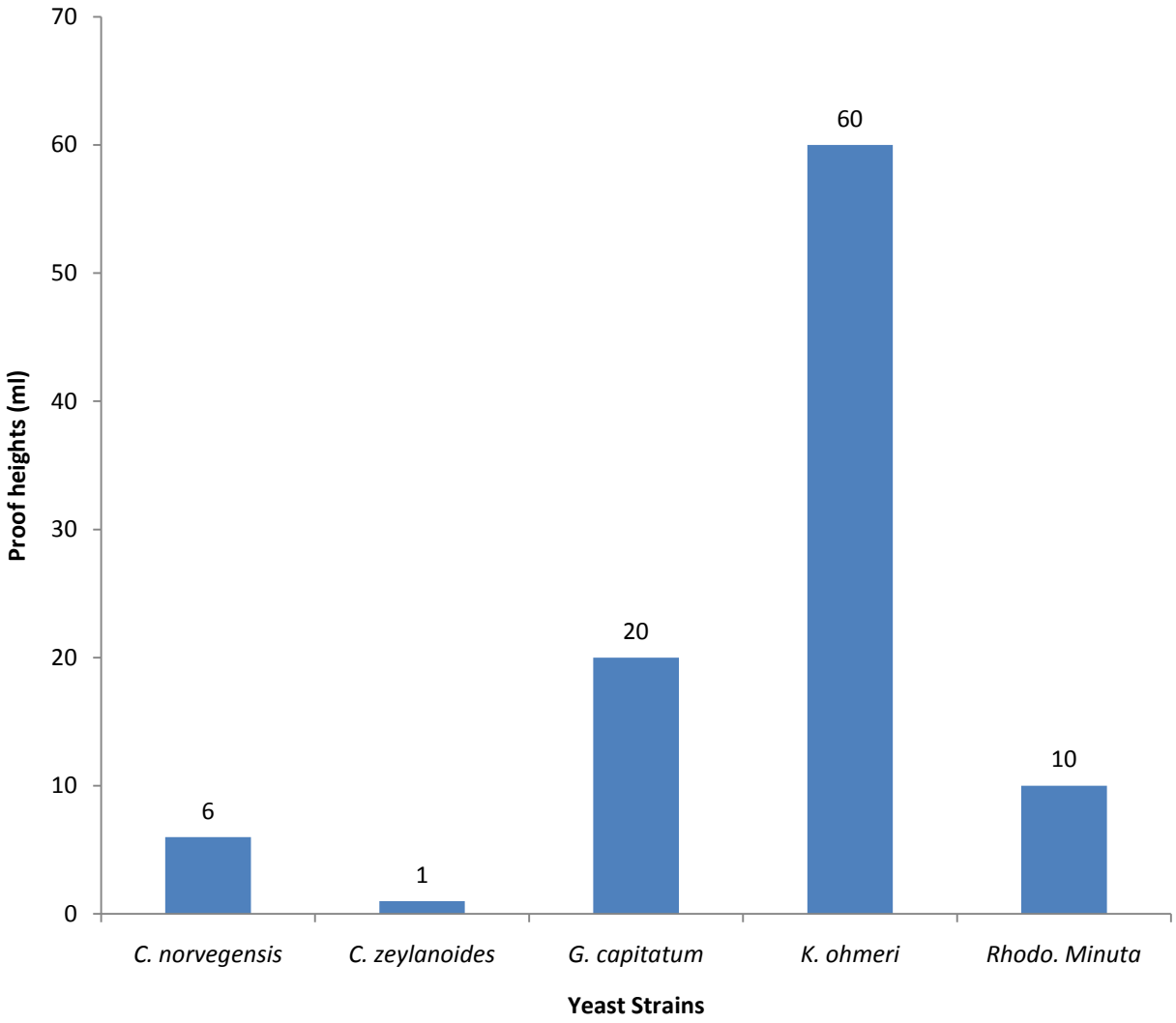


Fig 4.28k: Proof Heights of Sweetened Dough of UV Mutants at 25 mins

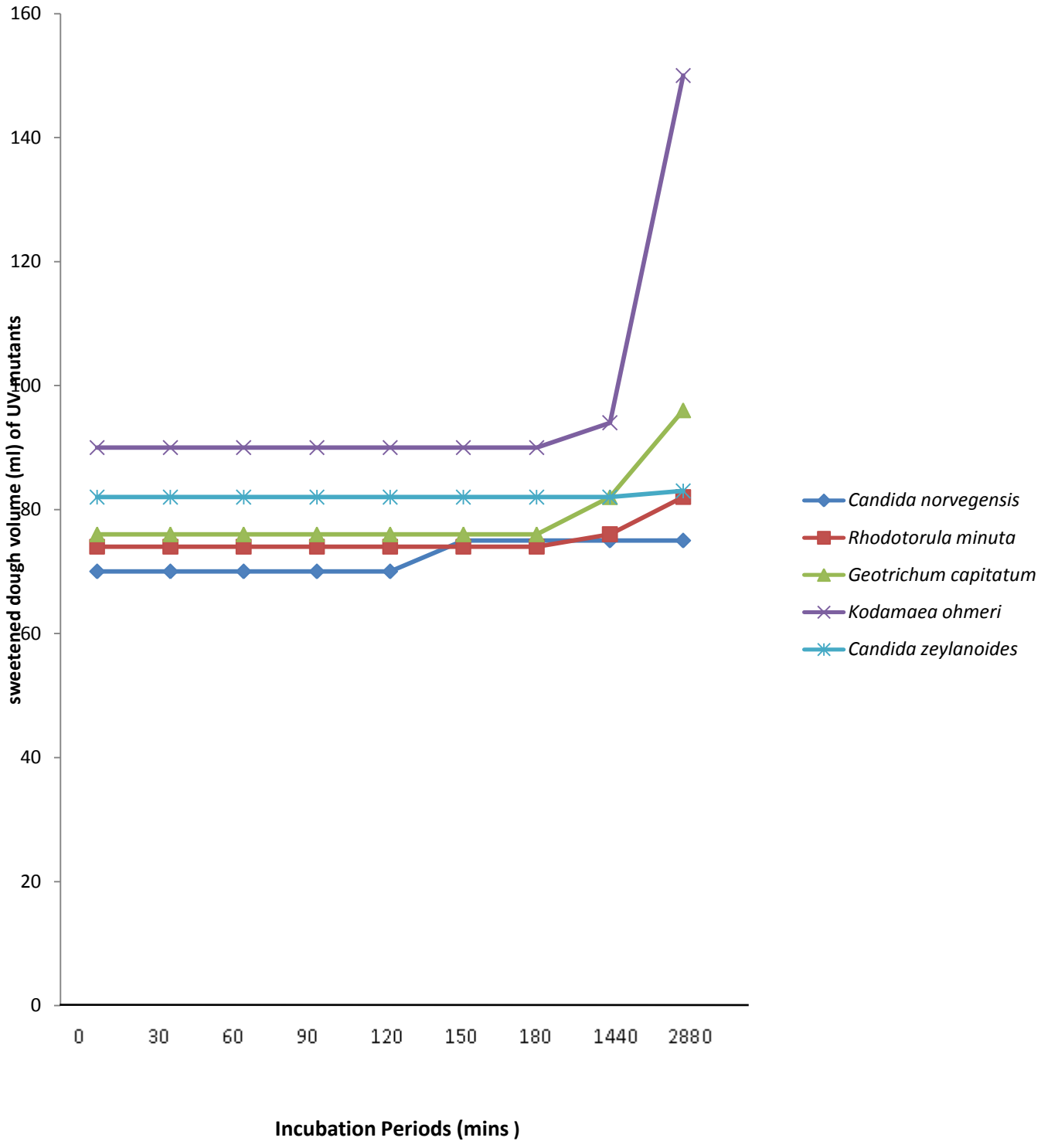


Fig 4.28l: Leavening Profile of Sweetened Dough for UV Mutants at 25 mins

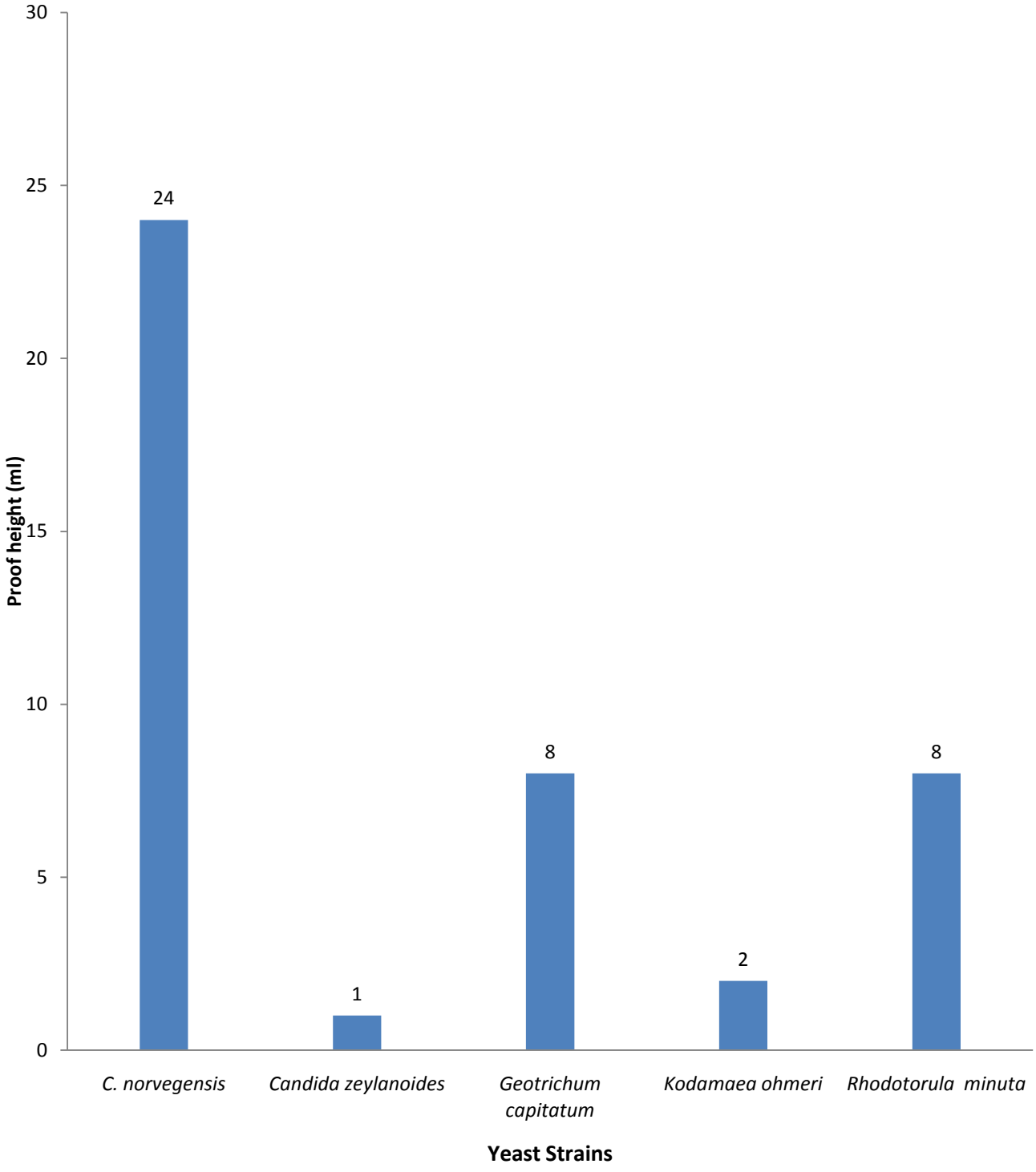


Fig 4.28m: Proof Heights of Sweetened Dough of UV Mutants at 30 mins

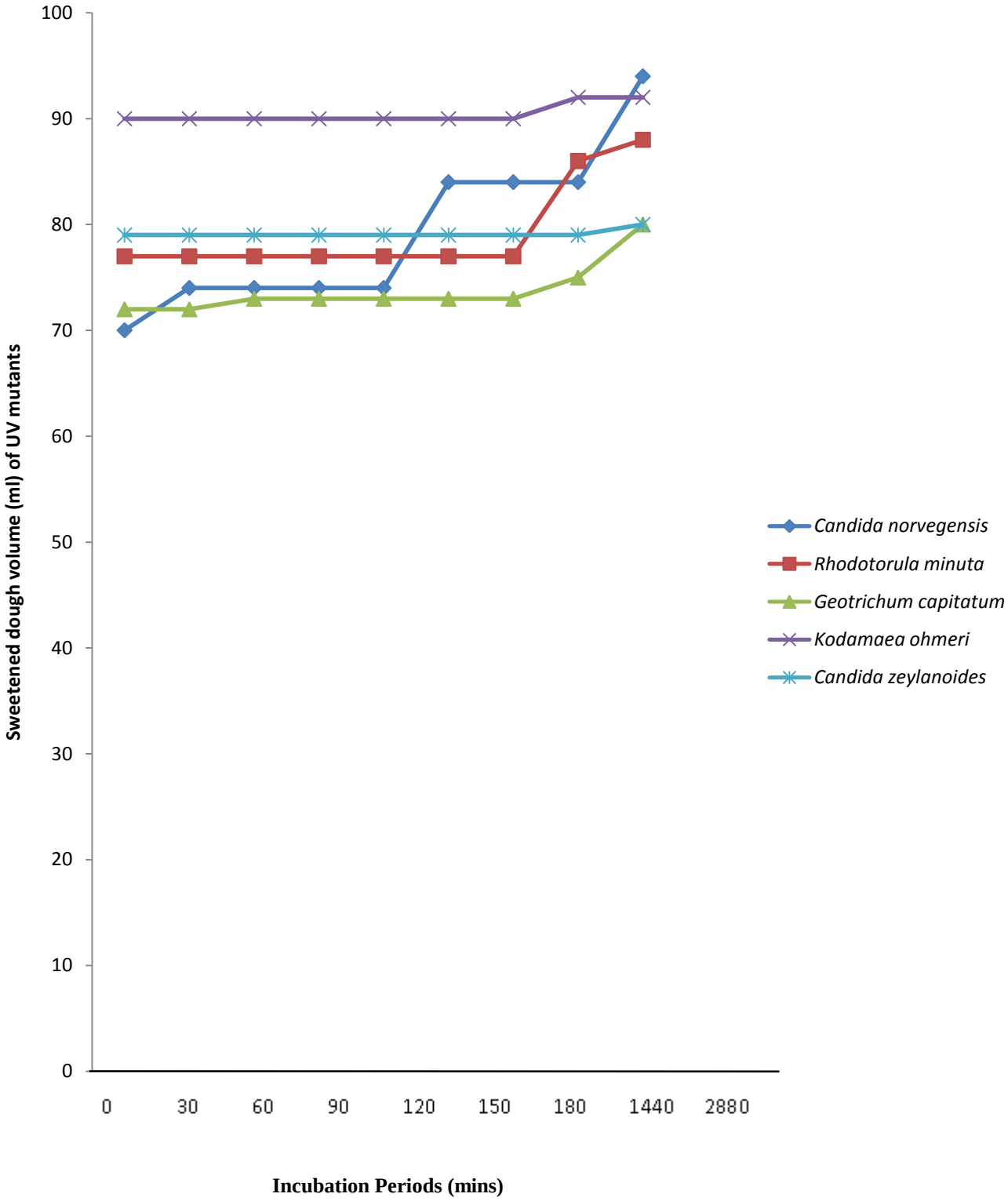


Fig 4.28n: Leavening Profile of Sweetened Dough for UV Mutants at 30 mins

The unsweetened dough leavening capacities of the different UV-light-induced mutants at various time intervals showed the following leavening activities with the highest and the least respectively as follows; at 4 minutes, *Kodamaea ohmeri* (105ml), the least (50ml) for *Geotrichum capitatum* and *Rhodotorula minuta* (Fig 4.29a). At 6 minutes, *Candida norvegensis* (110ml), the least, *Rhodotorula minuta* (40ml) (Fig 4.29c). At 10 minutes, *Candida zeylanoides* (125ml), the least *Candida norvegensis* (10ml) (Fig 4.29e). At 15 minutes, *Kodamaea ohmeri* (154ml), the least *Candida norvegensis* (15ml) (Fig 4.29g). At 20 minutes, *Geotrichum capitatum* (130ml), the least*Candida norvegensis* (20ml) (Fig 4.29i). At 25 minutes, *Geotrichum capitatum* (100ml), the least, *Candida norvegensis* (08ml) (Fig 4.29k). At 30 minutes, *Geotrichum capitatum* (113ml), the least *Candida zeylanoides* (80ml) (Fig 4.29m). From the result, it was observed that the unsweetened dough favoured better leavening capacities with the various mutants than the sweetened dough at various time intervals. (Appendices XVIII andXIX). For the sweetened dough, the mutants with the highest leavening activities were *Candida norvegensis* at 4 minutes (25ml). *Kodamaea ohmeri*, at 6 minutes (46ml), 20 (36ml) and at 25 (60ml), followed by *Geotrichum capitatum* at 6 minutes (37ml) and at 30 minutes *Candida norvegensis* (24ml). But for the unsweetened dough, the mutants with the best leavening performance at 4, 6, 10, 15 minutes were*Kodamaea ohmeri,* 4(105ml), 6(106ml), 10(118ml), 15(154ml), *Geotrichum capitatum*, 10(118ml), at 6 and 10 minutes *Candida norvegensis* (110 and 125ml) respectively. This is followed by *Geotrichum capitatum* at 20 minutes (130ml), 25minutets (100ml), 30 minutes (113ml) and at 20 minutes *Rhodotorula minuta* (105ml). The result of the UV-light-induced mutants showed a better leavening ability in the lean or unsweetened dough when compared with the sweetened dough fermented with the wild type. This observation was made in the wild type yeasts and are stated as follows:*Rhodotorula minuta* (27- 105 ml), *Candida zeylanoides* (68 - 125ml) and *Geotrichum capitatum* (60 - 130ml) which had a lower leavening capacity at 10 and 20 minutes mutation timeintervals respectively (Appendices X VII and XIX).

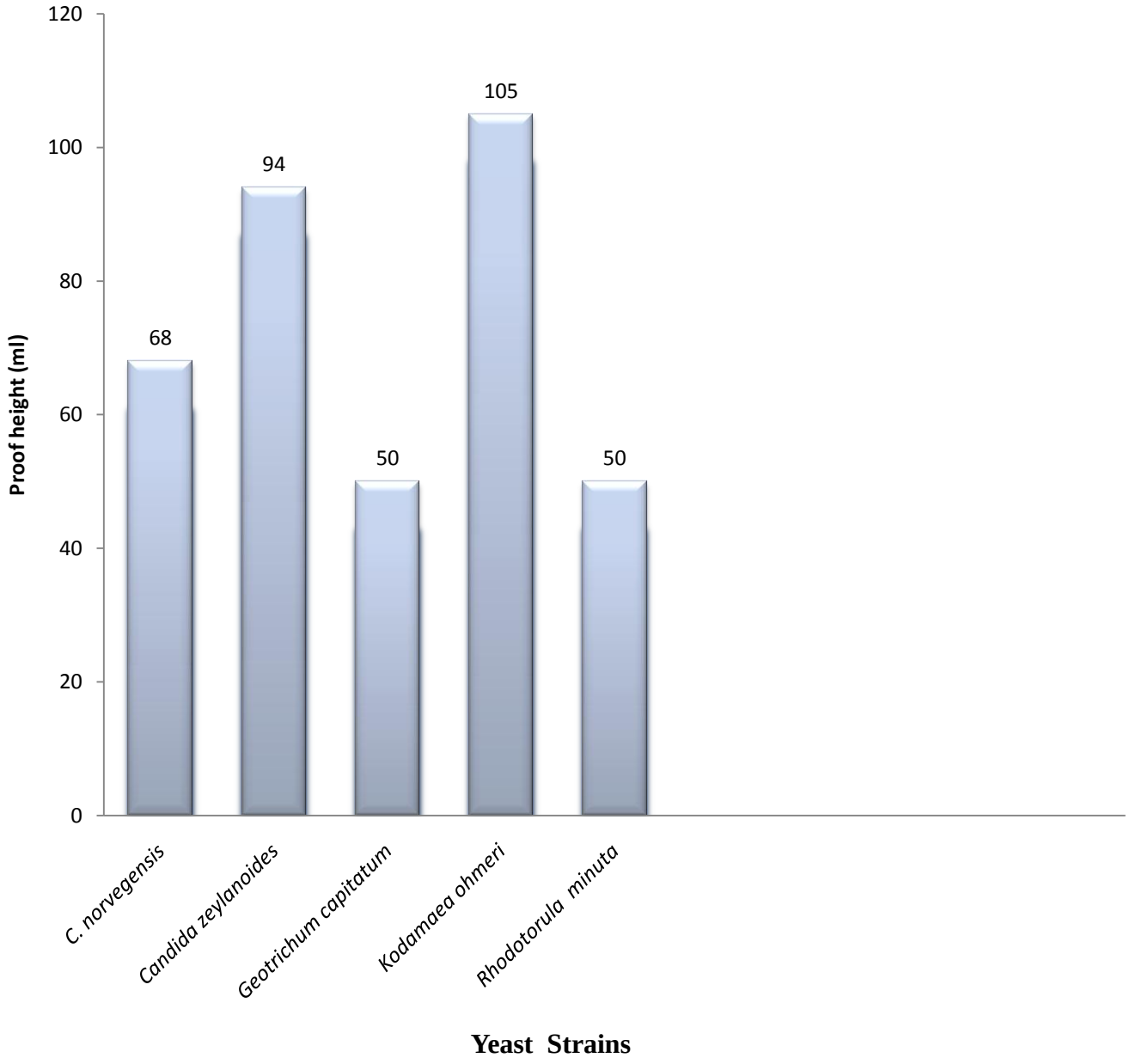


Fig 4.29a: Proof Heights of Unsweetened Dough of UV Mutants at 4mins

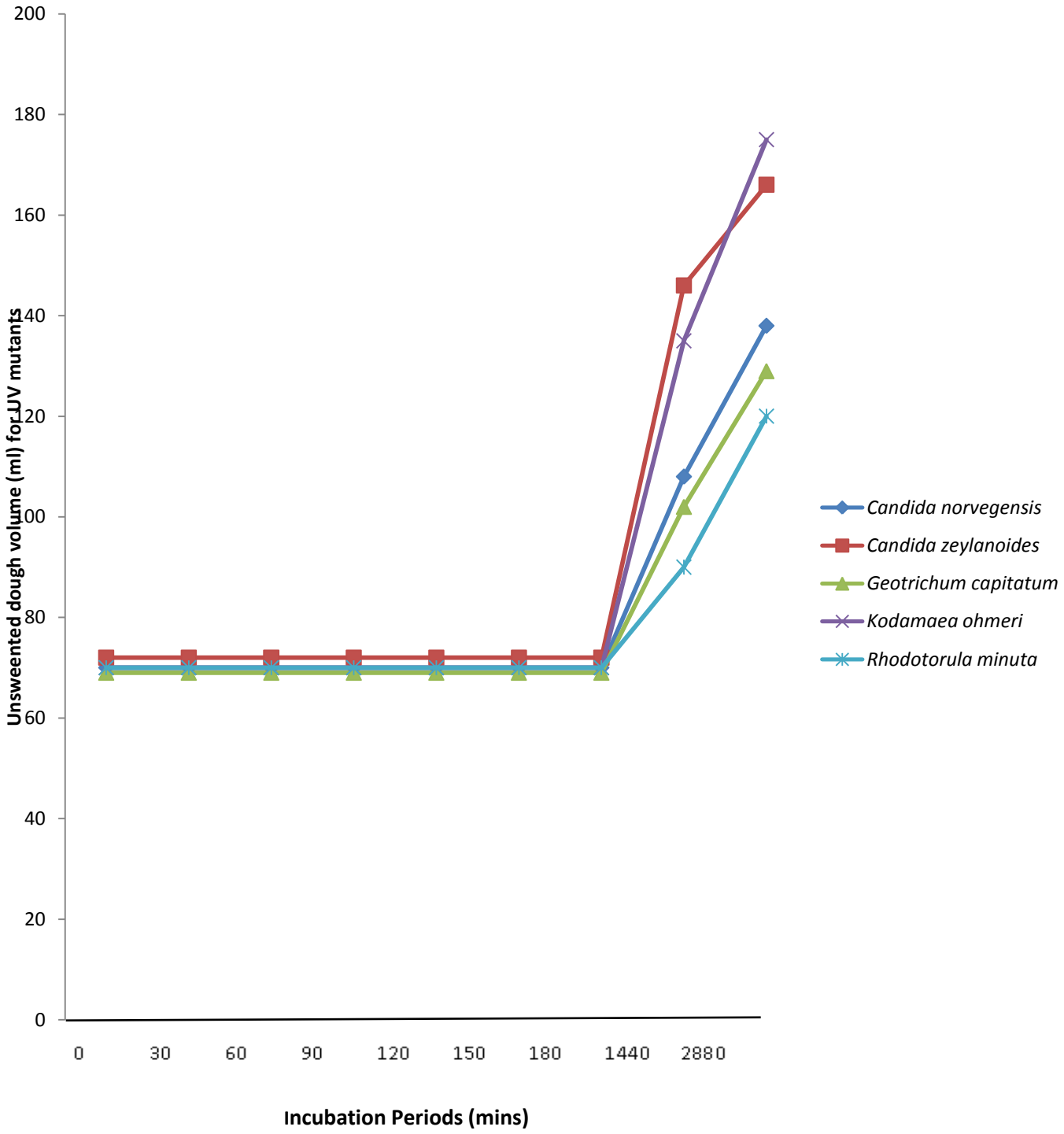


Fig 4.29b: Leavening Profile of Unsweetened Dough for UV Mutants at 4 mins.

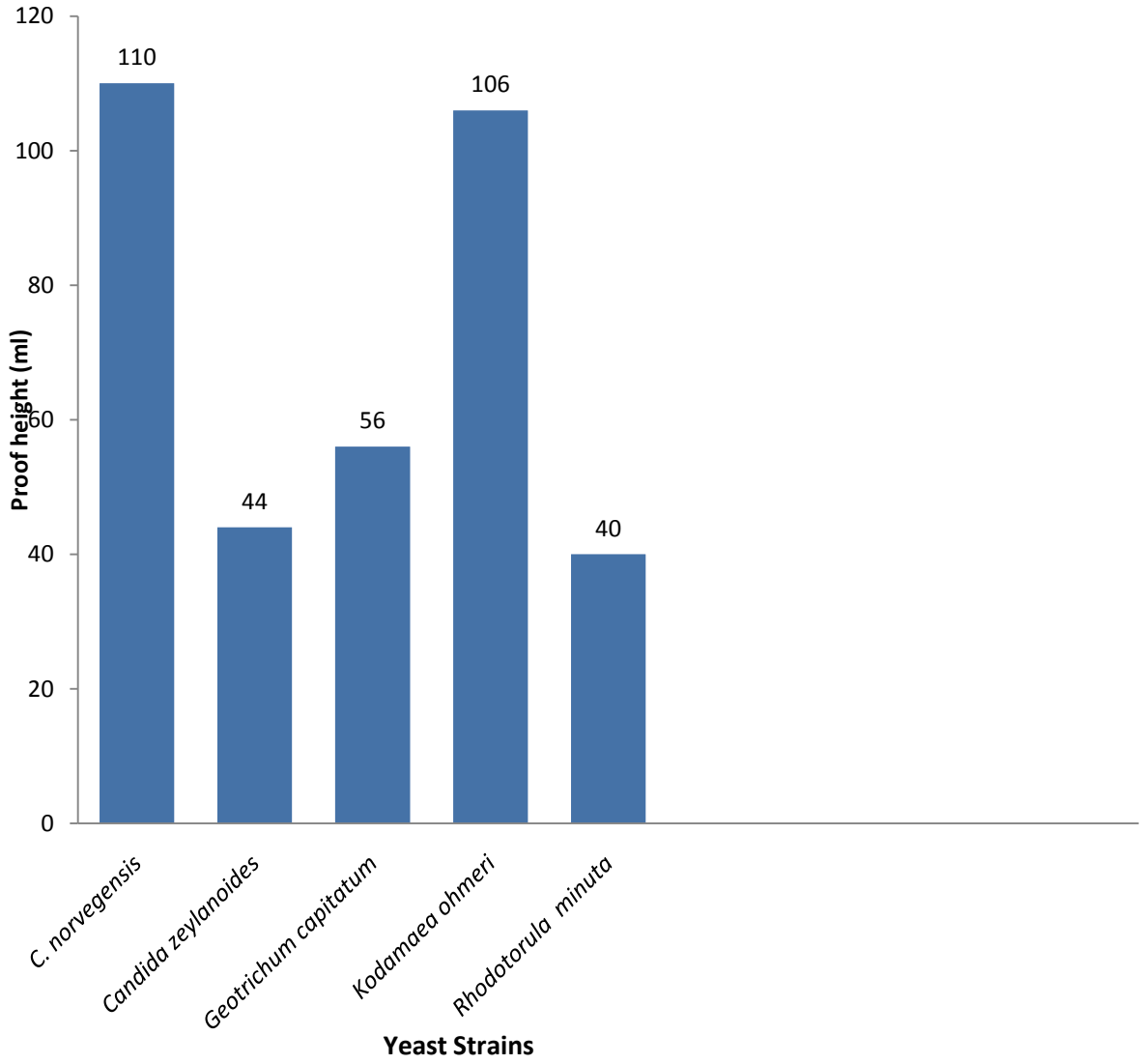


Fig 4.29c: Proof Heights of Unsweetened Dough of UV Mutants at 6 mins

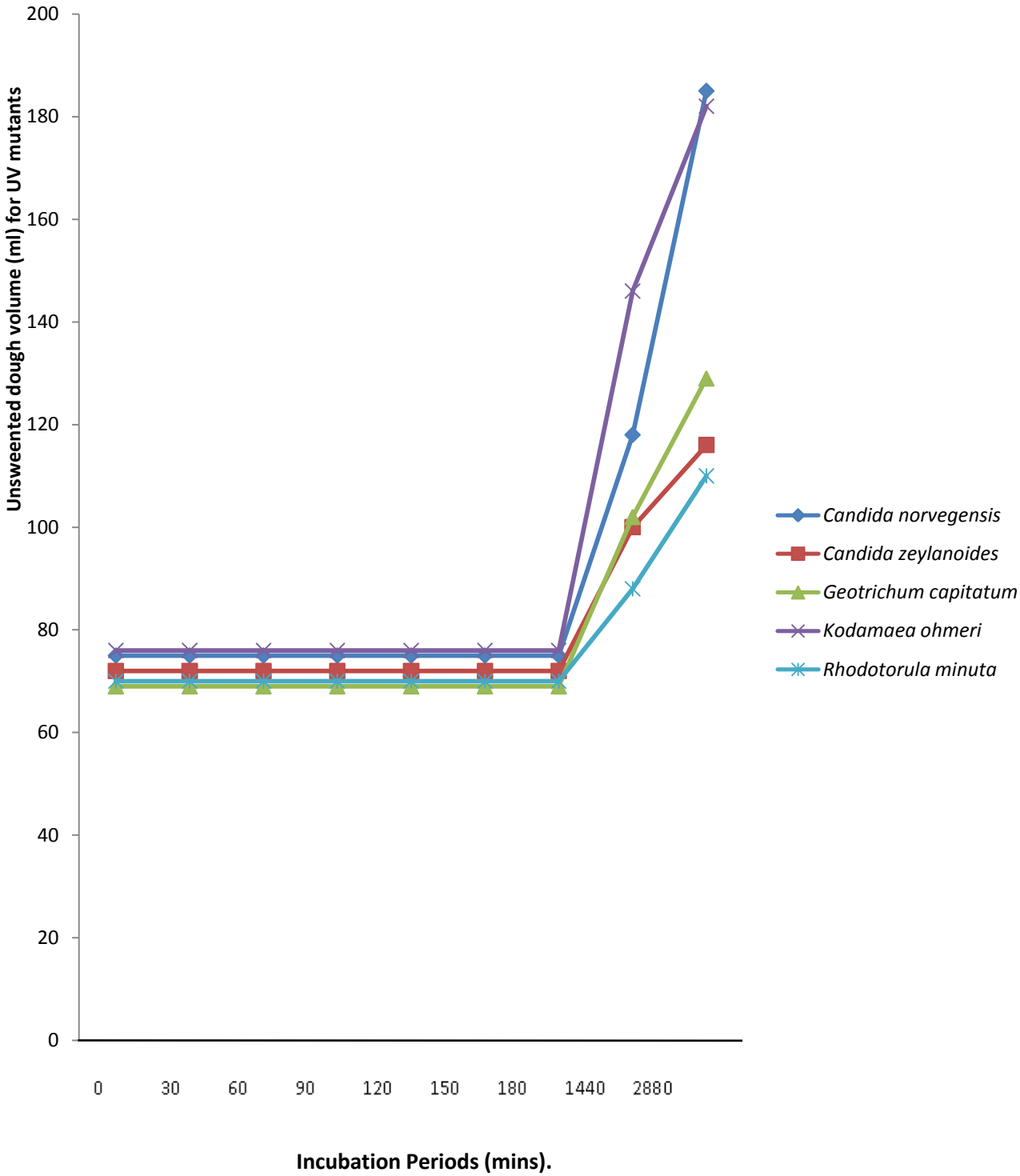


Fig 4.29d: Leavening Profile of Unsweetened Dough for UV Mutants at 6 mins.

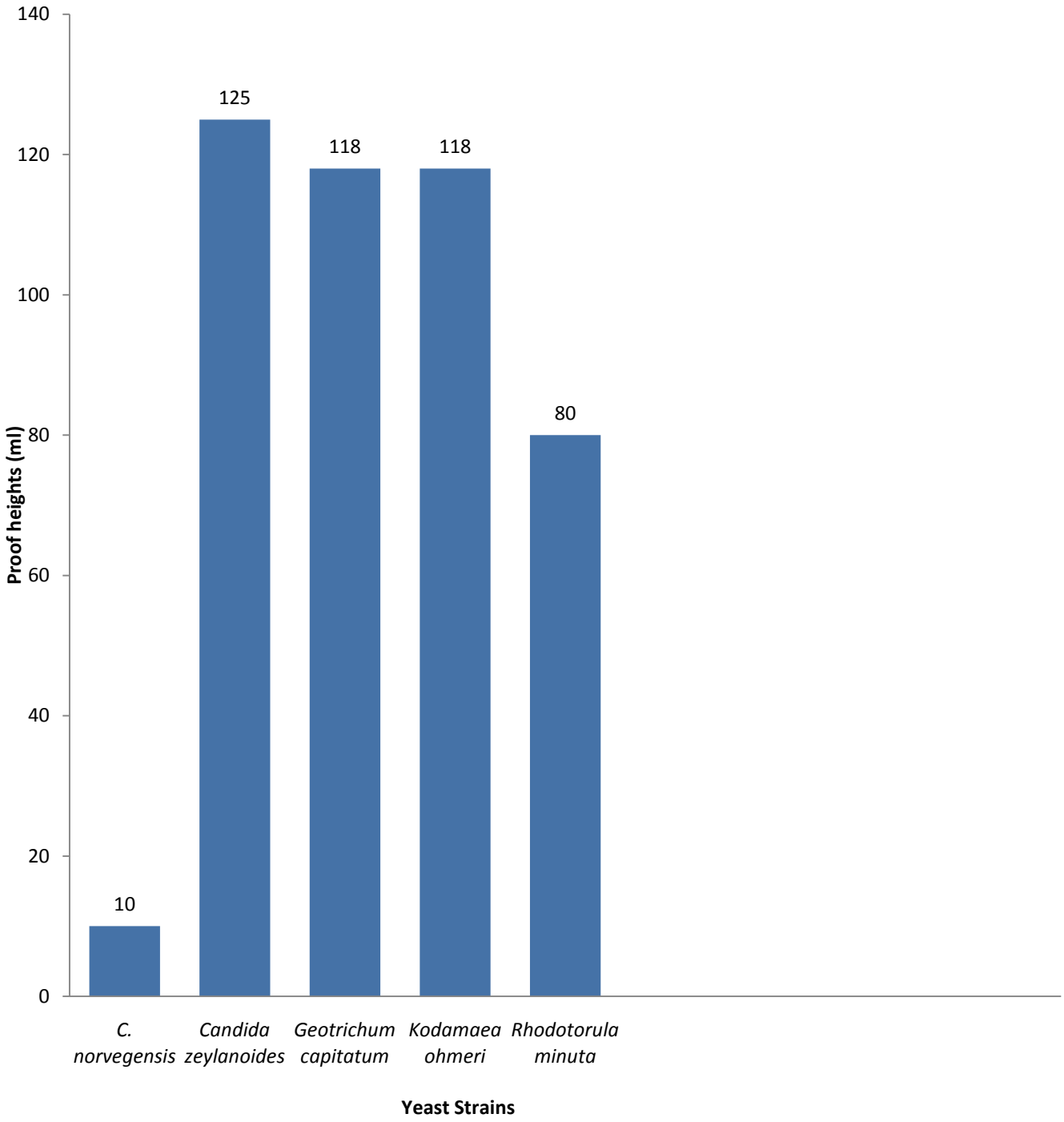


Fig 4.29e: Proof Heights of Unsweetened Dough of UV Mutants at 10 mins

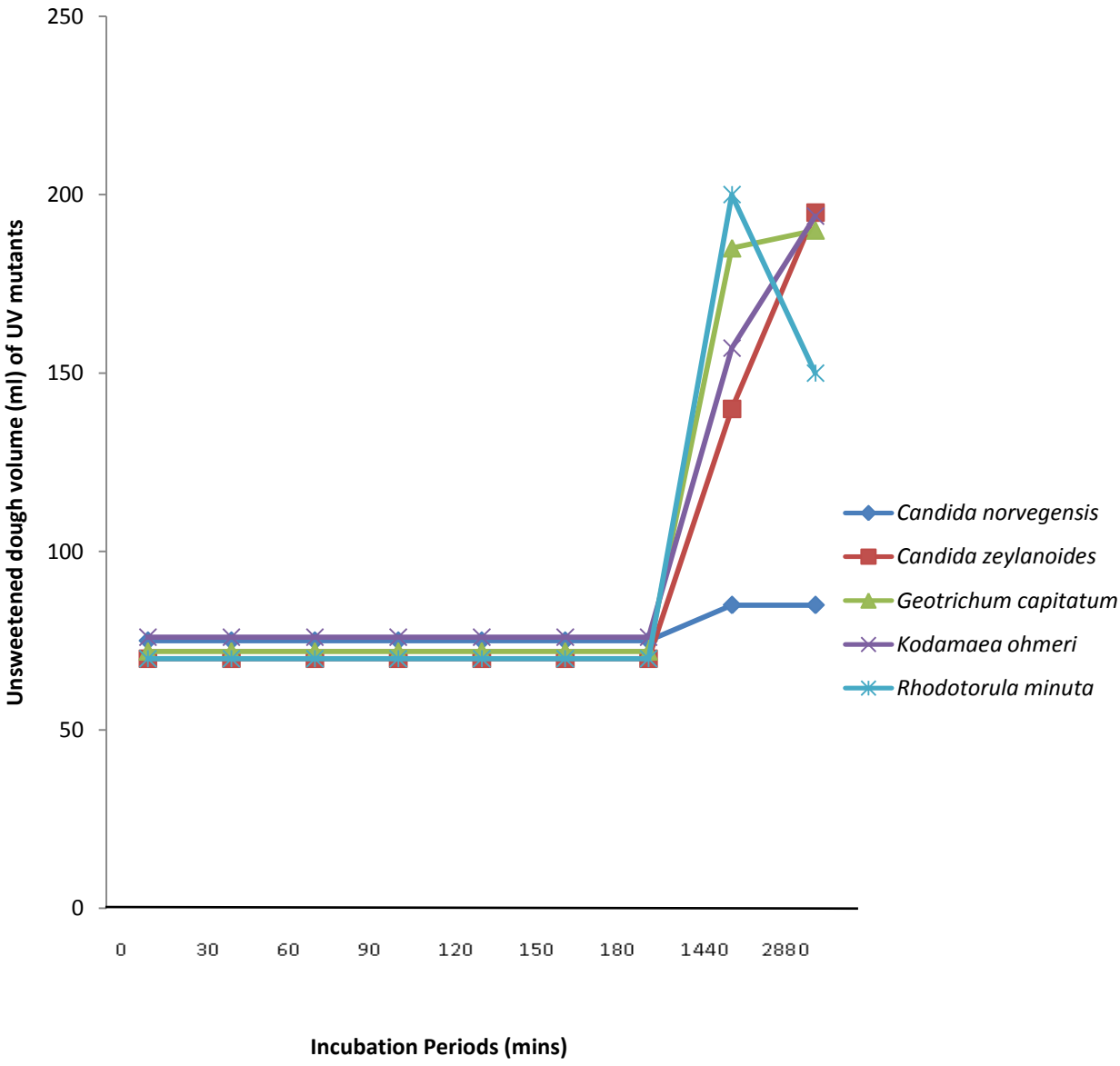


Fig 4.29f: Leavening Profile of Unsweetened Dough for UV Mutants at 10 mins.

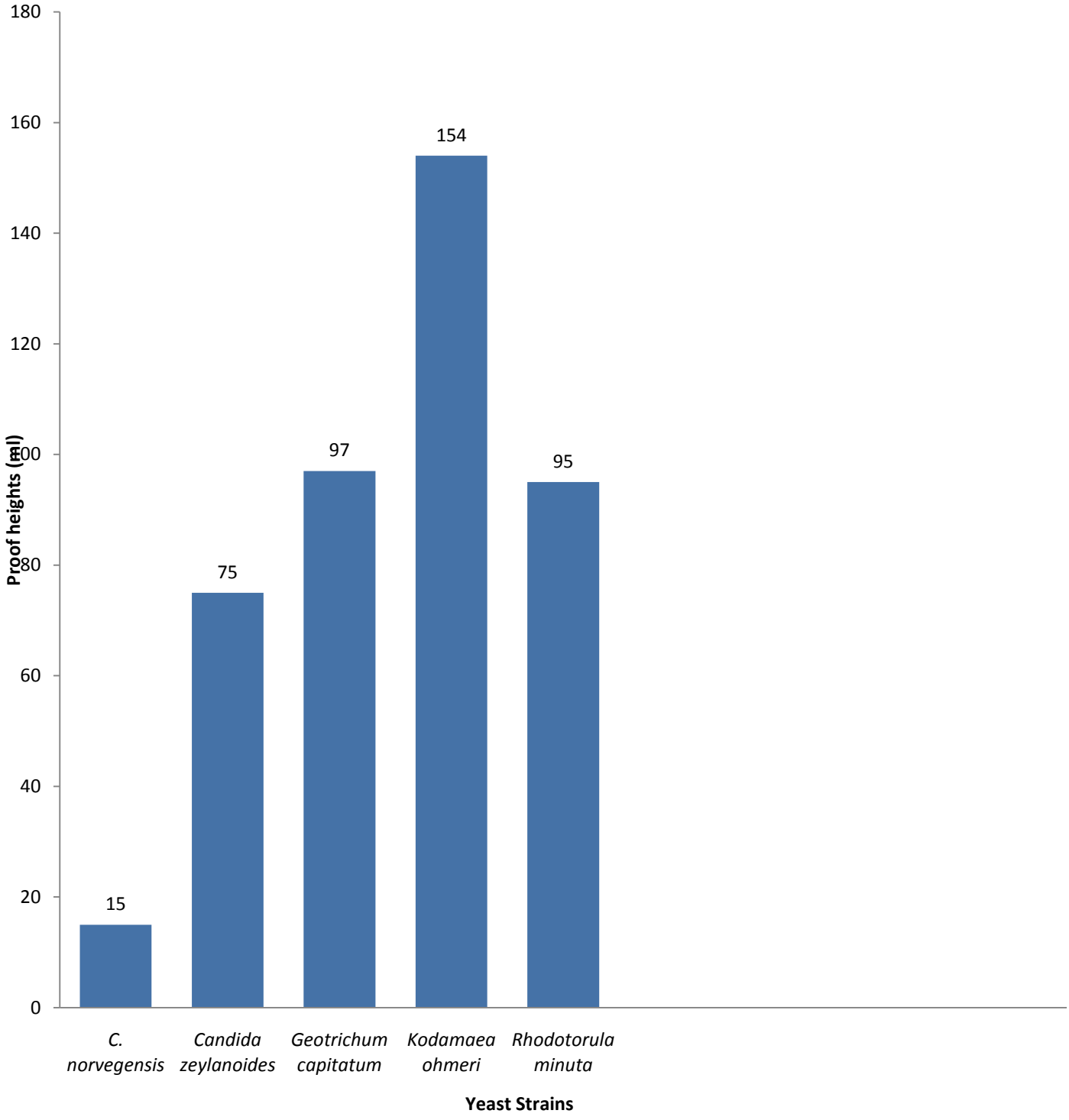


Fig 4.29g: Proof Heights of Unsweetened Dough of UV Mutants at 15 mins

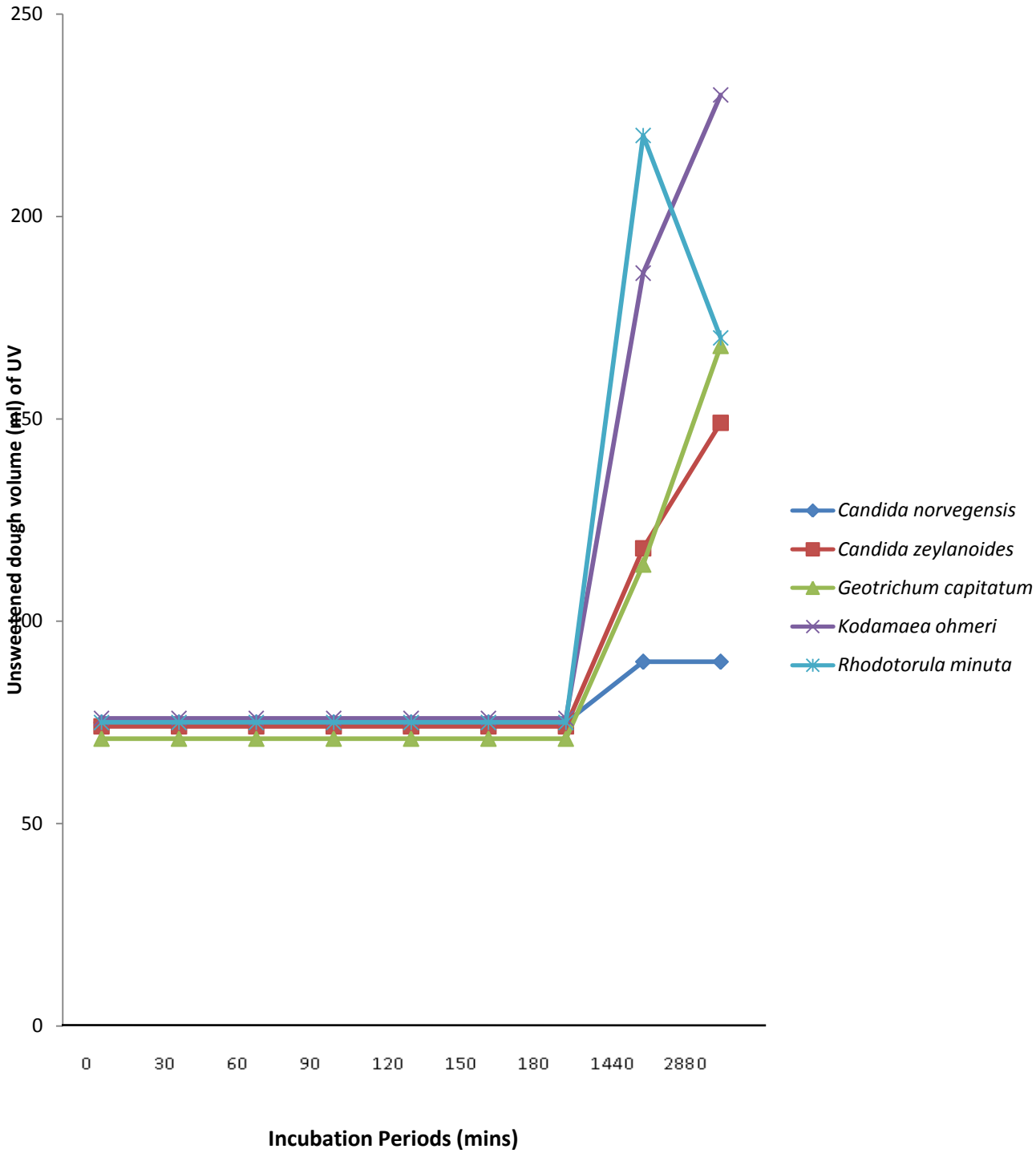


Fig 4.29h: Leavening Profile of Unsweetened dough for UV Mutants at 15 mins.

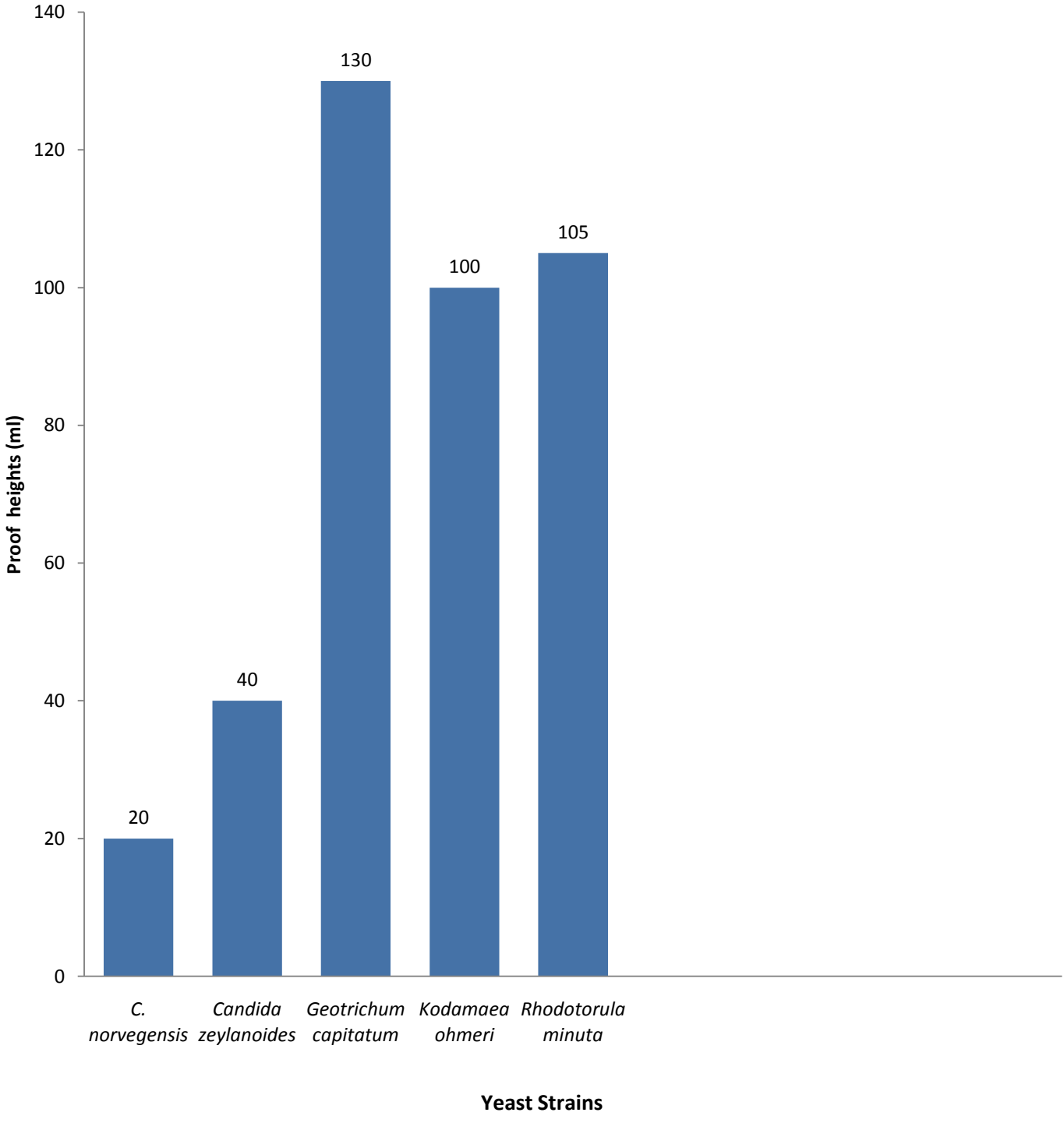


Fig 4.29i: Proof Heights of Unsweetened Dough of UV Mutants at 20 mins

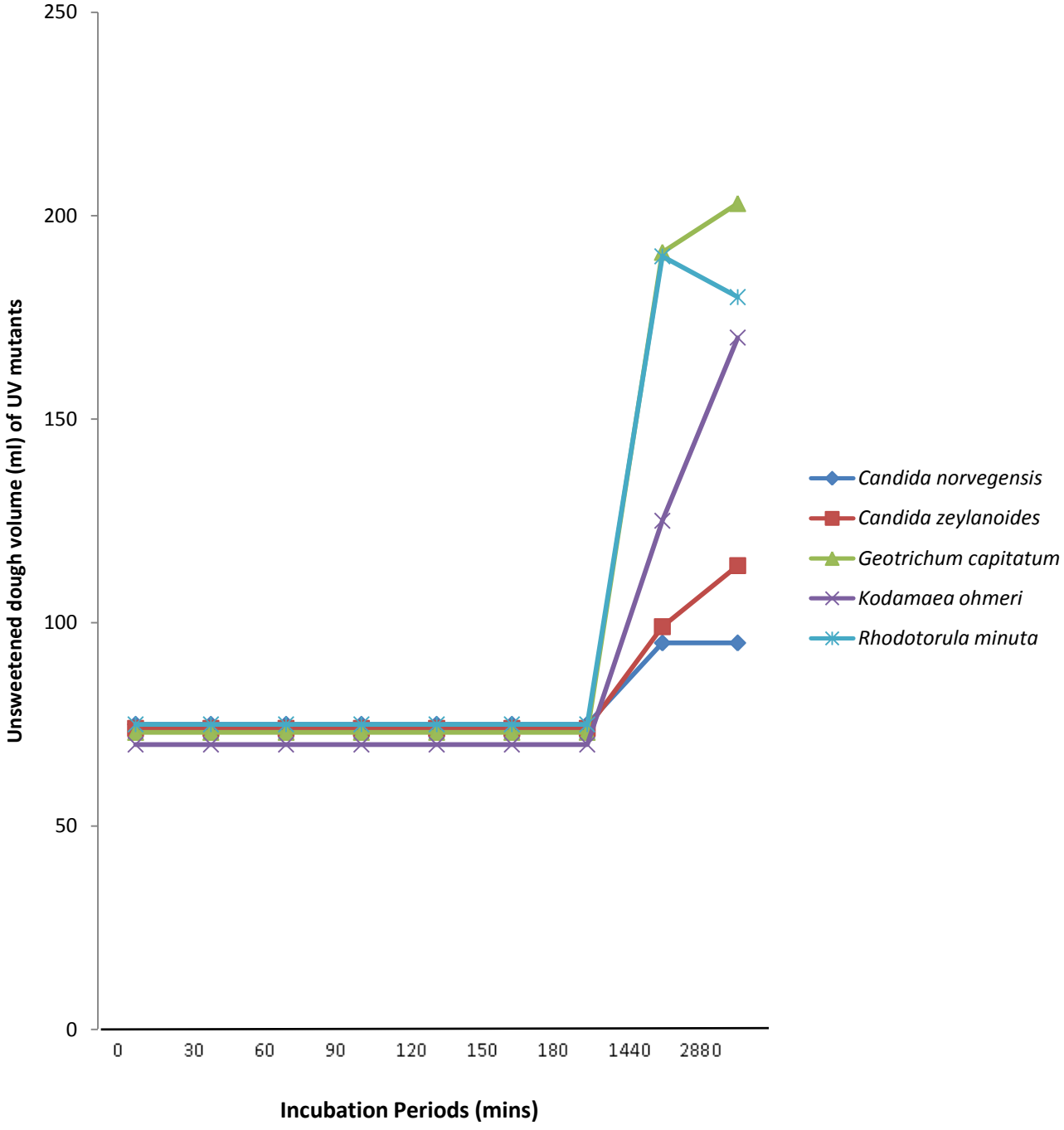


Fig 4.29j: Leavening Profile of Unsweetened Dough for UV Mutants at 20 mins.

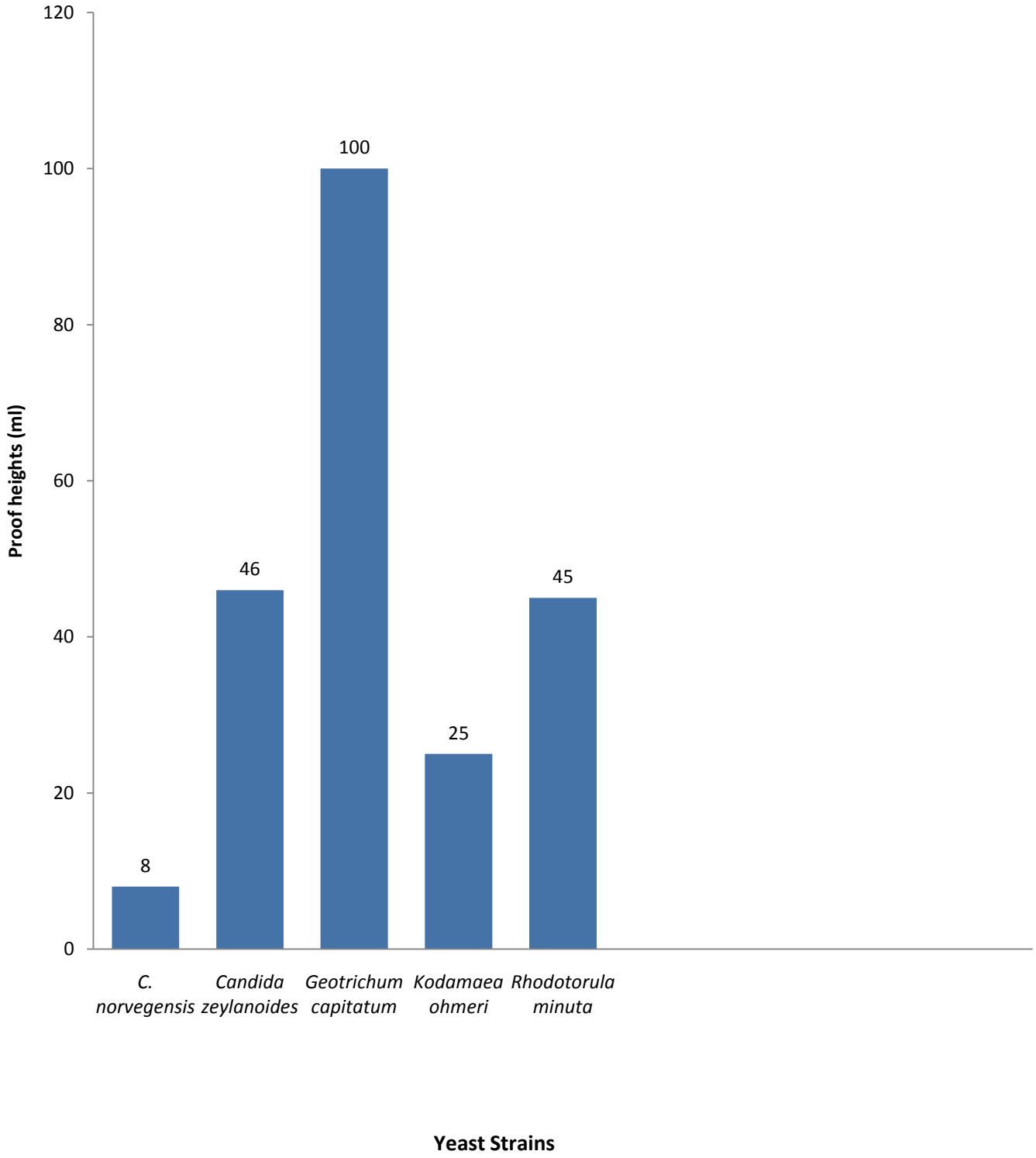


Fig 4.29k: Proof Heights of Unsweetened Dough of UV Mutants at 25 mins

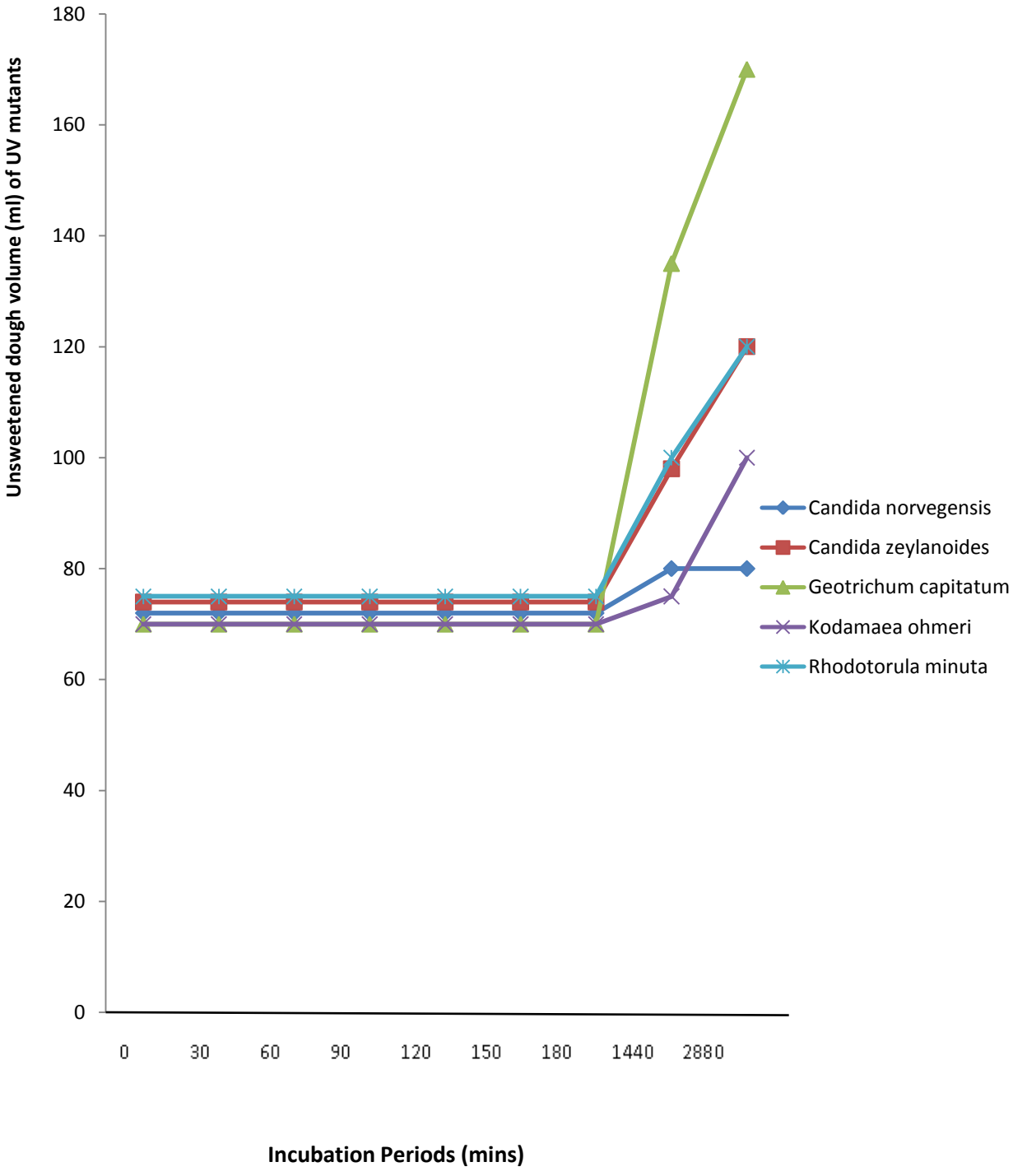


Fig 4.29l: Leavening Profile of Unsweetened Dough for UV Mutants at 25 mins.

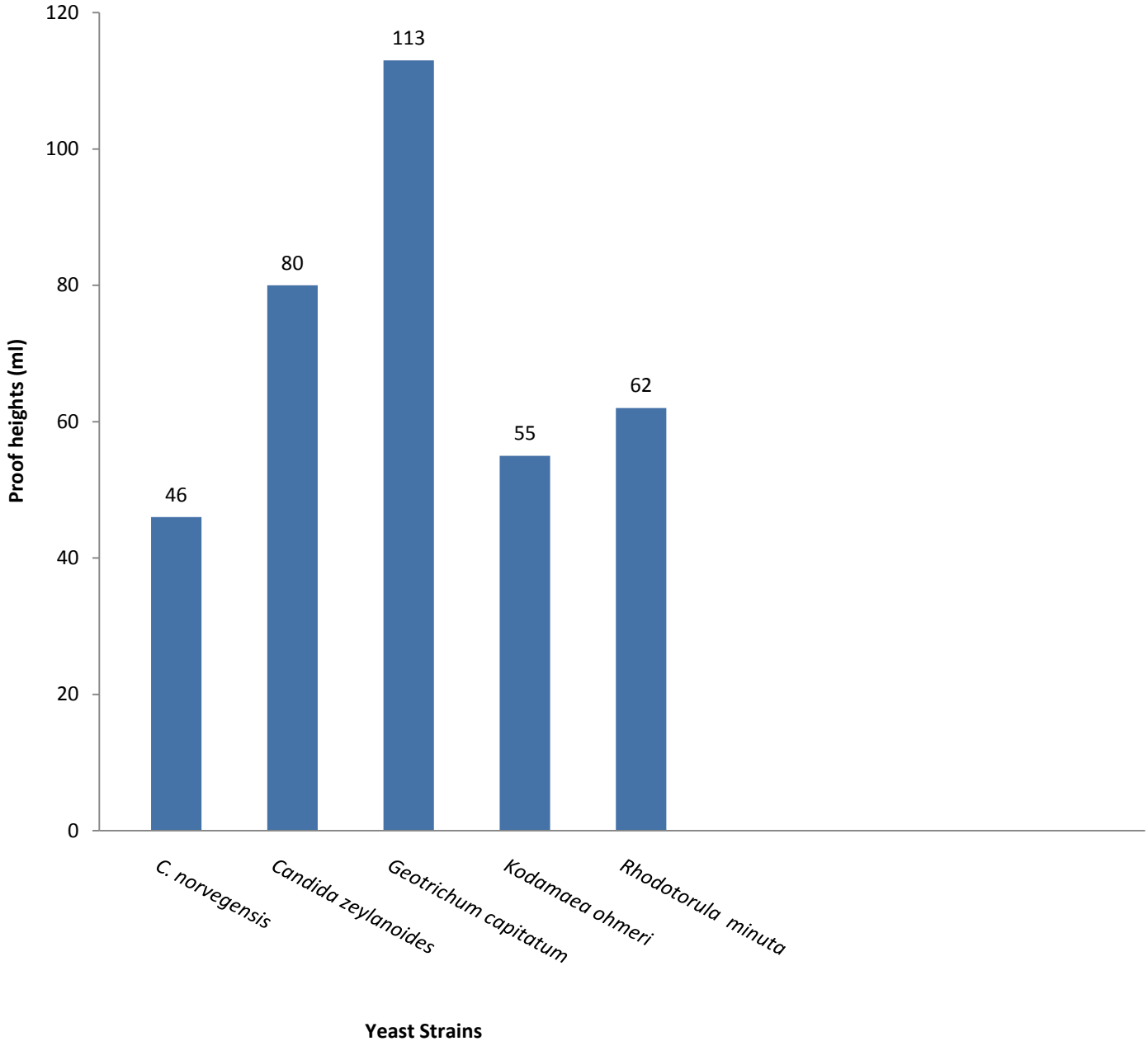


Fig 4.29m: Proof Heights of Unsweetened Dough of UV Mutants at 30 mins

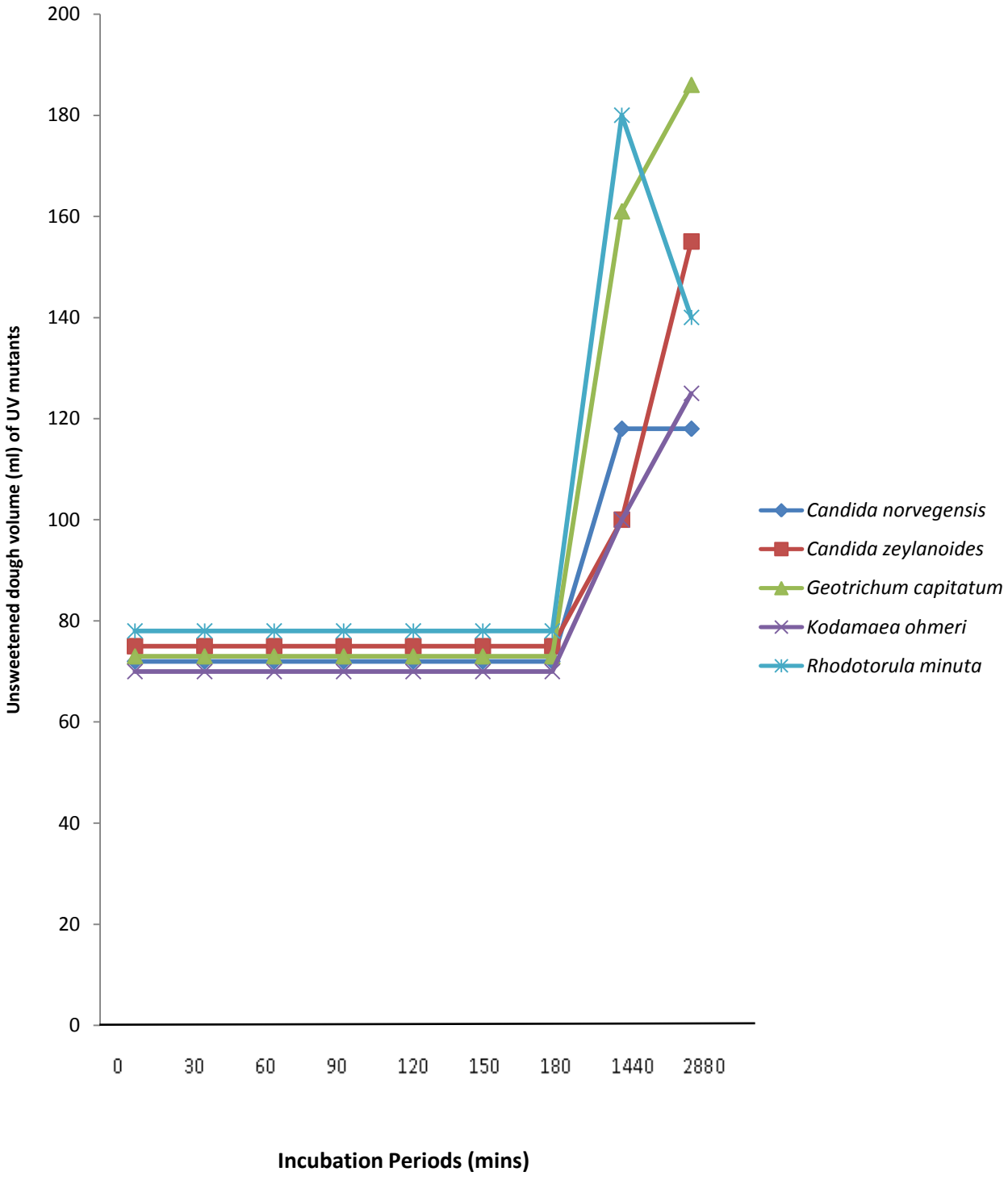


Fig 4.29n: Leavening Profile of Unsweetened Dough for UV Mutants at 30 mins.

4.17 Sensory Evaluation

The sensory evaluation result presented in Table 4.13 shows the mean scores for the parameters used in assessing the quality of the bread samples. These are colour, texture, aroma and overall acceptability. Bread samples were considered acceptable if their mean value for overall acceptability were equal or above 3 based on the hedonic scale of 1(lowest)-5(highest) (Table 4.13). (The higher the value, the higher the consumer acceptability).

The results statistically showed that there was a significant difference between the mean values of the parameters ($p \leq 0.05$) both in the wild, mutants and control samples of bread for both sweetened and unsweetened (Tables 4.13and 4.14). DS20 mutant bread sample was the best with regard to the four parameters assessed with the mean values (4.25^{a}, 4.00^{a}), followed by 7F9 (4.00^{a}, 3.50^{b}) for both sweetened and unsweetened bread samples. Others include DS6 (3.75^{a}, 3.75^{a}), 7F8 (3.75^{a}, 3.75^{a}), and 2F36 ($3.50^{a,}$ 3.50^{b}) (Table 4.14). In the case of wild yeast bread samples all the samples tested were acceptable and the best was DS20 with a mean value (4.00^{a}) in sweetened sample, followed by DS6 (3.75^{a}) and the least was 2F36 (3.50^{b}) for both sweetened and unsweetened bread samples (Table 4.14). These samples tested compared favourably, if not even better than the control samples which showed a mean value of (3.56^{a}, 3.3^{b}, 3.50^{a}, 4.50^{a}) for both sweetened and unsweetened bread samples in relation to the four parameters tested (Tables 4.13 and 4.14) i.e. for mutants of nitrous acid, wild and baker's yeast bread samples.The ultraviolet (UV) mutant bread samples were all preferred by the 30 man panelists based on the consumer acceptability assessment values at various intervals of mutation both for sweetened and unsweetened (Table 4.15).Considering the four parameters assessed in terms of the organoleptic properties, the following bread samples were ranked as the best in accordance with their mean value; for texture, colour, aroma and overall acceptability. The sweetened bread samples were ranked as the best in relation to time as follows ;texture, DS6 (3.57^{a}) at 15 minutes, colour DS10 (4.15^{a}) at 20 minutes. Aroma7F8 (4.43^{a}) at 6 minutes,overall

acceptability, DS10 (4.15^{a}) at 20 minutes. This is followed by 7F9 (3.15^{a}) at 10 minutes, for texture, colour DS6 and DS20 (3.86^{a}) at 15 minutes and 20 minutes, aroma DS6 and DS10 (3.43^{bc}) at 10 minutes and 15 minutes and overall acceptability, DS6 and DS20 (3.86^{a}) at 15 minutes and 20 minutes.

The following unsweetened bread samples showed the best results 7F9 (4.43^{a}) at 10 minutes for texture, colour, 7F9 (5.00^{a}) at 10 minutes, Aroma, 7F9 and DS6 (3.29^{ba}) at 15 minutes and 20 minutes and overall acceptability 7F9 (4.43^{a}) at 10 minutes. This was followed by 2F36 for texture, (4.00^{ba}) at 4 minutes, colour (4.86^{a}) for DS14, at 30 minutes; Aroma, 2F36 (3.00^{ba}) at 4 minutes (Table 4.15).

Table 4.13: Analysis of Variance of Organoleptic Attributes of Sweetened and Unsweetened Bread Samples Made with Wild Yeast and Its Mutant Species and Commercial Baker's Yeast.

Parameters	Type of Sample					
	A	**B**	**C_1**	**C_2**	**D1**	**D_2**
Texture	3.22^{b}	3.40^{b}	3.38^{ba}	2.88^{b}	3.22^{b}	3.40^{b}
Colour	4.44^{a}	4.90^{a}	4.38^{a}	4.13^{a}	4.44^{a}	4.90^{a}
Aroma	2.56^{b}	3.00^{b}	2.50^{b}	3.00^{b}	2.56^{b}	3.00^{b}
Overall acceptability	3.56^{a}	3.30^{b}	3.63^{a}	2.75^{b}	3.56^{a}	3.30^{b}

$P \leq 0.05$ DMRT

Key:

A = Sweetened mutant bread

B = Unsweetened mutant bread

C_1 = Wild yeast sweetened bread

C_2 = Wild yeast unsweetened bread

D_1 = Control, sweetened bread sample made with commercial baker's yeast

D_2 = Control unsweetened bread sample made with the commercial baker's yeast

Means with different superscripts are significantly different by Duncans Multiple Range Test (DMRT) across row.

Table 4.14:Comparative Analysis of the Mean Performance of the Wild Yeasts, Its Mutants and Commercial Baker's Yeast on Bread Samples

Bread samples	Sample code/Yeast strains					
	Mutants		Wild		Control (Baker's yeast)	
	A	B	C	D	E	F
DS20	4.25[a]	4.00[ab]	4.00[a]	2.50C	3.50[a]	4.50[a]
7F9	4.00[a]	3.50[b]	3.25[a]	3.25[a]	3.50[a]	4.50[a]
DS6	3.75[a]	3.75[a]	3.75[a]	3.75[a]	3.50[a]	4.50[a]
7F8	3.75[a]	3.75[a]	3.75[a]	3.75[a]	3.50[a]	4.50[a]
2F36	3.50[a]	3.50[b]	3.50[a]	3.00[b]	3.50[a]	4.50[a]

$P \leq 0.05$ DMRT

Key

A = Sweetened mutant bread

B = Unsweetened mutant bread

C = Wildyeast sweetened bread

D = Wild yeast unsweetened bread

E = Control sweetened bread made with commercial baker's yeast

F = Control unsweetened bread made with commercial baker's yeast

Mean values with different superscripts are significantly different by Duncan Multiple Range Test (DMRT) across the row ($P \leq 0.05$).

Table 4.15: Analysisof Variance of Organoleptic Attributes of Sweetened andUnsweetened Bread Samples with Mutant Yeasts (Ultra Violet light)

Sample code	Parameters					Mutation time (minutes)			
	UVT (mins)	Texture mean score	Colour mean score	Aroma mean score	Overall acceptability mean score	UVT mean score texture	UVT mean score colour	UVT mean score aroma	UVT mean score overall acceptability
A									
2F36	4	2.86^{a}	3.51^{a}	3.71^{ba}	3.57^{a}	2.38^{a}	3.38^{a}	3.63^{a}	3.38^{a}
7F8	6	2.15^{a}	3.29^{a}	4.43^{a}	3.29^{a}	3.38^{a}	3.75^{a}	3.50^{a}	3.75^{a}
7F9	10	3.15^{a}	3.57^{a}	3.00^{a}	3.57^{a}	3.23^{a}	4.13^{a}	3.25^{a}	4.13^{a}
DS6	15	3.57^{a}	3.86^{a}	3.43^{a}	3.86^{a}	3.38^{a}	3.75^{a}	3.50^{a}	3.75^{a}
DS10	20	2.29^{a}	4.15^{a}	3.43^{a}	4.15^{a}	2.75^{a}	4.00^{a}	3.00^{a}	3.38^{a}
DS20	25	2.71^{a}	3.86^{a}	3.00^{a}	3.86^{a}	2.25^{a}	3.38^{a}	3.00^{a}	3.38^{a}
DS14	30	2.43^{a}	3.71^{a}	2.71^{c}	3.71^{a}	2.75^{a}	3.63^{a}	3.38^{a}	3.63^{a}
B									
2F36	4	4.00^{ba}	4.86^{a}	3.00^{ba}	3.71^{a}	3.29^{a}	4.51^{a}	3.29^{a}	3.57^{a}
7F8	6	2.88^{bc}	4.43^{a}	2.57^{ba}	3.71^{a}	3.13^{a}	4.43^{a}	3.15^{a}	4.43^{a}
7F9	10	4.43^{a}	5.00^{a}	3.29^{ba}	4.43^{a}	3.13^{a}	5.00^{a}	3.00^{a}	3.86^{a}
DS6	15	2.71^{c}	3.00^{b}	3.29^{ba}	4.15^{a}	3.29^{a}	4.15^{a}	3.00^{a}	4.14^{a}
DS10	20	3.43^{bac}	5.00^{a}	2.57^{ba}	4.00^{a}	4.00^{a}	4.86^{a}	2.57^{a}	3.71^{a}
DS20	25	3.57^{bac}	4.71^{a}	2.43^{b}	3.43^{a}	3.29^{a}	4.29^{a}	2.71^{a}	4.00^{a}
DS14	30	3.29^{bac}	4.86^{a}	3.43^{a}	4.29^{a}	4.15^{a}	4.57^{a}	3.43^{a}	3.86^{a}

Key:

A = Sweetened mutant bread sample.
B = Unsweetened mutant bread sample.
UVT = Ultra violet irradiated time in minutes.
2F36, 7F8 and 7F9 = Samples from fresh fermented orange juice.
DS10, 14 and 20 = Samples from defective orange juice. Mean values with different superscripts are significantly different by Ducan Multiple Range Test (DMRT) across row ($p \leq 0.05$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Morphological and Physiological Characteristics of Yeast Strains Isolated

Morphological characteristics such as colonial morphology (colonial pigmentation, size and surface appearance), vegetative morphology (cell shape and arrangement), presence of pseudomycelium or true mycelium and pellicle formation are presented in (Table 4.1). Yeasts capable of growing on solid surfaces tend to form colonies with distinctive morphology; this is because individual species often form colonies of characteristic size and appearance (Prescott *et al*., 2008). The result obtained in this research agrees with the report of Prescott *et al*. (2008) since all the yeast strains isolated were similar in colonial appearance and vegetative morphology in that they share the same oval, spherical shape and budding as a means of asexual reproduction.

The application of yeast in traditional biotechnology such as brewing, baking and wine involves their exposure to various environmental stresses, which can affect the vitality and viability of the culture as reported by James *et al*. (2003). This is evident in the growth of the yeast strains obtained in this study when they were able to grow at temperatures slightly higher than room temperature (Table 4.1). Also, a most important factor influencing the effect of temperature on growth is temperature sensitivity of enzyme-catalysed reactions. At low temperatures, a temperature rise increases the growth rate because as the rate of each reaction increases, metabolism as a whole is more active at higher temperatures and the microorganism grows faster (Prescott *et al*., 2008). The major components of cells such as proteins and plasma membrane are dramatically affected when cells are exposed to elevated temperatures (Berney and Gervais,

2001). Nevertheless, under defined conditions most eukaryotic (Mager and Moradas, 1993) and prokaryotic (Morozov *et al*., 1997) microorganisms are able to acquire a certain degree of thermotolerance, so as to protect their components. Yeasts exposed to a mild preconditioning heat treatment acquired a certain degree of thermotolerance, which is related to heat shock proteins (HSPs) which are major factors in the acquisition of thermotolerance (Mager and Moradas, 1993; Morano *et al*., 1998; Parsell *et al*., 1994). The thermotolerance of isolates obtained in this research probably also depended on the kinetics of temperature variation (Table 4.4) which is related to membrane permeability (Gervais and Martinez de Maranon, 1995; Martinez de Maranon *et al*., 1999). The survival of all the wild yeast strains obtained in this study grown at physiological temperatures (room temperature) and then subjected to mild heat shock (35 – 37^0C) and lethal heat shock (42^0C) (Table 4.1 and 4.4) agrees with the report of Piper (1993) that prior induction of heat shock responses enables cells to survive subsequent exposure to lethal high temperatures. Thermotolerant capacity exhibited by all the wild yeast strains obtained in this research compared to the industrial strain (Table 4.4) is due to the expression of a set of proteins known as heat shock proteins (HSPs) and are rapidly induced when cells are subjected to elevated temperatures (Westerheide and Morimoto, 2005) (Table 4.1 and 4.4).

The diversity of yeast isolated from the fresh and spoilt single strength orange juice utilised for this research showed that most citrus juices employ the whole range of natural flora that could function under the varied environmental conditions, which can affect the vitality and viability of the cultures as reported by James *et al*. (2003) (Fig 4.1 and appendix I).Thus, the result of this study revealed that *Candida* species were more in number. *Candida krusei* had the highest prevalence of (56.86%), *Rhodotorula minuta* (19.6%), *Candida zeylanoides* (7.84%), *Candida*

parasilosis (5.88%), *Geotrichum capitatum* (3.92%), *Candida lusitaniae*, *Candida norvegensis* and *Kodamaea ohmeri* (1.96%) respectively (Fig 4.1).

5.2 Carbon Assimilation Profile

Carbon assimilation is an important criterion in the taxonomy and identification of yeasts which depends on organic carbon sources for their energy supply and growth (Table 4.2). Galactose is a non-conventional nutrient for yeasts, but it can be used as a sole carbon source when glucose is absent from the medium. Thus, the ability of yeast strains of *Kodamaeaohmeri*, *Candidalusitaniae* and *Candidaparapsilosis* isolated in this study to assimilate galactose indicated that the strains have the GAL genes responsible for galactose fermentation (Yun *et al*., 2001) (Table 4.2 and 4.6). Furthermore, the ability of these isolates to also ferment maltose shows that they possess uptake mechanism that involves two systems; an energy – dependent maltose permase (ATP $\leftrightarrow$ ADP) which transports the maltose intact across the cellular membrane and a maltose (alpha – glucosidase) which hydrolyses maltose internally to yield two glucose units (Stewart, 2006). Thus, the mechanism is mediated genetically by three maltose utilisation genes (MAL genes) which are involved in the operation of the high-affinity maltose transporter (Lodolo *et al*., 2008) (Table 4.2 and 4.5).

The ability of the isolates of*Candida lusitaniae*, *Candida parapsilosis* and *Rhodotorula minuta* to metabolise xylose shows that the strains possess xylose reductase and xylitol hydrogenase genes responsible for xylose fermentation (Kosman, 2003) (Table 4.2). Thus, these make them suitable for ethanolic fermentation of hydrolysates of cellulosic materials. All of the yeast strains obtained in this research metabolised glycerol except *Candida norvegensis*. This indicated that these strains possess the glycerol kinase gene (GuT1) and a gene for mitochondrial glycerol 3-

phosphate dehydrogenase (GuT2) responsible for glycerol assimilation during fermentative growth (Pavlik *et al*., 1993; Ronnow and Kielland – Brandt, 1993) (Table 4.2).

An unconventional storage dissacharide found in yeast is trehalose (α,α – 1, 1 – diglucose) which exists in high concentration in resting and stressed cells. The yeast strains of *Candida lusitaniae, Candida parapsilosis, Kodamea ohmeri* and *Rhodotorula minuta*utilised trehalose and this showed that they have the gene responsible for synthesis of trehalose enzyme because the breakdown of trehalose to glucose is mediated by trehalose enzyme and both synthesis and degradation are regulated via cAMP (Versele and Thevelein, 2001). But *Candida krusei* and *Candida norvegensis* did not assimilate trehalose and this indicated that they lack this gene (Table 4.2). Thus, it can be inferred that most of the yeasts isolated here employed different sugars as their main carbon, and hence, energy sources.

5.3 Flocculation Profile

Yeasts which ferment simple sugars and which show appreciable abilities to flocculate, to produce high levels of ethanol and to tolerate relatively high temperatures and high carbohydrate concentrations are potentially useful for industrial ethanol production. Yeast flocculation rate analysed in this research showed that flocculation is characterised by intense interaction among the yeast cells which results in the formation of large cell aggregates or floc (Table 4.3). Variation in the flocculation agrees with the report of Barly *et al*. (2005) which stated that flocculation frequently occurs during late-logarithmic or stationary phases of growth upon depletion of carbon source and this might be partly due to increased transcription of flocculation genes at the post-diauxic shift. Some processes have replaced centrifugation by the natural ability of certain yeast strains to flocculate (Kondo *et al*., 2002), or by the introduction of flocculation

genes into non-flocculant strains. For instance, the ethanol industry in Brazil typically uses the batch process and yeast is separated from the medium by centrifugation which is the most expensive and complex step in ethanol production (Watari *et al*., 1990) but recently, cell separation is manually obtained by natural cell sedimentation (Schwan *et al*., 2001). The variation in yeast flocculation rate is due to the process employed by natural yeast strains presenting the FLO genes, in which the flocculins are inhibited by sugars leading to sedimentation at the end of fermentation (Cunha *et al*., 2006) (Table 4.3). The highly flocculent yeast strains obtained in this research can be useful at an industrial scale because flocculating yeast cells have been employed only in the later phase of primary beer fermentation to separate biomass from the fermented broth as reported by Domingues *et al*. (2001) (Table 4.2). Furthermore, the non-flocculant strain as was observed in one of the strains isolated. *Candida norvegensis* in this research can be useful in biotechnology because the possibility of inducing controlled flocculation in a naturally non-flocculant strains provides great potential for improving brewing, wine making, baking and ethanol-producing yeast (Denquin, 2001) (Table 4.3).

5.4 Ethanol Tolerance Profile

Ethanol tolerance is very important in brewing, wine-making and especially in the biosynthesis of industrial ethanol. Because it is so critical and has been studied extensively (Ameh *et al*., 1989; Singh and Mishra, 1993).

Variation in ethanol tolerance of budding yeasts can be explained in terms of many factors such as lipid composition of the plasma membrane (You *et al*., 2003; Takagi *et al*., 2005), accumulation of trehalose (Lucero *et al*., 2000) or heat shock proteins (HSp104) (Piper, 1995),

activity of plasma membrane H+-ATPase (Supply *et al*., 1995, Aguilera *et al*., 2006) and the mitochondrial stability (Ibeas and Jimenez, 1997). The ability of most yeast strains obtained in this research to withstand 18% v/v ethanol concentration (Table 4.3) may be due to the effect of an increase in fatty acid unsaturation in cellular membrane which increases ethanol tolerance (Alexandre *et al*., 1994). The variations in the ethanol tolerance level of the different yeast strains obtained from this research (Table 4.3) agrees with the report of Gibson *et al*. (2007). They stated that the stress induced by increasing amounts of ethanol accumulated to toxic concentrations during ethanolic fermentation is the major factor responsible for reduced ethanol production and eventually stuck fermentation. Thus, yeast strains that can tolerate stress imposed by high ethanol concentration are highly desired. Ethanol is well known as an inhibitor of microbial growth and its toxic effects on yeast cells involve loss of cell viability (Alexandre and Charpentier, 1998). The result obtained in this study agrees with this finding , when tolerance to 10% v/v ethanol was tested quantitatively in terms of relative viabilities of the yeast strains after various incubation periods. The yeast strains exhibited various degrees of poor ethanol tolerance at this concentration (Table 4.3).

5.5 Fermentation Profile

Sugar fermentation is a pre-requisite for industrial ethanol production. The other attributes enhance productivity and minimise cost of production: for example, flocculence facilitates sedimentation, centrifugation, and recycle of cells; thermotolerance reduces cooling costs, and osmotolerance permits fermentation of high gravity substrates (Stewart *et al*., 1984). All the yeast strains obtained in this research with the exception of *Geotrichum capitatum* appear to have considerable potential for industrial application. They combine four attributes necessary for such application namely; flocculation ability, tolerance to 8 – 18% ethanol and to growth at 37 – 42^{0}C

and ability to ferment 10 – 40% (w/v) glucose (Table 4.4 and 4.5). Yeast possessing such a combination of attributes appear to be rated high in published literatures (e.g. Hacking *et al*., 1984; Benitez *et al*., 1983; Ameh *et al*., 1989; Alexandre *et al*., 1994; Westerheide and Morimoto, 2005) (Table 4.1 and 4.4).

Most of the yeast strains obtained in this study exhibited relatively high fermentation rates on hexose sugars namely: glucose, galactose, maltose and sucrose (Table 4.4 and 4.5). Thus, they could be used in fermentations when molasses, malted cereals or hydrolysates of other fermentation substrates such as whey and starchy products are used. This result is consistent with the report of Ameh *et al*. (1989) and Oke and Ijebor, (1997).

5.6 Nutritional Requirements of Mutants

Nutritionally exacting mutant strains are identified as such by their inability to grow on the simplest chemically defined medium (‘minimal’) sufficient for growth of the wild strain. They can, however, grow on a complex medium (‘complete’) containing a large variety of known and unknown organic compounds (Pronk, 2002). The isolates obtained in this study, particularly the mutant strains showed differences from the wild strains in having additional growth-factor requirements (Table 4.7). All the wild yeast strainshowever grew in the absence of each of the tested amino acids in the minimal basal growth medium. The result obtained from this study agrees with the report of Pronk,(2002); Idise *et al*., (2010) thereby confirming the mutagenicity of the yeast strains obtained in this research.

5.7 Genetic Modification of the Characterised Yeast Strains

Ultraviolet (UV) irradiation has been shown to cause various mutations in cells, which often lead to other problems including cell death (Russel, 2006;Michele,2012).Most of the yeast strains obtained in this study were seriously affected by the UV light, with some having little (if any) growth after exposure depending on the level of exposure in relation to time (Fig 4.2 – 4.9).

Low doses of radiation may not produce any adverse effect on the cells (Fig 4.2 & 4.3). But if one lengthens the exposure time, or increases the intensity of UV light, an increase in the number of unpaired dimers and an increase in mutation probably occurred. If a mutation occurred in an essential gene, the cell may die and is said to contain a lethal mutation (Claus, 1989). The result obtained from this study also showed that nitrous acid-induced mutant strains at a high exposure time had a decrease in the percentage survival rate (Fig.4.9). Both results of the UV irradiated and nitrous acid-induced mutant yeast strains showed high percentage of kill with low cell survival rate at exposure time of 20 minutes for nitrous acid and 25 – 30 minutes for UV. This trend is in agreement with the observation of Ado, (2004) and Idise *et al* (2010) that for a mutagenic agent to be successful, it should have a high rate of kill (Fig 4.7-4.8).

Microbial inactivation from UV treatment is illustrated by a sigmoidal shape curve (Sastry *et al*., 2000). The initial plateau indicates the commencement of cell injury. The result obtained in this study showed that at a lower dose of exposure with the UV light at 4 – 6 minutes a high rate of cell survival was obtained (Figs. 4.2 and 4.3). This result is in agreement with the findings of (Sastry *et al*., 2000). As exposure continues, maximum damage occurs to the cells and minimal additional treatment becomes lethal (Sastry *et al*., 2000). The survival numbers declined rapidly and a lag phase due to UV resistance and experimental components (i.e. suspended solids that would protect the microorganism from direct exposure) is noticed (Sastry *et al*., 2000).

Microbial inactivation from UV treatment is associated with photochemical changes that take place in proteins and nucleic acids when UV light is absorbed (Jay, 1996). Mutations occur that disrupt DNA transcription and replication, which ultimately causes death of the microorganisms (Miller *et al*., 1999). In some cases, wounded cells are able to repair damage caused by UV exposure and may be able to reactivate themselves through activity repair – enzyme (Parrotta and Bekdash, 1998). The response of some of the yeast strains obtained in this study with regards to the above are in agreement with this study as was observed in *Candida norvegensis* at 6 and 10 minutes exposure (Figs. 4.3-4.4) and *Candida parapsilosis* at 25 and 30 minutes exposure (Figs. 4.7-4.8).

5.8 Alcohol Producing Ability of the Wild, Modified and Brewers Yeast Strains

Yeasts are commercially valuable to a variety of industries. These organisms, mainly known in baking and brewing industries, play an important role in production of alcohols, animal nutrition (single-cell protein) and, more recently, enzymes. Organisms with desirable traits have been selected by screening culture collections or by mutagenesis and subsequent isolation of the desired strains (Alexander, 1986).

Modification of yeasts to increase ethanol production had also been achieved through improvement of strains that could overcome low tolerance to ethanol and high substrate concentrations, which are characteristics of most commercial yeast strains (Alexander, 1986). It is noteworthy that when the wild strains of yeasts were mutagenised using both nitrous acid and UV light mutants, there was an appreciable increase in yield of ethanol and drastic decrease in the length of fermentation (Tables: 4.8 and 4.9). This discovery agrees with the findings by different workers (Gong *et al.*, 1981; Jeffries, 1984; Lee *et al.*, 1986 and Shaheen *et al.*, 2009).

They also isolated a number of improved ethanol-producing mutants. The relationship between the fermentative ability and viability of yeast is intimate (Singh *et al.,* 2009; Yamada *et al.,* 2009; and Ghorbani *et al.,* 2011). The cell growth rate (cell dry weight, cell number (optical density), the ethanol output obtained in this study increased linearly with decrease in amount of sugarconsumed or residual sugars concentration (Figs 4.10- 4.20 & Table 4.7a-g). It is recognised that ethanol has three inhibitory effects; inhibition of cell multiplication, inhibition of fermentation and a lethal effect on cells (Moulin *et al.,* 1984). The decrease in cell growth rate and reduction in the length of fermentation exhibited by the yeast strains (Table 4.3 and 4.9) isolated in this study with respect to increase in ethanol concentration are similar to the observations made by Piper, (1995). The author had noted that the rising ethanol level during batch fermentation on high concentrations of sugar substrates acted initially to reduce growth and fermentation rates, and adversely affected cell viability. Thus, a high level of ethanol tolerance for a yeast strain is a prerequisite for high efficiency of fermentation and high yield of ethanol (Table 4.3 and Figs. 4.10 – 4.20).

The optimum lengths of fermentation were obtained on the second, third, fourth and fifth days for the mutants of UV light, nitrous acid and wild type (Table 4.8 and 4.9). These days of fermentation represented the phase of active sugar assimilation which favored ethanol production. Since the decreasing length of fermentation is desirable for ethanol yield as it reduces time input and labor costs, therefore mutants with such attributes should be adopted for industrial ethanol fermentation. These observations were consistent with the findings of (Ado *et al.,* 2010; Stanley *et al.,* 2010; Awatif, 2011; Razmovski and Vucurovic, 2011).

Genetic manipulation of brewing yeasts may be considered as producing process improvements, more flexibility in choice of raw materials or new products. Sturley and Young, (1986) reported

that the process of alcohol fermentation was improved by lowering the time taken for fermentation. They also reported that yeasts which ferment faster and possess the capability to withstand higher temperature and osmotic pressure would ferment more concerntrated substrate, thus increasing productivity (Sturley and Young, 1986). The wild and mutant yeast strains of *Candida, Kodamaea* and *Rhodotorula* speciesobtained in this research showed similar characteristics.

The quest for increased alcohol production demands the development of organisms that have tolerance to ethanol, high osmotolerance, and could degrade cellulose, starch and xylose. Such organism could make ethanol production more competitive (Alexander, 1986). The isolates, *Candida lusitaniae, Candida parapsilosis, Kodamaea ohmeri* and *Rhodotorula minuta,* obtained in this research are in agreement with this statement in having attributes of tolerance to ethanol, ability to ferment xylose and increase ethanol production (Tables 4.2, 4.5, 4.8 – 4.9).

One important area for yeast strain improvement, one that could substantially reduce ethanol production costs, is that of increasing the variety of carbohydrates that yeasts could economically ferment. Starch, cellulose and xylose are abundant carbohydrates found in plant materials. Other fungi apart from yeasts have been identified as being capable of breaking down these complex carbohydrates. Thus combining the genetic material of these various organisms could result in a unique individual capable of coding for the enzymes necessary to attack a number of carbohydrates simultaneously (Alexander, 1986). *Candida lusitaniae, Candida parapsilosis* and *Rhodotorula minuta* obtained in this research were able to ferment some of those complex carbohydrates such as xylose and starch, producing ethanol. Multiple studies have shown progress towards strain improvement by mutation (Gong *et al.,* 1983, Maleszka *et al*; 1983; Jeffries, 1984, 1985).*Candida* species that was mutated by UV irradiation produced five times

more ethanol than the native strain due to increased activity of xylulokinase and xylitol dehydrogenase (Gong et al; 1983). However, the major drawback with this method is to get the stable ethanol titers from mutagenic strains after every fermentation reaction. The findings obtained from this research which showed similar result agrees with the report of Chandel *et. al.*, (2011).

5.9 Dough Leavening Capacity of the Wild, Modifiedand Baker's Yeast Strains

The importance of strains of yeast of species *Saccharomyces cerevisiae* had been well recognised from ancient times for fermentation. Today, Baker's yeast is used for bread manufacturing throughout the world at Industrial scale. With the improvement of the baking industry, the use of starter culture increased tremendously. At present, the baking industry in Nigeria is solely dependent on import of yeast. So, there is a dire need to explore the potential of indigenous strains other than *Saccharomyces cerevisiae* in order to meet the national requirement and save foreign exchange.

There are different sources for isolation of yeast species. However, their presence was mostly from acidic foods. Among them, citrus (Arais *et al*., 2002) is considered to be one of the best. The species of yeast isolated in this study were of *Candida,Geotrichum, Kodamaea* and *Rhodotorula*. These are osmophilic in nature and had been reported by Ekwenye, (2000), as well as Okagbue and Ekejindu, (1998), to be used in the production of fermented foods and for baking purposes.

Keeping in view of the importance of yeast, the leavening ability of yeasts isolated from orange juice was investigated in this study. The wild type and mutants of yeast obtained in this study

showed a slower rate of dough fermentation when compared with the control,a commercial baker's yeast (*Saccharomyces cerevisiae*), within 180 minutes of incubation. From 24 - 48 hours, however, a fast dough fermentation period occurred. This could be attributed to the lag phase of growth, size of inoculum and rate of carbon(iv)oxide production by the different yeast strains used. Studies have shown that yeast produces carbon(iv)oxidefrom the sugar present in the dough and the more the yeast produces more carbon(iv)oxide would makethe dough fermentation "faster", while theless the yeast produces less carbon(iv)oxide and would makethe dough fermentation slower. (Plyer, 1988; Ana *et al*., 2001 and Rao *et al.*, 2006). Moreover, the baker's yeast used as the control also contains fast active ingredients as against the cream yeast which was used in this study.

The sweetened or sugar dough showed a better leavening capacity than the unsweetened dough for almost all the wild type, nitrous acid-induced mutants and the control, the commercial baker's yeast. However, there was a remarkable improvement in the leavening capacity of wild type *Rhodotorula minuta* on unsweetened or plain or lean dough.The addition of sucrose in sweetened dough provided additional source of energy and nutrient, thereby producing more carbon (iv) oxide from fermentation of the sugar and the rising of the dough. Also an important trait that influences the baking performance of yeast in sweet dough fermentation is the level of invertase activity (Evans, 1990). This enzyme catalyses the hydrolysis of sucrose into glucose and fructose as rapidly as possible, increasing osmotic pressure ,thereby enhancing the leavening of the dough fermentation. It is also noteworthy that the isolate of the wild type *Kodamaea ohmeri* showed a higher leavening ability when compared with the control (the commercial baker's yeast,*Saccharomyces cerevisiae*) in both sweetened and unsweetened dough (Figs. 4.24 and 4.25). This report is in agreement with the studies of other workers, where they reported

yeasts such as *Issatchenkia orientalis*, *Pichia membranaefaciens* and *Torulaspora delbrueckii* showed higher dough leavening abilities than *Saccharomyces cerevisiae* (Almeida and Pais, 1996; Harnandez-Lopez *et al*., 2003).

In dough without addition of sugar (lean or plain), the principal fermentable sugar for yeast is maltose, liberated from the starch of the flour by amylases (Oda and Ouchi, 1990; Higgins *et al*., 1999). The leavening ability of sponge dough is closely related to maltose fermentability (Blazquez *et al*., 1993; Higgins *et al*., 1999). It is generally accepted that a good baker's yeast should be able to rapidly ferment maltose, because if bread is made without added sugar, the "existing" free sugars are completely fermented within the first hour, leaving only the starch-derived maltose to sustain fermentation. The result obtained in this study on unsweetened dough fermentation showed an improved leavening capacity both in the wild type and mutant strains (Fig. 4.25) and is consistent with the report of other workers (Oda and Ouchi, 1990; Higgins *et al*., 1999). The most desirable property for mutants of industrial strains is genetic stability and physiological reproducibility (Oda and Ouchi, 1990). The mutants isolated in this study were stable and their fermentative capacities were in many cases improved with regard to wild type. The mutants with the best properties (following UV irradiation) were *Candida zeylanoides* and *Geotrichum capitatum.*These had a lower leavening capacity in their wild state but showed an increase in their leavening activities in lean or unsweetened doughs (Figs. 4.29e and 4.29i). The mutant of *Candida norvegensis* (nitrous acid treatment) also showed an improved fermentative capacity, that is, higher leavening ability on sweetened dough than the wild type and control (commercial baker's yeast,*Saccharomyces cerevisiae*) (Fig. 4.24). This finding is in agreement with the report of other studies (Oda and Ouchi, 1991 and Randez-Gil *et al*., 1995).

5.10 Sensory Evaluation of Bread Made with Wild Strains, Modified and Baker's Yeast

Bread manufacturers have on-going interest in strains of baker's yeast, especially those that increase dough fermentation rates and yield a high quality final product (Ana *et al*., 2001). The sensory quality of food products plays an important role in the choice of food. Hedonic testing is often used to determine consumer's acceptance of a new, or improving the existing, food product (Meilgard *et al*., 1991). The higher value presents higher consumer acceptability (Table 4.14 and 4.15). The result of the sensory evaluated yeast bread, statistically in terms of texture, colour, aroma and overall acceptability showed that all the tested bread samples were well accepted by the panelists. However, DS20 mutant bread samples were the best with regard to each of the parameters assessed when compared with the wild type and control samples in both sweetened and unsweetened bread (Table 4.15). This was followed by 7F9, DS6 and 7F8 mutant breads. These breads compared favourably, if not even better than, the commercial bread sample especially in the sweetened bread samples in relation to their organoleptic properties (texture, colour and aroma) (Table 4.14 and 4.15). This result is in agreement with findings of Ana *et al*., (2001); Wonghalaung and Boonyaratanakorkit, (2007) and Cheng-Chang *et al*., (2010). They showed that the product of bread baked with mutant baker's yeast had a better consumers' acceptance than that of its wild type.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

Most traditional fermentations such as fermented juices are characterised by numerous natural microflora of yeasts of varying functions that could be beneficial or detrimental to the fermentation processes: Mixed cultures that produce the blend of rich flavours and aromas of the product and some microorganisms that could accelerate spoilage, particularly in the finished products exist. Therefore, fermented products should be hygienically and microbiologically safe for human consumption.

The abundance and availability of the wild yeasts obtained in this study satisfied one of the basic requirements for use of microorganisms in fermentation industry. Industrial yeast strains can be divided into two groups, one that had high sweetened dough activity but low unsweetened dough activity and vice versa. The yeast strains from fermented juice and their mutants can be developed as novel strains capable of leavening both sweetened and unsweetened bread dough efficiently. This will reduce the necessity of carrying out the expensive procedure of producing multiple baker's yeast strains.

This study has also shown that strain improvement technology such as genetic improvement can be achieved through mutations. There should be no fear in developing genetically modified strains for increased productivity in the food industry. This is because the most desirable property for mutants of industrial stains is genetic stability and physiological reproducibility. The mutants obtained in this research were very stable, and yielded better bread quality. Their fermentative capacities were in many cases, improved upon with regard to the wild type when

compared with the baker's yeast. This study was also successful in demonstrating that a significant increase in the yield of ethanol can be achieved through mutations. Mutants with the desired characteristics were found and such mutants did accumulate more ethanol than the wild type as was observed in this research.

6.2 CONCLUSION

Yeasts are the most suitable microorganisms for ethanol production. The species of yeasts obtained in this research fermented both pentose and hexose sugars. This showed that these strains have the potentials for the conversion of cellulose, hemicelluloses and hydrolysate of other fermentation substrates such as whey and starchy products to fermentable sugars for bioethanol and ethanol production.

The yeast strains isolated from this study exhibited four attributes namely: flocculationability, tolerance to ethanol, thermotolerance and ability to ferment 10-40% glucose. These are desirable characteristics of ideal microorganisms required for industrial ethanol production. Viability of yeast strains in the presence of high ethanol, glucose concentration, and temperature as prerequisites for a high efficiency of fermentation for enhanced ethanol yield was achieved. Thus, selection of yeast strains with high resistance to ethanol, osmotic and thermal stress are of research importance for understanding the evolution of the organism and of economic value for traditional brewing and rapidly developing biofuel industries. The result obtained in this study showed that the mutant strains had high efficiency of fermentation, reduction in the length of fermentation and a high yield of ethanol than its wild type. Thus, these attributes make them suitable for industrial application.

Brewers and wine makers constantly struggle to understand the flocculation behavior of yeasts often without much success. Specifically adjusted flocculation properties of industrial adjusted microorganisms: which involves pH adjustment,addition of chemicalprecipitant, could indeed lead to significant improvement in the processing of biotechnology fermentation products such as foods, biofuels and industrially produced peptides. For industrial applications, a system capable of converting specific yeast strains which predominate at each industrialplant to conditioned flocculant strainsmust be developed.This is because the yeast strains obtained in this research showed that the development of industrial yeast strains capable of flocculation would permit the separation of the cells at the end of fermentation in a natural manner without the need for mechanical processing.

The improvements of sweet and unsweetened baker's products fermented with wild and its mutant yeast strains wasdescribed in this study. The strains of yeast obtained in this research both wild and its mutant matched baker's yeast in their ability to utilize maltose and showed a better leavening capability than the commercial yeast. Bell *et al* (2001), presumably linked this to the long period of domestication of yeast and the relative rarity of maltose as a sugar in the environment. Osmotolerance was widely distributed in the wild, mutant and baking strains.

The wild and mutant strains of yeast obtained in the study have sufficient potential to be produced commercially, because their fermentative abilities on sweetened dough and unsweetened dough were improved. In sensory evaluation, the quality of the baked products in terms of texture and colour of bread was excellent. Therefore, it is very feasible to produce leavened bread using wild and mutant yeasts obtained from fermented orange juice as the sole leavening agent for optimum loaf height and volume. This is possible as most of the strains efficiently produced carbon(iv)oxide in their respective dough types.

6.3 RECOMMENDATIONS

Based on the findings of this study the following recommendations are made:

1. Flocculation Profile

Ethanol tolerance significantly improves when yeast cells self flocculated and form the flocs compared with the free yeast cells. The process is simpler and economically competitive compared with the yeast cell immobilisation by supporting materials.

The yeast flocs during fermentation can be recovered by sedimentation rather than by centrifugation which is widely used in the recovery of free cells, saving the capital investment for centrifuges operation.

2. Ethanol Tolerance Profile

High ethanol-tolerant yeast strains are recommended in order to overcome the problem with inhibition during fermentation because the amounts and types of inhibitory compounds vary strongly between different raw materials. Ethanol tolerance profile is also recommended in ethanol production rather than applying a fed-batch mode of substrate addition with proper feed protocol and control variables in which the levels of inhibitors are kept at an acceptable level.

3. Temperature Profile

Exposure of yeast cells to elevated temperatures increases the ability of cells to survive exposure to lethal temperatures during ethanol production. Thermotolerance is important for simultaneous saccharification and fermentation (SSF) in order to allow fermentation at temperatures closer to the optimal temperature for the enzymes.This is because in SSF a compromise between the optimal temperatures for the cellulolytic enzymes and the yeast is required. Since most of the strains isolated tolerated 42^0C and the optimal temperature for cellulolytic enzyme activity is 45-50^0 C, thus, fermentation at elevated temperatures is recommended.

4. Fermentation Profile

One area for strain improvement and one that could substantially reduce ethanol production costs, is that of increasing the variety of carbohydrates that yeasts will economically ferment. Starch, cellulose and xylose are abundant carbohydrates found in plant materials. Some fungi have been identified as being capable of breaking down these complex carbohydrates. Thus, combining the genetic materials of various yeast strains obtained in this research is recommended, this will result in unique individual capable of coding for the enzymes necessary to attack a number of carbohydrates simultaneously.

5. The wild strain and mutant strains obtained from this research can be further developed using the following number of proven techniques:

- Strains improvement by hybridisation (breeding) using sphaeroplast fusion is recommended; this has greater relevance in genetic manipulation of yeast genera that have no sexual phase to their life cycle (*Candida* spp)
- genetic enhancement, cloning and expression
- DNA karyotyping to ensure trueness to type and purity of strain is recommended.

6. Pure cultures of the yeast strains from fermented orange juice should be generated as novel baker's yeast strains that exhibit strong maltose utilisation and hyperosmotic resistance.

7. Optimisation of potential fermentation parameters to enhance the production of ethanol can be carried out using the wild and mutant strains obtained from this research. a

REFERENCES

Association of Official Analytical Chemists. (2000). Official methods of analysis of AOAC International 17th edition. Vol. II, Gaithenbug, M.D., USA, Official method. pp. 20-57.

Aboada,O.O.sand Obkpolor,E.A.(2010).The leavening ability of baker'syeast on dough prepared with composite flour(wheat/cassava).*African Journal of Food Science*,**4**(6): 325-329.

Ado, S.A. (2004). Studies on genetics improvement of *Aspergillus niger*-CA-3 for amylase production.PhD thesis, Department of Microbiology, Ahmadu Bello University, Zaria.

Ado, S.A; Olukotun, G.B., Ameh, J.B. and Yabaya, A.(2010). Bioconversion of cassava starch to ethanol in a simultaneous Saccharification and fermentation process by co-cultures of *Aspergillus niger*and *Saccharomyces cerevisiae*.*Journal of Pure and Applied Microbiology*,**4**(1):55-61.

Aguilera, F., Peinado, R.A., Millan, C., Ortega, J.M. and Mauricio, J.C. (2006).Relationship between ethanol tolerance, H+ - ATPase activity and the lipid composition of the plasma membrane in different wine yeast strains.*International Journal of Food Microbiology*, **110**:34-42.

Akinyemi, O.M. (1990). Studies on developing a starter culture for fermentation of "Burukutu" beer. M.Sc thesis. Department of Microbiology, Ahmadu Bello University Zaria, Nigeria pp27

Alexander, N.J. (1986). Genetic manipulation of yeasts for ethanol production from xylose. *FoodTechnology*,**40**(10):99-130.

Alexandre, H. and Charpentier, C. (1998).Biochemical aspects of stuck and sluggish fermentation in grape musk.*Journal of Industrial Microbiology and Biotechnology*, **20**: 20-27.

Alexandre, H., Rousseaux, I. and Charpentier, C. (1994).Ethanol adaptation mechanism in *Saccharomyces cerevisiae*.*Journal of Biotechnology and Applied Biochemistry*, **20**:173-183.

Almeida, M.J. and Pais, C.S. (1996). Characteristics of the yeast populations from traditional corn and rye bread doughs. *Letters of Applied Microbiology*, **23**:154-156.

Ameh, J.B. and Umaru, L. L. (2000). Screening of yeast isolates for use in bread making. *Spectrum Journal*,**7**(2); 150.

Ameh, J.B., Okagbue, R.N. and Ahmad, A.A. (1989).Isolation and characterisation of local yeast strains for ethanol production.*Nigerian Journal of Technology Resources*, **1**:47-52.

Aminetzach, Y.T., Macpherson, J.M. and Petrov, D.A. (2005).Pesticide resistance via transposition-mediated adaptive gene truncation in *Drosophila. Science*,**309** (5735); 764-767.

Ana, R. M., Antonio, C.C., Francisco, C. and Tahia, B. (2001).Improved properties of Baker's yeast mutants resistant to 2-Deoxy-D-Glucose. American Society for Microbiology, pp. 1-7. Springer link.

Anderson, P.J., Mcneil, K. and Watson, K. (1986). High efficiency carbohydrate fermentation to ethanol at temperatures above 40^{0C} by *Kluyveromyces marxianus var. maxianus* isolated from sugar mills. *Applied and Environmental Microbiology*, **51**(6):1123-1133.

Anestis, M. (2006). AP Bio logy. 2nd edition.McGraw-Hill Professional,England. pp. 61

Anonymous. (2001).Citrus summary 1999-2000. Florida agricultural statistics services, Orlando, Fla.Retrived 20/09/2001.

Anonymous. (2002a).Understanding baking: the art and … Google books (Http://books. google.co.UK) Retrived 09/09/2002

Anonymous.(2002b).Ethanola Biofuel.http://www.eia.doe.gov/kids /energy_facts/ sources /ren e wable/ ethanol.html.Retrived 05/02/2002.

Anonymous. (2006a)."The Microbial World: Yeasts and yeast-like fungi" (http:/ /heilos.bto.ed.ac.UK/bto/microbes/yeast.html).Institute of cell and Molecular Biology. Retrieved 24/12/2006.

Anonymous. (2006b). Handbook of brewing-Google books (Http://books.co.UK) Retrieved22/02/2006.

Anonymous. (2007a). "Biofuel cell", (http://automation. tkk.fi/Research /Bioprocess Automation).Helsinki University of Technology. Retrieved 11/28/2007

Anonymous. (2007b). Mutations.Available at File://mutation % 2001.htm Retrieved 22/02/2007.

Anonymous. (2008). "Appendix I :Indo-European Roots"(http://ww.bartley.com/61/roo ts/IE598. html). Retrieved 16/11/2008.

Anonymous. (2009a). "What are yeasts?"(http://www.yeastgenome.org./VL-what_are_yeast.html). Yeast Virtual library. Retrieved 28/11/2009.

Anonymous. (2009b). "Fuel Ethanol Production: GSP systems Biology Research". Retrived from (http://genomicsgtl.energy.gov/biofuels/ethanolproduction.shtml).Genome Science program.U.S. Department of Energy Office of Science. Retrieved 28/11/2009.

Anonymous. (2009c). How stuff works (http://Science.howsuffwork.com/dictionary/famous-scientists/chemists/louis-pasteur-info.html Retrived 04/01/2011.

Anonymous. (2009d). Ultraviolet radiation. Enyclopedia Britannica. Utimate Reference suite. Chicago: Encyclopedia Britannica.

Anonymous. (2010a). The Sommelier Prep Course: An … Google books (http://books.g oogle. co.UK). Retrieved12/07/2010.

Anonymous. (2010b). "Le comite des fabricants de levure" (http://www.cofalec.com/contentO.aspx2 progress). Retrieved 21/02/2010.

Anonymous. (2011a). Eukara! Vancouver Scientist takes the headache out of red wine http://www.vancouversun.com./health/4281742/story.html. Retrieved 24/03/2011

Anonymous. (2011b). (Http://www.umm.edu/altmed/articles/brewers-yeast-000288.html). Retrived 24/03/2011

Anonymous.(2011c).A brief History of fermentation, East and West (http://www.**soyinfocenter**.com.Retrieved 04-01-2011.

Anonymous. (2011d). Accomplishments of Louis Pasteur (http://www.fjcolla20.com/fjc-publishings/documents/LPasteurRpt.html).Retrieved on 04-01-2011

Anonymous. (2011e). Studies on Fermentation; The disease of Beer, their causes, and the means of preventing them.New York.Macmillan publishers.pp 66.

Anonymous. (2013), Wild Flavour inc. www.wildflavours.com. Retrieved 02/04/2013.

Arias, C.R., Burns, J.K., Friedrich, L.M Goodrich, R. M. and Parish, M.E. (2002). Yeast species associated with orange juice. Evaluation of different identification methods.*Applied Environmental Microbiology*,**68**:1955-1961.

Awatif, A. J. (2011).Effect of some fermentation parameters on ethanol production from beet molasses by *Saccharomyces cerevisiae*CAM13. *American Journal ofAgricultural andBiological sciences*,**6**(2):301-306.

Balasubramanian, M.K., Bi, E. and Glotzer, M. (2004).Comparative analysis of cytokinesis in budding yeasts, fission yeast and animal cells. *Journal of current Biology*,**14**(18): 806-818.

Bankar, A.V., Kumar, A.R., and Zinjarde, S. S. (2009). Enivironmental and Industrial application of *Yarrowia lipolytica*. *Applied Microbiology and Biotechnology*,**84** (5); 847-865.

Bankar, A.V., Kumar, A.R., and Zinjarde, S. S. (2009). Removal of chromium (VI) ions from aqueous solution by adoption onto two marine isolates of *Yarrowia lipolytica*.*Journal of Hazardous materials*,**170** (1): 487-494.

Barley,S.(2010).Stinky flower is kept by yeast partner.http://www.newsc ientis t.com/article/mg20527473.900- stinky-flower-is-kept- warm-by-yeast-partner.html).

Barnett, J.A. (2003). Beginnings of Microbiology and Biochemistry: the contribution of yeast research. *Microbiology* (Reading, Engl.**149** (pt 3): 557-567.

Bass, D., Howe, A., Brown, N. Barton, H., Demidova, M., Michelle, H., Li L., Sanders, H., Watkinson, S.C., Willcock, S. and Richards, T.A. (2007). Yeast forms dominate fungal

diversity in the deep oceans. *Proceeding of Biological sciences/ The Royal society*,**274**(1629): 3069-3077.

Barly, J.C., Douglas, L.M., Pretorius, I.S., Bauer, F.F. and Dranginis, A.M. (2005). Characteristics of Floll – dependent flocculation in *Saccharomyces cerevisiae,FEMS Yeast Research Journal*, **5**:1151-1156.

Bell, A.A., Ough, C.S., and Kliewer, M.W. (1999). Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis vsinifera var.* Thompson Seedless Grapevines. *American Journal of Enoli Vitic*,**30** (2): 124-129.

Bell, P.J.L., Higgins, V. J and Attifield, P.V. (2001).Comparison of fermentative capacities of industrial baking and wild type yeast of different sugar media. *Letters in AppliedMicrobiology*,**32**;227.

Benabdesselem,F.M.(2010).Malt factor induced flocculation in *Saacharomyces uvarum.Scientific Research and Eassys* ,**5**(17) : 2595-2602.

Berney, L. and Gervais, P. (2001). Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Journal of Applied Microbiology and Biotechnology*,**57**:34-42.

Benitez, T., Delcastillo, L., Aguilera, A., Conde, J. and Oimedo, E.C. (1983).Selection of wine yeast for growth and fermentation in the presence of ethanol and sucrose. *Applied and Environmental Microbiology*,**45**(5):1429-1436.

Bernton, H., Kovarik, W. and Sklar, S. (1982). The Forbidden fuel: Power alcohol in the twentieth century. New York: Boyd Griffin, Inc.pp 90-99.

Berg, L. R. and Cengage, L. (2007). Introductory Botany: plants, people and the environment. 2nd edition . McGraw-Hill Professional,England.pp.86.

Bertram, J (2000). The molecular biology of cancer *Molecular Aspects of Medical*,**21** (6): 167-223.

Beth, A.M. (1998). Mutation, Mutagens, and DNA Repair.Ph.D thesis.Division of Biology, Kansas State University.

Bisson, L. F., Cooris, D.M., Kruckeberg, A.L. and Lewis, D.A. (1993). Yeast sugar transporter. *Critical Reviews in Biochemistry and Molecular Biology*, **28**(4): 259-308.

Blazquez, M.A., Lagunas, R., Gancedo, C. and Gancedo, J. (1993).Trehalose-6-phosphate, a new regulator of yeast glycolysis that inhibits hexokinases. *FEBS letter*,**329**:51-54.

Brat, D., Boles, E. and Wiedemann, B. (2009).Functional expression of a bacterial xylose Isomerase in *Saccharomyces Cerevisiae.Applied and Environmental Microbiology*,**75**(8): 2304-2311.

Brookline, M. A. (2004). Cook's illustrated magazine, Baking Illustrated.Boston common Press, pp 47-52.

Burrus, V. and Walder, M. (2004). Shaping genomes with interactive and conjugative elements. *Research Microbiology*, **155** (5): 376-386.

Calleja, G.B. (1987). Aggregation. *In*The Yeast by Ross,J.P. and Harrison, J.S, ed. Academic Press London.pp.86

Centina, S.G and Sierra, B.G. (1994).Therapeutic evaluation of *Saccharomyes boulardii* in children with acute diarrhea. *Annals of Pediatrics*, **41**:397-400.

Cerveny, K.L., McCaffery, I.M. and Jensen, R.E. (2001). Division of mitochondria requires a novel DMNI interacting protein, Net2p. *Journal of Molecular and Cellular Biology*, **12**:309-321.

Chandel, A.K., Chandrasekhar, G., Radhika, K., Ravinder, R. and Ravindra, P. (2011). Bioconversion of pentose sugars into ethanol: *A review and future directions*,**6**(1):008-020.

Cheng-chang, L., Pao-chuan, H., Der-feng, T. and Shang-Jung, W. (2010). Evaluation of the New fusant improving Bread Textural characteristics. *Journal of food Technology*,**8**(3):95-98.

Christopher, W. (2006). Flocculation Basics. *Yeast*,**8**:25-38.

Claus, G.W. (1989). Effects of UV radiation on bacteria DNA, cell viablility and mutation frequency.A laboratory textbook for Microbiology. New York: W.H. Freeman and Co. pp333-346.

Corredor, D.Y. (2006). Effect of decorticating sorghum on ethanol production and composition of DDGS. Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS. pp 2-8.

Corriher, S. (2008). Bakewise: The hows and ways of successful baking with over 200 Magnificent Recipes. New York: Scribner. pp32.

Covadonga, R.A., Jacqueline, K.B., Lorrie, M.F. Renee, M.G., and Mickey, E.P., (2002). Yeast species associated with orange juice: Evaluation of different identification methods. *Applied and Environmental Microbiology*, **68**:4:1955-1961.

Cunha, A.F., Silvia, K.M., Luiz, H.G., Sergio, F.R., Gonc, A. and Pereira, G. (2006). Control by sugar of *Saccharomyces cerevisiae* flocculation for industrial ethanol production. Federation of European Microbiological Societies (FEMS). *Microbiology Review Yeast Research*, **6**:280-287.

Cyr, N., Blanchette, M., Price, S.P. and Sheppard, J.D. (2007). Vicinal Diketone Production and Amino acid uptake by two Active Dry Lager Yeasts During Beer Fermentation. *Journal of American Society of Brewing Chemistry*, **65**(3): 138 – 144.

Deak, T. and Beuchat, L.R. (1992). Yeasts associated with fruit juice concentrates. *Journal of Food Protein*,**56**:777-782.

Dequin, S. (2001). The potential of genetic engineering for improving brewing, wine-masking and baking yeasts. *Journal of Applied Microbiology and Biotechnology*, **56**;577-588.

Dickinson, J.R. (1999). Carbon Metabolism.*In*: Dickinson, J.R. and and Schwizer, M. *TheMolecular Physiology*of *Saccharomyces cerevisiae.* Philadelpha, P.A., Taylor and Francis.

Dien, B.S., Bothast, R.J., Iten, L.B., Barrios, L., and Eckhoff, S.R. (2002). Fate of Bt protein and influence of corn hybrid on ethanol production. *Cereal chemistry*,**79**: 582-585.

Domingues, L., Lima, N. and Teixeira, J.A. (2001). Alcohol production from cheese whey permeate using genetically modified flocculant yeast cells. *Journal of Biotechnology and Bioengineering*, **72**:507-514.

Doniger, S. W., Kin, H.S., and Swain, D. (2008).A catalog of neutral and deleterious polymorphism in yeast. Palestine Liberation Organisation Genetics. **4**(8): e1000183.

Drake, J.W. and Holland, J.W. (1999). Mutation rates among RNA viruses. *Proceedings ofNational AcademicScience*,U.S.A.**96** (24): 13910-13913.

Dunn, B., Levine, R.P. and Sherlock, G. (2005). Microarray Karyotyping of commercial wine yeast strain reveals shared, as well as unique, genomic signatures. *BMC genomics*,**6**(1):53.

Edward, B. and Cornish, B. (1997).New beer in an old bottle, the growth of Biochemical Knowledge.Athel. Universite de Valencia.

Ekwenye, U.N. (2000). Screening of osmophilic yeast for ethanol tolerance. *Nigerian Journal of Biotechnology*,**11**(1):18.

Ellis, N.A., Ciocci, S. and German, J. (2001). Back mutation can produce phenotype reversion in bloom syndrome somatic cells.*Human Genetics*,**108** (2): 167-173.

Espinel, I., Stockman, A.L., Roberts, G., Pincus, D., Pollack, J. and Marker, J. (1998).Comparison of rapid yeast plus system with API20C system for identification of common, new and emerging yeast pathogens.*Journal of Clinical Microbiology*, **36**:883 – 886.

Esser, K., Hiurich, J. and Hues, U. (1987).Genetic control of flocculation of yeast with respect to application in biotechnology.*Process Technology*, **4**:383 – 398.

Evans, I.H. (1990). Yeast strains for baking. In:Spencer, J.T.F. and Heidelberg, D.M. (eds), Yeast Technology, pp:14-54. Springer Verlag.New York.

Eyre-Walker, A. and Keightley, P. (2007). The distribution of fitness effects new mutations. Nature Review*Genetics*,**8**(8):610-618.

Fickers, P., Benetti, P. H., Wache, Y., Marty, A., Mauersherger, S., Smit, M.S. and Nicaud, J.M. (2005). Hydrophobic substrate utilisation, by the yeast *Yarrowia lipolytica*, and its potential application.*FEMS Yeast Research*,**5**(6-7): 527-543.

Fleet, G. H. and Praphailong, W. (2001). Yeasts, In: Spoilage of processed foods: Causes and Diagnosis, AIFST. South wood Press.Tallahassee,South Africa. pp. 343-397.

France, W.J., and Wragg, B.H., (1988). Up to date Bread Making.A Complete Guide to Craft. Maclarin and Sons Ltd.London. pp. 53-59.

Gervais, P. and Martinez de Maranon, I. (1995). Effects of the kinetics of temperature variation on *Saccharomyces cerevisiae* viability and permeability.*Biochemistry and Biophysiology Journal*,**1235**:52-56.

Ghorbani, F., Tounesi, H., Sari, A.E. and Najafpour, G. (2011).Cane molasses fermentation for continuous ethanol production in immobiliszed cells reactor by *Saccharomyces cerevisiae.Renew Energy*,**36**:503-509.

Gibbons, W.R. and Westby, C.A. (1986). Solid phase fermentation of fodder beets for ethanol production: effect of grind size. *Journal of Fermentation Technology*,**64**: 175-179.

Gibson, B.R., Lawrence, S.J., Leclaire, J.P., Powell, C.D. and Smart, K.A. (2007). Yeast responses to stresses associated with industrial brewery handling. Federation of European Microbiological Societies (FEMS).*Microbiology Review*, **31**: 535-569.

Gong, C.S., McCracken, L.D. and Tsao, G.T., (1981).Direct fermentation of D-xylose to ethanol by a xylose-fermenting yeast mutant, *Candida*species.XF217, *Biotechnology Letter*,**3**:245-250.

Gong, C.S., Claypool, T.A., McCracken, K.D; Mann, C.M; Ueng, P.P. and Tsao, G.T, (1983), Conversion of Pentoses by Yeast.*Biotechnology Bioengineering*, **25**: 85-104.

Gonzalez, T. A., Jubany, S., Carrau, F.M. and Gaggero, C. (2001). Differentiation of Industrial wine yeast strains using microstatellite marker. *Letters in applied Microbiology***33**(1); 71-75.

Govender, P., Domingo, J.L., Bester, M.C., Pretorius, I.S. and Bauer, F.F. (2008).Controlled expression of the dominant flocculation genes FLO1, FLO5 and FLO11 in *Saccharomyces cerevisiae*.*Journal of Applied and Environmental Microbiology*, **74**:6041-6052.

Griffin, M. A, and Gisslen, W. (2005). Professional baking (4^{th}ed). New York. John Wiley.pp 10.

Hacking, A. J. Taylor, I. W. F. and Hamas, C. M. (1984).Selecting of Yeasts able to produce Ethanol from Glucose at 40^{0C}. *Applied Microbiology and Biotechnology*,**19**: 361-363.

Henahun, S. (2009).“Complete DNA sequences of Yeast” Science up dates.Http://www.accessexcellence.org/WN/SUA07/yeast496.html. Retrived. 28/11/2009.

Hatcher, W.S., Pasrish, M.E., Weihe, J.L., Splittstoesser, D.F. and Woodward, B.B. (2000).Fruit beverages, pp.565-568. *In* Downes, F.P. and Ho, K. (ed). Methods for microbial examination of food.American Public Health Association, Washington DC.

Hernandez-Lopez, M.J., Prieto, J.A. and Randez-Gil, F. (2003).Osmotolerance and leavening ability in sweet and frozen sweet dough.Comparative analysis between *Torulaspora delbrueckii*and *Saccharomyces cerevisiae*baker's yeast strains. *Antonie Van Leuwenhoek,* **84**:125-134.

Herrera, C. and Maria, I.P. (2010). Nectar yeasts warm the flowers of a winter-blooming plant. *Proceedings of the Royal Society Biological,***277** (1689): 1827-1834.

Hidekatsu, I., Hideo, T., So-ichi, Y. and Kunio, T. (2001).Functional co between the nuclear localization of fhtlp and its flocculation and heat tolerance activities in budding yeast *Saccharomyces cerevisiae*.*Journal of Biometric science,* **8**(5): 416-420.

Higgins, V.J.M., Braidwood, P., Bissnger, I.W.D. and Attfield, P.V. (1999). Genetic evidence that high non-induced maltase and maltose permease activities determines efficiency of gas production by baker's yeast in un-sugared dough. *Applied and Environment Microbiology,* **65**:680-685.

Ho, N.W., Chen, Z. and Brainard, A.P. (1998). Genetically engineered *Saccharomyces* yeasts.Yeast capable of effective co-fermentation of glucose and Xylose. *Applied and Environmental Microbiology,***64**(5): 1852-1859.

Ho., N.W., Ladisch, M. and Tolan, J.S. (2004). Genetically engineered Saccharomyces yeasts.*Purdue News Service,* (**765**) 494 – 2096.

Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichiol, S. and Vandepol, S. (1982).Rapid evolution of RNA genomes. *Science,***215** (4540); 1577-1585.

Hurley, R., Louvois, J. D., and Mulhall, A. (1987). Yeast as Human and animal pathogens, In: Rose, A.H. and Harrison, J.S. editor. The Yeasts, Vol. 1. Academic press, Inc., New York, N.Y. pp 207-281.

Hussain, T., Salhi, O., Lematre, J. Charpentier, C, Bonali, R. (1986).Comparative studies of flocculation and deflocculating of *Saccharomyces uvarum* and *Kluyveromyces bulgaricus. Applied Microbiology Biotechnology, **23**: 269-273*

Hutkins, R.W. (2006). Microbiology and technology of fermented foods.1st edition, Blackwell Publishing.

Huxley, A. (2009). "Discourses: Biological and Geological (volume VII): yeast" (http://alephO.Charku.edu/huxtey/CE8/yeast.html).

Ibeas, J.I. and Jimenez, J. (1997). Mitochondrial DNA loss caused by ethanol in *Saccharomyces* flor yeasts. *Journal of Applied Environmental Microbiology,* **63**:7-12.

Idise, O.E., Ameh, J.B, Yakubu, S.E., Okuofu, C.A. and Ado, S.A. (2010). Determination of genetic marker of mutagenised strains of *Pseudomonas aeruginosa* and *Bacillus cereus* isolated from effluent of petroleum refinery. *Bayero Journal of Pure and Applied Sciences,***3**(1): 180-182.

Irfan, H. (2007). Sensory Techniques.18th Annual IAOM Comference (MEA District) Muscatoman. pp. 1-30.

Jain, M.R., Zinjarde, S.S., Deobagkar D.D. and Deobagkar, D.N. (2004). 2,4,6- trinitrotuene transformation by a tropical marine yeast, *Yarrowia lipolytica* NCIM 3589. *Marine Pollution Bulletin*,**49**(9-10): 783-788.

James, J. (2006). Ethanol from sugar. United States Department of Agriculture.http://www.rurdev.usda.gov/rbs/pub/sep06/ethanol.htm

James, T.C., Campbell, S., Donnelly, D. and Bond, U. (2003).Transcription profile of brewery yeast under fermentation conditions.*Journal of Applied Microbiology*, **94**:432.

Jay, J.M. (1996). Modern Food Microbiology, 5th ed. Chapman and Hall, New York.

Jean-Luc, L., Didier, M., Jean-Marie, C., and Francis, K. (2007). Bread, Beer and wine: *Saccharomyces Cerevisiae*diversity reflects human history. *Molecular Ecology*,**16**(10): 2091-2012.

Jeffries, T.W. (1984). Mutants of *Pachysolen tannophilus* showing enhanced rates of growth and ethanol fermentation from D-Xylose. *Enzyme Microbiology Technology*,**6**:254-258.

Jeffries, T.W. (1985). Emerging technology for fermenting D-xylose.*Trends Biotechnology*,**3**(8):320-325.

Jenifer, B. (1999). Fertile ground for investment New Administration Raises agriculture to number one priority. A special International Report prepared by Washington Times Advertising Department-Published on September 30.pp 6-10.

Jin, Y.L. and Speers, R.A. (1998). Flocculation of *Saccharomyces cerevisiae*constitutes a new subtelomeric gene family. *Yeast,***11**: 1001-1113.

Johnson, B.F., Walker, T., Calleja, J.B., and Seligy, V.L. (1988). Sexual co-flocculation and asexual self flocculation in budding and fission yeast: experimental establishement of fundamental difference. *Candida Journal of Microbiology*,**34**: 1105-1107.

Jonathan, D., and Errol, W.H. (2000). Factors affecting apple aroma/flavor volatile concentration: *Review, New Zealand Journal of Crop and Horticultural Science*,**28**: 155-173.

Jones, R.P., Pamment, N. and Greenfield, P.F. (1981).Alcohol fermentation by yeasts.The effect of environmental and other variables.*Process Biochemistry*, **16**(3):42-49.

Joshi, V.K., Sandhu, D. K. (2000). Quality Evaluation of Naturally Fermented Alcoholic Beverages, Microbiological Examination of Source of Fermentation and Ethanol Productivity of the Isolates.*Journal of Applied Microbiology*,**29**: 323-334

Kaplan, S.L (2006). Good Bread is Back: A contemporary History of French Bread, the way it is made, and the people who make it. durham/Londom: Duke University Press.

Klein, D, W., Lansing, M. and Harley, J. (2004).Microbiology. 6thed. New York: McGraw-Hill. pp 50-53.

Klieger, P. C. (2004). The Fleischmann yeast family (http://books.goggle.com/?id).Arcadia Publishing. pp.13Retrived21/02/2010.

Kodama, Y., Kielland Brandt, M.C. and Hansen, J. (2005).Lager Brewing Yeast.*Topics in current genetics*,**15**: 145-164.

Kollaritsch, H., Kemsner, P., Wiedermann, G. and Scheiner, O. (1989).Prevention of traveller's Diarrhea. Comparison of different non-antibiotic preparations. Travel Medicine International pp: 9-17.

Kondo, A., Shigechi, H. and Abe, M. (2002). High-level ethanol production from starch by a flocculent *Saccharomyces cerevisiae* strain displaying cell-surface glucoamylase. *Journal of Applied Microbiology and Biotechnology*, **58**:291-296.

Kosman, D.J. (2003). Molecular mechanism of iron uptake in fungi.*Journal of Molecular Microbiology*, **47**:1185-1197.

Kourkoutas Y. Kanellaki M., Koutinas, A. A., Tzia, C. (2005). Effect of fermentation conditions and immobilisation supports on the wine making *Jounal ofFoodEngineering*,**69**: 115-123.

Kunkee, R.E. (1984). Selection and modification of yeasts and lactic acid bacteria for wine fermentation.*Food Microbiology*, **1**:317 – 327.

Kurtzman, C.P. (2006). Detection, Identification and enumeration methods for spoilage yeasts. In: Blackburn, C. de.W.editor.Food spoilage Microorganisms. Cambridge, Woodhead publishing. pp. 28-54.

Kurtzman, C.P., (1994).Molecular taxonomy of the yeasts. *Yeast*,**10**(13): pp27-40.

Kurtzman, C.P., and Fell, J.W. (2005). Biodiversity and Ecophysiolology of yeasts *In*: The yeast handbook, Gabor p, de la Rosa (l.eds.) Berlin: Springer. pp 11-30.

Kurtzman, C.P., and Fell, J.W. (2006).Yeast systematic and phylogeny-implications of molecular Identification methods forstudies in ecology. (http://www.ars.usda.gov/research/publications/htm?) Retrived 07/01/2007.

Kurtzman, C.P., and Piskur, J. (2006). Taxonomy and phylogentic diversity among the yeasts (in comparative Genomics: Using Fungi as Models.) Berlin: springer. pp 29-46.

Kurugol, Z. and Koturoglu, G. (2005).Effect of *Saccharomyces boulardii* in children with acute diarrhea. *Acta of Pediatrica*,**94**:44-47.

Kutty, S.N. and Philip, R. (2008). Marine yeasts-a review. *Yeasts*,**25** (5): 465.

LagerKvist (2005). The enigma of ferment: from the philosopher's stone to the first biochemical Nobel prize. ULF.World Scientific Publishers.Singapore .pp. 7.

Lalette Richard and Horst W. Doelle (1989). Yeast (*Saccaromyces cerevisiae)*. MIRCEN-Biotechnology, Department of Microbioloy, University of Queenland, St. Lucia, 4067 Brisbane, Australia.pp 86-96.

Lee, H., James, A.P., Zahab, D.M., Mahmourides, G., Malszka, R. and Schneider, H. (1986).Mutants of *Pachysolen tannophilu*with improved production of ethanol from D-xylose.*Applied Environmental Microbiology*,**51**(6):1252-1258.

Lee, J.G. (2009). South East Asia under Japanese Occupation-Harukoe (Haruku).Children (and families) of the Far East prisoners of war. (Http://www.cofepow.org.UK/pages/asia-_haraku2.html). Retrived 28/11/2009.

Legras, J.L. Merdinoglu, D. Cornuet, J.M. and Karst, F. (2007). Bread, beer and wine: human history. *Journal of Molecular Ecology*,**16**(10): 2091-20102.

Lezinous, V., Christakopoulos, P., Li, L.W., Kekos, D., and Macris, B.J. (1995).Study of a single and mixed culture for direct bio-conversion of sorghum carbohydrates to ethanol.*Applied Microbiology Biotechnology*,**43**:412-415.

Lin, Y and Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. *Journal of Applied Microbiologyand Biotechnology*,**69**: 627-642.

Little, A. and Cameron, C. (2008). Black yeast symbionts compromise the efficiency of antibiotic defenses in fungus-growing ant *Journal of Ecology*,**89**(5): 1216-1222.

Liu S. Q. (2002). Malolactic fermentations in Wine-Beyond de-acidification. *Journal of AppliedMicrobiology*,**92**: 589-601.

Lodolo, E.J., Kock, J.L.F., Axcell, B.C. and Brooks, M. (2008).The yeast *Saccharomyces cerevisiae*, the main character in beer brewing.*FEMS Yeast Research*,**8**:1019-1036.

Lopitz, O.F., Rementeria, A., Elguezabel, N. and Garaizar, J. (2006). Kefir: A symbiotic yeast-bacteria community with alleged healthy capabilities. *Revista/bereomericantacleMicologia,* **23**: 67-74.

Loureiro, V. and Malfeito, F. M. (2003). Spoilage yeasts in the wine industry. *International Journal of Food Microbiology*,**86**(1-2):23-50.

Lucero, P., Penalver, E., Moreno, E. and Lagunas, R. (2000). Internal trehalose protects endocytosis from inhibition by ethanol in *Saccharomyces cerevisiae.Journal of Applied Environmental Microbiology*, **66**:4456-4461.

Madigan, M.T., Martinko, J.M., John, M. and Parker, J. (1996). Brock Biology of Microorganisms (http://cwx.prenhall.com/bookbind/pubbooks/boock/). 8th ed. Prentice Hall.Retrived 08/04/2011.

Mager, W.H. and Moradas, F.P. (1993). Stress response of yeast. *Biochemistry Journal*, **290**:1-13.

Maleszka; R. and Schneider, H. (1983).Concurrent production and consumption of ethanol by cultures of *Pachysolen tannnophilus growing on D-xylose.Applied EnvironmentalMicrobiology*,**44**:909-913.

Martinez de Maranon, I., Chaudanson, N., Joly, N. and Gervais, P. (1999). Slow heat rates increases yeast thermotolerance by maintaining plasma membrane integrity. *Journal of Biotechnology and Bioengineering*, **65**(2):176-181.

Martini, A. (1992). Biodiversity and conservation of Yeasts. *Biodiversity and Conservation* 1: 324-333.

Mason, R.L. and Nottingham, S.M. (2013), Sensory Evaluation Manual.www.scribd.com/doc/1348150 8/569067.Retrived 09/04/2013. pp. 1-190.

Maupas, J., Champemont, P. and Delforge, M. (1983). Treatment of irritable bowel syndrome with *Saccharomyces boulardii*: a double blind, placebo-controlled study. *Medicine Chirurgie Digestives*,**12**(1): 77-79.

McBryde, C., Gardner, J.M., de Barro Lopes, M. and Jiranek, V. (2006). Generation of Novel wine yeast strains by Adaptive Evolution. *American Journal of Enoli Vitic*,**57**: 423-430.

McFarland, L., Suramicz, C. and Greenberg, R. (1994).A randomised placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *Journal of the American MedicalAssociation*,**271** (24); 1913-1918.

McFarland, L., Suramicz, C. and Green Berg, R. (1995). Prevention of β-lactam associated diarrhea by *Saccharomyces boulardii* compared with placebo. *Journal of the AmericanMedical Association*,**90** (3); 439-448.

Meilgard, M., Civille, G.V. and Carr, B. T. (1991).Sensory Evaluation Techniques. 2ed. USA, Boca Roton, CRC Press Inc. pp. 354.

Michelle C.A. (2012). The effects of UV light on bacterial growth. www.eHow.com.Retrived 08/12/2011.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Journal of Analytical Chemistry*,**31**: 426-428.

Miller, R., Jeffery, W., Mitchell, D. and Elasri, M. (1999). Bacterial responses to ultraviolet slight, *American Society forMicrobiology*, **65**(8):535-541.

Moghaddas, J., Truant, A. L., Jordan, C. and Buckley, H. R. (1999).Evaluation of the Yeast Plus System for the Identification of YeastDiagonastic*MicrobiologyInfect*ious *Disease*,**35**:271-273.

Moore, L.E. (1996). Fundamental of fungi. Englewood Cliffs, New Jersey: Prentice Hall. pp 533-534.

Moulin, G; Boze, H. and Galzy, P. (1984). Inhibition of Alcoholic Fermentation. *Biotechnolo*gy *and Genetic Engineering Reviews*,**2**:365-382.

Morano, K.A., Liu, P.C. and Thiele, D.J. (1998). Protein chaperones and the heat shock response in *Saccharomyces cerevisiae*. *Current Opinion on Microbiology*, **1**:197-203.

Morozov, I.I., Petin, V.G. and Dubovick, B.V. (1997). Influence of tonicity and chloramphenicol on hyperthermic cytotoxicity and cell permeability under various heating rates. *International Journal on Hyperther*, **13**:49-57.

Nagodawithana, T.W. and Trivedi, N. (1990). Yeast selection for baking. In: Panchal, C.J. (ed.), Yeast strain selection. pp: 139-184. New York: Maarcel Dekker.

Najafpour, G.D., and Lim, J.K., (2002). Evaluation and isolation of ethanol producer strain SMP-6, Regional symposium on Chemical Engineering, pp 202-211.

Nancy, S. (1996). Breads from the La Brea Bakery. Villard. pp 20-21.

Neiman, A.M. (2005). Ascospore formation in yeast *Saccharomyces cerevisiae*. *Journal ofMicrobiology an*d *Molecular Biology Reviews*,**69**(4): 565-584.

Nigam, J. N. Gogoi, B.K., and Bezbarauh, R.L. (1998).Alcoholic fermentation by agar-immobilised yeast cells.*World Journal of Microbiological Biotechnology*,**14** (3): 457-162.

Oda, Y. and K. Ouchi, (1990). Role of Yeast maltose fermentation in carbon (iv) oxide production rate from sponge dough.*Food MicrobiologyJournal***, 7**:43-47.

Oda, Y. and Ouchi, K. (1991).Construction of a sucrose-fermenting baker's yeast incapable of hydrolysing frutooligosaccharides. *Enzyme Microbiology Technology*,**13**:495-498.

Okagbue, R.N. and Ekejindu, G.O.C.(1988). Leavening activity of *Candida*speciesisolated from Nigerian palm wine. Bulletin Abstract.*Biotechnology Society of Nigeria*. pp. 27-28.

Oke, T.A. and Ijebor, J.A. (1997). Comparative studies of yeast isolates of "burukutu" and palmwine table wine production using oranges. *Nigerian Journal of Biotechnology*,**4**: 60-68

Okunowo, W.O., Okotore, R.O. and Osuntoki, A.A. (2005). The fermentative efficiency of indigenous yeasts strains of different origin on orange juice. *African Journal of Biotechnology*,**4**(11):1290 – 1296.

Orebe,F.,Fagbemi,T.N. and Adeyemi,E.O.(2008).Fruit juice industry; problems and prospects.www.the nation onlineng.net/archive2/tblnews-Detail.php?id=63167.

Ostergaard, S., Olsson, L., and Neilsen, J. (2000).Metabolic engineering of *Saccharomyces cerevisiae.Microbiology and Molecular BiologyReviews*,**64** (1): 34-50.

Oswal, N., Sarma, P.M., Zinjarde, S.S. and Pant, A. (2002).Palm oil mill effluent treatment by tropical marine yeast. *Bioresources Technology*,**85** (1): 35-37.

Oyeka, C.A. and Ugwu, L.O. (2002).Fungal flora of human toe webs. *Mycoses*,**45** (11-12) 488-491.

Panchal, C. J. and Stewart, G. G. (1981).Ethanol Production by a highly Flocculent Yeast Strains. *Development in Industrial Microbiology*,**22**: 711-717

Panchal, C. J., Peacock, L. and Stewart, G. G. (1982). Increased Osmotolerance of Genetically modified Ethanol Producing Strains of *Saccharomyces species*. *Biotechnology Letter*,**4**(10): 639-644.

Parish, M. E. and Haggins, D.P. (1989).Yeast and Moulds Isolated from spoiling citrus product and by products. *Journal of Food Protein*,**52**: 261-263.

Parrotta, M.J. and Bekdash, R. (1998).UV disinfection of small ground water supplies. *Journal AWWA*, **90**(2):71-81.

Parsell, R.A., Kowal, A.S., Singer, M.A. and Lindguist, S. (1994). Protein disaggregation mediated by heat shock protein HSp104. *Journal of Nature*, **372**:475-478.

Paula, I. F. (2010). How Baking Works: Exploring The Foundamentals Of Baking Science. New York: Wiley. pp 9-11.

Pavlik, P., Simon, M. and Ruis, H. (1993). The glycerol kinase gene (GuT1) of *Saccharomyces cerevisiae*: Cloning and Characterisation. *CurrentGenetics*, **24**:21-25.

Pearson, D. (1981). Chemical Analysis of Foods 8th ed. Church hill Livingstone, New York pp. 436.

Pedersen, O., Anderson, T. and Christensen, C. (2007).Carbon (IV) oxide in planted aquaria. *TheAquatic Gardener*,**20**(3): 24-33.

Perpete, P., Santre, G., Bodart, E., and Collin, S. (2005). Uptake of Amino Acids during Beer production: The concept of a critical Time Value. *Journal ofAmerican Society ofBrewery Chemistry*,**63** (1); 23-27.

Phillips, T. (1999).Planets in Bottle: More about yeast.(http://scie nce.nasa.gov /newhome/ headline/msadibmar 99 Ib.html).

Philips, S. (2013). Yeast fermentation:yeast proofing techniques/craftbaking…Baking 911.www.craftybaking.com/how to/yeast.Retrived 09/04/2013.

Pierce, J.S. (1987). The role of nitrogen in brewing.*Journal of Institute of Brewery*, **93**:378 – 381.

Pilone, G.J. (1985). Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: Collaborative study. *Journal of Association official Analytical Chemistry*,**68***(*2): 188-190.

Piper, P.W. (1993). Molecular events associated with acquisition of heat tolerance by the yeast *Saccharomyces.*Federation of European Microbiological Societies (FEMS).*Microbiology Review*, **11**:339 – 355.

Piper, P.W. (1995). The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. Federation of European Microbiological Societies (FEMS). *Microbiology Letter*, **134**:121-127.

Piskur, J., Rozpedowska, E., Polakova, S., Merico, A and Compagno, C. (2006). How did *Saccharomyces* evolve to become a good Brewer?. *Trends in Genetics*, **22** (4).

Pitt, J.I., and Hocking,A.D**.** (1997). Fungi and Food spoilage, 2nd ed. Blackie Academic and Professional, London. pp.66-100.

Ponk,J.T.(2002).Auxanographic yeast strains in fudamental and applied reasearch.*Applied* and *Environmental Microbiology*,**68**(5):2095-2100.

Prescott, L.M., Harley, J.P. and Klein D.A. (2008).Microbiology 7th ed. McGraw-Hill, New York.pp 1088.

Purves, Williams, K., Sadava, D., Orians, and Gordon, H. (2004).Life, the Science of biology.7th edition.Macmillan Publishers.Australia. pp. 139-140.

Pyler, E.J. (1988). Baking science and Technology 3rd. Vols.I and II. Sosland Publishing Company. Kansas city.pp120-135.

Randez-Gil, F. and Sanz, P. (1994). Construction of industrial baker's yeast strains able to assimulate maltose under catabolite repression conditions. *AppliedMicrobiologyBiotechnology*. **42**:581-586.

Randez-Gil, F., Prieto, J.A. and Sanz, P. (1995). Deoxyglucose-resistant (DOG^{R1} and DOG^{R2}): two genes from *Saccharomyces cerevisiae*that confer 2-deoxyglucose resistance when over-expressed. *Yeast,***11**: 1233-1240.

Rao, A.S. and Asoke, K.G. (2006). Introduction to microbiology published by Prentice-Hall of India Private limited, New Delhi pp. 66-67.

Rao, R.S., Prakasham, R.S., Prasad, K.K., Rjesham S., Sarma, P.N. and Rao, L. (2004). Xylitol production by *Candida sp*.: parameter optimisation using Taguchi approach". *Journal ofprocess Biochemistry*,**39**: 951-956.

Razmovski, R. and Vucurovic, V. (2011). Ethanol production from sugar beet molasses by *Saccharomyces cerevisiae*entrapped in an alginate-maize stem ground tissue matrix. *Enzyme Microbiology Technology*,**48**:378-385.

Reinhart, P. (2001). The Bread Bakers Apprentice: Mastering the Art of Extraordinary Bread. Ten Speed Press, pp. 160-166.

Reinhart, P. (2009). Reinhart's Artisan Breeds Every Day. Berkeley, Calif: Ten Speed Press. pp 207 – 209. ISBN 1-58008-998-4.

Ricon, A.M., Codon, A.C., Casterejon, F and Beritez, T. (2001). Improved properties of bakers yeast mutant resistant to 2-deoxy-D-glucose. *Applied and EnvironmentalMicrobiology*. **67**: 4279-4282.

Ramachandra, H.G.R. and Arum, H. K. (2005). Spray drying of Mango juice-butter milk blends. *Journal of Diary Science*,**85**: 395-404.

Roehr, M. (2001). The biotechnology of Ethanol: Classical and future Application. Willy-VCH Verlag GumbH (ed). NewYork.pp 61-65.

Ronnow, B and Kielland-Brandt, M.C. (1993).GuT2, a gene for mitochondrial glycerol 3 – phosphate dehydrogenase of *Saccharomyces cerevisiae*.*Yeast*,**9**:121-130.

Ross, J.P. (1997). "Going wild: Wild yeast in winemaking" (http://www.findarticles .com/p/articles).Retrived 28/11/2009.

Russel, C.R. (2006). Effects of UV radiation on yeast genetics.Department of Biology, Health Sciences, Tennessee Technology University, Cookveille, Tennessee 38505.

Saddiq M., Harte J., Dolan, K., Khan, A., Collins, J.K. and Perkins Veazie, P.M. (2006).The use of Lycopene-rich Watermelon Juice or Juice Concentrate in Different Food Systems (abstract).International Food Technology Meeting Abstracts.pp.223.

Sadosky, P. and Schawrz, P.B.(2002). Effect of araboxylans, beta-glucans and dextrins on the viscosity and membrance filterability of a beer model solution. *Journal ofAmericanSociety. Brewing Chemistry*, **60**(4): 153-162.

Saint-Marc, T., Blehaut, H., Musial, C. and Touraine, J, (1995).AIDS related Diarrhea; a double-blind trial of *Saccharomyces boulardii*. Sem Hosp Pans,**7**1: 735-741.

Sandhu, D. and Manjit, W. (1985). Yeasts Associated with pollinating Bees and Flower Nactar. *Journal of Microbial Ecology*, **11**: 51-58.

Sastry, S.K., Datta, A.K. and Worobo, R.W.(2000).Ultraviolet light. *Journal of FoodSupplemen*t. **1**:90 – 92.

Sawyer, S.A., Parsch, J., Zhang, Z. and Hertl, D. L. (2007).Prevalence of positive selection among nearly neutral amino acid replacements in drosophilia.*Proceedings of National. Academic Science*, U.S.A.**104** (16); 6504-6510.

Schwan, R.F., Mendonca, A.T., da Silva, J.J. Jr., Rodrigues, V. and Wheals, A.E. (2001). Microbiology and Physiology of cachaca (aguardante) fermentations.*Anthony Van Leeuwenhoek Journal*, **79**:89-96.

Serna-Saldivar, S.O., and Rooney, L.W. (1995).Structure and chemistry of sorghum and millets. *In*: Sorghum and Millets Chemistry and Technology, D.A.V. Dendy (Ed). American Association of Cereal chemists, St. Paul, MN.

Shaheen, A., Hafeezur, R.M., Farman, A.S., Rajoka, M.I. and Suhail, A.S. (2009). Production of ethanol by indigenous wild and mutant strain of thermotolerant *Kluyveromyces marxianus.* Under optimised fermentation conditions. *Pakistan Journal of Analytical chemistry,* **10**(1&2):25-33.

Shakhashiri and Kevin, S. (2009). Ethanol the Dose, Effects, and side Effects of the World's second most popular Drug.WWW.Scifun.org.Retrived 05/02/2009.

Siegfried, A. (2009). Yeast rises to a new occasion (http://www.ag ricultue.purdue.e du/agcom m/Agcom /news/ backgrd/9808.Ho.yeast.html).Retrived 28/11/2009.

Singh, N., Pradeep, S. and Mishra, P.K (2009).Studies on Ethanol production using immobilized cells of *Kluyveromyce thermotolerans* in a packed bed reactor. *Journal of Science Industrial Research*, **68**: 617-623.

Slavikora, E and Vadkertiova, R. (2003).The diversity of yeasts in the agricultural soil. *Journalof Basic Microbiology*, **43**(5): 430-436.

Sniegowski, P., Gerrish, P., Johnson, T. and Shaver, A. (2000). The evolution of mutation rates: separating causes from consequences. *Bioessays*, **22** (12): 1057-1066.

Soares, E.V., Teixeira, J.A and Mota, M. (1994).Effects of cultural and nutritional conditions on the control of flocculation expression in *Saccharomyces cerevisiae.*Candida Journal of Microbiology, **40**: 851-857.

Speers, R. A., Wan, Y. Q., Jin, Y.L. and Stewart, R.J. (2006). Effects of fermentation parameters and cell wall properties on yeast flocculation. *Journal of Institute Brewery*,**112**: 246-254.

Spiegel-Roy, P and Goldschmidt, E.E. (1996).Biology of citrus.Cambridge University Press, Cambridge.pp.45-99.

Stan, S. (2002). On the doublet formation in the flocculation process of the yeast cells.Http://arxiv.org/ps/physics/0100602vi.

Stanley, D; Bandara, A; Fraser, S; Chambers, P.J. and Stanley, G.A. (2010). The ethanol stress response and ethanol of *Saccharomyces cerevisiae*. *Journal of Applied Microbiology* **109**:13-24.

Stanley, P. C. (2009). Stanley, P. C. and Linda, S. Y. Ed. The ICC Handbook of Cereals, Flour, Dough And Product Testing: Methods and Applications. Pennsylvania: DEStech Publications, Inc. pp 69.

Stewart, G. G., Murray, C.R., Panchal, C.J., Russel, I. and Sillis, A.M. (1984). The Selection and Modification of Brewery Yeast Strains. *Food Microbiology*,**1**: 289-302.

Stewart, G.G. (2006). Studies on the uptake and metabolism of wort sugars during Brewing fermentations. *Technical Quality Master BreweryAssociationofAmerica*, **43**: 265-269.

Stewart, G.G., Russel, J. (1995). Characterisation of affinity-purified cell wall binding proteins of *Saccharomyces Cerevisiae.*Possible role in flocculation. *Journal of American Society of Brewery Chemistry*, **53**: 111-116.

Stratford, M. (1989). Evidence for two mechanisms of flocculation in *Saccharomyces cerevisiae.Yeast*, **5**: 441-445.

Stratford, M. (1991). Yeast flocculation: reconciliation of physiological and genetic view point. *Yeast*, **8**: 25-38

Stratford, M. (1996).Induction of flocculation in brewing yeast by change pH value.*FEMS Microbiology Letters*, **136**: 13 – 18.

Strauss, C.J., Kock, J.L.F., Vanwyk, P.W.J., Viljoen, B.C., Botes, P.J. and Hgam, S. (2003). Inverse flocculation patterns in *Saccharomyces Cerevisiae* U0FSY-2330. *Journal of Institute Brewery*, **109** (1): 3-7

Straver, M. H., Klije, J. W. and Smith, G. (1993).Cause and control of flocculation in yeast. *Journal of Biotechnology*,**11**:228-232.

Stoyan, T. and Carbon, J. (2004).Inner kinetochore of the pathogenic yeast *Candida glabrata*.*Eukaryotic cell*,**3**(5):1154 – 1163.

Stryer, L. (1975). Biochemistry. W. H. Freeman and Company Limited.San Francisco.pp 116-120.

Sturley, S.L. and Young, T.W. (1986). Genetic Manipulation of commercial yeast strains *Biotechnology and Genetic engineeringReviews*,**4**:1-38.

Suh, S.O., McHugh, J.V., Pollock, D.D and Blackwell, M. (2005). The beetle gut a hyperdiverse source of novel Yeasts. *Mycological Research*,**109** (pt3): 261-259.

Supply, P., Dexaerde, A.D., Roganti, T., Goffeau, A. and Foury, F. (1995). In frame recombination between the yeast H + - ATPase isogenes Pma1 and Pma2: insights the mechanism of recombination initiated by a double stranded break. *Journal of Molecular and Cellular Biology*, **15**:5389-5395.

Takagi, H., Takaoka, M., Kawaguchi, A. and Kubo, Y. (2005).Effect of L–proline on sake brewing and ethanol stress in *Saccharomyces cerevisiae*.*Journal of Applied Environmental Microbiology*, **71**:8656-8662.

Taylor, N. W. and Orton, W. I. (1978).Aromatic compounds and sugars in flocculation of *Saccharomyces cerevisiae*.*Journal of Institute Brewery*,**84**:113-114.

Teoh, A.L., Heard, G. and Cox, J. (2004).Yeast ecology of Kombucha fermentation. *International Journal of FoodMicrobiology*,**95**(2): 119-126.

Teramoto,Y., Sato.R.and Ueda(2005).Characteristics of fermentation of yeast isolated from traditional Ethiopian honey wine ,ogol .*African Journal of Biotechnology*,**4**(2):160-163.

Teuniseen, A. and Steensma, H.Y. (1995). The dominant flocculation genes of *Saccharomyces cerevesiae* constitute a new sub-telomeric gene family. *Yeast*, **11**:1001 – 1113.

Turhollow, A.F., and Heady, E.O. (1986).Large-scale ethanol production from corn and sorghum and improving oil conversion technology.*Energy in Agriculture*,**5**:309-319.

Ulfshtahl, U. and Donalies, E.B. (2008).Food Biotechnology.Ecke Nevoight Science.Albania. pp 260-269.

Ursula, G. (1978).Principles of Genetics. 2nd ed. Publisher: Library of congress cataloging in publication data.New York . pp 174-180.

Verachtert, H. and Iserentent, D. (1995) Properties of Belgian acid beers and their microflora. The production of gueuze and related refresing acid beer.*Cerevesia*,**20** (1): 37-42.

Versele, M. and Thevelein, J.M. (2001).Lrel affects chitinase expression, trehalose accumulation and heat resistance through inhabitation of the CbKI protein Kinase in *Saccharomyces cerevisiae*. *Journal of Molecular Microbiology*, **41**:1311-1326.

Versteepen, K.J., Derdeline Kx, G. and Delvaux, F.R (2000).Late fermentation expression of FLOI in *Saccharomyces cerevisiae.Journalof American Society BreweryChemistry*, **59** (2): 69-7b.

Voet, D. and Voet, and Judith, G. (1995).Biochemistry (2nded.) New York. NY: John Wiley and Sons.pp.44-98.

Walker, K., Skelton, H., and Smith, K. (2002).Cutaneous lesions showing giant yeast forms of *Blastomyces dermatitidis*. *Journal of Cutaneous pathology*,**29**(10): 616-618.

Wang, H.L., Swain, E. W., and Hesseltine, C.W. (1980). Phytase of Molds Used in Oriental Food Fermentation. *Journal of Food Science*,**45**: 1262.

Watari, J., Kudo, M., Nishikawa, N. and Kamimura, M. (1990).Construction of flocculant yeast cells (*Saccharomyces cerevisiae*) by mating or protoplast fusion using a yeast cell containing the flocculation genes FLO5.*Journal of Agricultural Biology and Chemistry*, **54**:1677-1681.

Weigel, J. (1980). Formation of ethanol by bacteria.A pledge for the use of extreme thermophilic anaerobic bacteria in industrial ethanol fermentation process.*Experientia*, **36:**1434-1446.

Westerheide, S.D. and Morimoto, R.I. (2005).Heat shock response modulators as therapeutic tools for disease of protein confirmation.*Journal of Biological Chemistry*, **280**:33097-33100.

Williams, N. (1996). Genome project: Yeast genome sequence ferments new research.*Science*,**272** (5261): 481.

Wongkhalaung, C. and Boonyaratanakornkit, M. (2007).Characterisation of New Baker's Yeast strains and their Leavening Ability in Bread Dough. *Kasetsar*t *JournalofNature Science,**41**:751-763.*

Wood, V., Givilliam, R. and Rojadream, M.A. (2002). The genome sequence *Schizosaccharomyces pombe.Journal of Nature science,***415**(6878): 871-880.

Yamada, R.Y., Bito, T., Adachi, T., Tanako, T. and Ogino, C. (2009). Efficient production of ethanol from raw starch by mated diploid *Saccharomyces cerevisiae*with integrated α-amylase and glucoamylase genes. *Enzymes MicrobiologyTechnology***44**:344-349.

Yeong, F. M. (2005). Severing all ties between mother and daughter: cell separation in budding yeast. *Journal of Molecular Microbiology,***55(**5): 1325-1331.

You, K.M., Rosenfield, C.L. and Knipple, D.C. (2003). Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. *Journal of Applied Environmental Microbiology,* **69**:1499-1503.

Yun, C.W., Bauler, M., Moore, R.E., Klebba, P.E. and Philpott, C.C. (2001).The role of FRE family of plasma membrane reductase in the uptake of siderophore iron in *Saccharomyces cerevisiae*.*Journal of Biological Advances*, **3**:294-306.

APPENDICES

Appendix I: Identity of Yeasts Isolated From Fresh and Spoilt Single Strength Orange Juice.

S/NO	SAMPLE CODE	IDENTIFICATION CODE	INFERENCE/IDENTITY GENUS	SPECIES
1	7F9	0000004	*Geotrichum*	*G.capitatum*
2	Ds10	0000004	*Geotrichum*	*G.capitatum*
3	2F36	6154170	*Kodamaea*	*K.ohmeri*
4	7F8	2000004	*Candida*	*C.norvegensis*
5	2F9	6666175	*Candida*	*C.parapsilosis*
A	2F37	6650175	*Candida*	*C.parapsilosis*
7	7F40	6342175	*Candida*	*C.parapsilosis*
8	2F28	6700165	*Rhodotorula*	*Rhodo.minuta*
9	2F6	6670165	*Rhodotorula*	*Rhodo.minuta*
10	7F1	6700165	*Rhodotorula*	*Rhodo.minuta*
11	7F19	6700165	*Rhodotorula*	*Rhodo.minuta*
12	7F35	6700140	*Rhodotorula*	*Rhodo.minuta*
13	7F41	6700120	*Rhodotorula*	*Rhodo.minuta*
14	7F47	6700140	*Rhodotorula*	*Rhodo.minuta*
15	DS2	6700120	*Rhodotorula*	*Rhodo.minuta*
16	DS6	6710121	*Rhodotorula*	*Rhodo.minuta*
17	DS24	6700120	*Rhodotorula*	*Rhodo.minuta*
18	2F4	6100I04	*Candida*	*C.zeylanoides*
19	2F5	6100I04	*Candida*	*C.zeylanoides*
20	DS14	6100100	*Candida*	*C.zeylanoides*
21	DS20	6100I00	*Candida*	*C.zeylanoides*
22	2F3	6000104	*Candida*	*C.krusei/inconspicua*
23	2F7	6000104	*Candida*	*C.krusei/inconspicua*
24	2F8	6000104	*Candida*	*C.krusei/inconspicua*
25	2F20	2000104	*Candida*	*C.krusei/inconspicua*
26	2F21	6000104	*Candida*	*C.krusei/inconspicua*
27	2F24	6000104	*Candida*	*C.krusei/inconspicua*
28	2F31	60001O4	*Candida*	*C.krusei/inconspicua*
29	2F34	6000104	*Candida*	*C.krusei/inconspicua*
30	7F3	6000104	*Candida*	*C.krusei/inconspicua*
31	7F10	6000104	*Candida*	*C.krusei/inconspicua*
32	7F6	6000104	*Candida*	*C.krusei/inconspicua*
33	7F12	6000104	*Candida*	*C.krusei/inconspicua*
34	7F14	6000104	*Candida*	*C.krusei/inconspicua*
35	7F15	6000104	*Candida*	*C.krusei/inconspicua*
36	7F18	6000104	*Candida*	*C.krusei/inconspicua*
37	7F39	6000104	*Candida*	*C.krusei/inconspicua*
38	DS1	6000104	*Candida*	*C.krusei/inconspicua*

39	DS3	6000104	*Candida*	*C.krusei/inconspicua*
40	DS4	6000104	*Candida*	*C.krusei/inconspicua*
41	DS7	6000104	*Candida*	*C.krusei/inconspicua*
42	DS8	6000104	*Candida*	*C.krusei/inconspicua*
43	DS9	6000104	*Candida*	*C.krusei/inconspicua*
44	DS11	6000104	*Candida*	*C.krusei/inconspicua*
45	DS12	6000104	*Candida*	*C.krusei/inconspicua*
46	DS16	6000104	*Candida*	*C.krusei/inconspicua*
47	DS18	6000104	*Candida*	*C.krusei/inconspicua*
48	DS19	6000104	*Candida*	*C.krusei/inconspicua*
49	DS23	6000104	*Candida*	*C.krusei/inconspicua*
50	DS30	6000104	*Candida*	*C.krusei/inconspicua*
51	2F2	6574175	*Candida*	*C. lustianiae*

Footnote

1. Fresh samples = 2F and 7F.
2. Defective samples = DS
3. Number of identified organisms

- *Geotrichum* = 2
- *Kodamaea* = 1
- *Rhodotorula* =10
- *Candida* = 38

a. *C.norvegensis* =1
b. *C.parapsilosis* =3
c. *C.krusei*/*inconspicua* =29
d. *C. zeylanoides* =4
e. *C lustianiea* = 1

Appendix **II: Isolation and Prevalence Rate of Yeast Strains Isolated From Fresh and Spoilt Single Strength Orange Juice**

Yeast strains	**Isolation rate**	**Prevalence rate(%)**
Rhodotorula minuta	10	19.60
Candida krusei	29	56.86
Kodamaea ohmeri	1	1.96
Candida zeylanoides	4	7.84
Candida lusitaniae	1	1.96
Candida parapsilosis	3	5.88
Candida norvegensis	1	1.96
Geotrichum capitatum	2	3.92

Appendix III: <u>Panel Rating Score Sheet, Bread</u>

DATE _____ SAMPLE CODE _____

Please score by putting a tick (√) in the appropriate box, the impression which you think best describes the sample.

Quality parameters	Descriptive terms for different score				
	1	2	3	4	5
Texture feel	Smooth	Rough	Coarse	Fine	Crispy
Appearance colour	Dark brown	White	Dark	Creamy	Brown
Odour/Smell Bread	Nil	Slight	Moderate	Strong	Very strong
Personal preference (Acceptability)	Bad	Poor	Average	Good	Excellent

Comment on sample _____

Name of panelist _____ Signature _____

Appendix IVa: Effects of Irradiation of Yeast from Fresh and Spoilt Single Strength Orange Juice (Fermented) with UV Light at 4 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 4mins	Reduction VC (CFU/ml)	% Kill	% Survival
Candidakrusei/inconspicua	2.3×10^7	1.38×10^9	0.92×10^9	40	60
C. norvegensis	2.1×10^9	1.29×10^9	0.8×10^9	38.57	61.43
C. lusitaniae	1.81×10^9	9.0×10^8	0.91×10^9	50.28	49.72
C. parapsilosis	2.44×10^9	1.30×10^9	1.14×10^9	46.72	53.28
C. zeylanoides	2.2×10^9	1.32×10^9	0.88×10^9	40	60
G. capitatum	2.23×10^9	1.41×10^9	0.82×10^9	36.77	63.23
K. ohmeri	2.2×10^9	1.12×10^9	1.08×10^9	49	51.0
Rhodo. Minuta	2.15×10^9	1.8×10^9	0.35×10^9	16.28	83.72

KEY: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix IVb: Effects of Irradiation of Yeast Strains with UV Light at 6 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 6mins	Reduction VC (CFU/ml)	% Kill	% Survival
Candida krusei	**2.3×10^9**	**1.13×10^9**	**1.17×10^9**	**50.87**	**49.13**
C. lusitaniae	**1.81×10^9**	**8.3×10^8**	**0.98×10^9**	**54.15**	**45.85**
C. norvegensis	**2.1×10^9**	**9.5×10^8**	**1.15×10^9**	**54.76**	**45.24**
C. parapsilosis	**2.44×10^9**	**1.25×10^9**	**1.19×10^9**	**48.77**	**51.23**
C. zeylanoides	**2.2×10^9**	**1.13×10^9**	**1.07×10^9**	**48.64**	**51.36**
G. capitatum	**2.23×10^9**	**1.39×10^9**	**0.84×10^9**	**37.67**	**62.33**
K. ohmeri	**2.2×10^9**	**1.0×10^9**	**1.2×10^9**	**54.55**	**45.45**
Rhodo. minuta	**2.15×10^9**	**1.65×10^9**	**0.5×10^9**	**23.25**	**76.75**

Key: VC= Viable count (CFU/ml), Values are means of duplicate count.

Appendix IVc: Effects of Irradiation of Yeast Strains with UV Light at 10 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 10mins	Reduction VC(CFU/ml)	% Kill	% Survival
Candida krusei	2.3×10^9	1.1×10^9	1.2×10^9	52.17	47.83
C. lusitaniae	1.81×10^9	1.10×10^9	0.71×10^9	39.22	60.78
C. parasilosis	2.44×10^9	7.8×10^8	1.66×10^9	68.03	31.97
C. zeylanoides	2.2×10^9	1.11×10^9	1.09×10^9	49.55	50.45
G. capitatum	2.23×10^9	1.36×10^9	1.87×10^9	39.02	60.98
K. ohmeri	2.2×10^9	1.0×10^9	1.2×10^9	54.55	45.45
C. norvegensis	2.1×10^9	1.36×10^9	0.74×10^9	35.23	64.77
Rhodo. minuta	2.15×10^9	1.27×10^9	0.88×10^9	40.93	59.07

Key: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix IVd: Effects of Irradiation of Yeast Strains with UV Light at 15 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 15mins	Reduction VC (CFU/ml)	% Kill	% Survival
CandidaKrusei	2.3×10^9	9.0×10^8	1.4×10^9	50.28	49.72
C. lusitaniae	1.81×10^9	5.5×10^8	1.26×10^9	69.62	30.38
C. norvegensis	2.1×10^9	8.7×10^8	1.23×10^9	58.57	41.43
C. parapsilosis	2.44×10^9	6.0×10^8	1.84×10^9	75.41	24.59
C. zeylanoides	2.2×10^9	9.2×10^8	1.28×10^9	58.18	41.82
G. capitatum	2.23×10^9	1.29×10^9	0.94×10^9	42.16	57.84
K. ohmeri	2.2×10^9	1.0×10^9	1.2×10^9	54.55	45.45
Rhodo. minuta	2.15×10^9	1.65×10^9	1.1×10^9	51.17	48.83

Key: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix IVe: Effects of Irradiation of Yeast Strains with UV Light at 20 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 20mins	Reduction VC (CFU/ml)	% Kill	% Survival
Candida krusei	2.3 X 10^9	7.4 X 10^8	1.6 X 10^9	67.83	32.17
C. lusitaniae	1.81 X 10^9	4.8 X 10^8	1.33 X 10^9	73.48	26.52
C. norvegensis	2.1 X 10^9	6.9 X 10^8	1.41 X 10^9	67.14	32.86
C. parapsilosis	2.44 X 10^9	5.3 X 10^8	0.91 X 10^9	78.28	21.72
C. zeylanoides	2.2 X 10^9	8.9 X 10^8	1.31 X 10^9	59.55	40.45
G. capitatum	2.23 X 10^9	7.9 X 10^8	1.44 X 10^9	64.57	35.43
K. ohmeri	2.2 X 10^9	1.0 X 10^9	1.2 X 10^9	54.55	45.45
Rhodo. minuta	2.15 X 10^9	5.8 X 10^8	1.6 X 10^9	73.02	26.98

Key: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix IVf: Effects of Irradiation of Yeast Strains with UV Light at 25 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 25mins	Reduction VC (CFU/ml)	% Kill	% Survival
Candida krusei	2.3×10^9	4.6×10^8	1.84×10^9	80	20.0
C. lusitaniae	1.81×10^9	3.1×10^8	1.5×10^9	94.48	5.52
C. norvegensis	2.1×10^9	5.5×10^8	1.6×10^9	73.81	26.19
C. parapsilosis	2.44×10^9	4.0×10^8	2.04×10^9	95.91	4.09
C. zeylanoides	2.2×10^9	8.3×10^8	1.4×10^9	62.27	37.73
G. capitatum	2.23×10^9	6.8×10^8	1.6×10^9	69.51	30.49
K. ohmeri	2.2×10^9	8.0×10^8	1.4×10^9	63.64	36.36
Rhodo. minuta	2.15×10^9	4.0×10^8	1.8×10^9	81.4	18.60

Key: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix IVg: Effects of Irradiation of Yeast Strains with UV Lightat 30 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 30mins	Reduction VC (CFU/ml)	% Kill	% Survival
Candida krusei	2.3 X 10^9	3.6 X 10^8	1.94 X 10^9	84.35	15.65
C. lusitaniae	1.81 X 10^9	2.2 X 10^8	1.59 X 10^9	87.85	12.15
C. norvegensis	2.1 X 10^9	5.3 X 10^8	1.57 X 10^9	74.77	25.23
C. parapsilosis	2.44 X 10^9	3.1 X 10^8	2.13 X 10^9	87.3	12.70
C. zeylanoides	2.2 X 10^9	5.3 X 10^8	1.67 X 10^9	75.91	24.09
G. capitatum	2.23 X 10^9	6.3 X 10^8	1.6 X 10^9	71.75	28.25
K. ohmeri	2.2 X 10^9	7.0 X 10^8	1.5 X 10^9	68.18	31.82
Rhodo. minuta	2.15 X 10^9	2.8 X 10^8	1.9 X 10^9	86.98	13.02

Key: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix V: Effects of the Treatment of Yeast Strains with Nitrous Acid at 20 Minutes.

Yeast strains	Initial VC (CFU/ml)	VC with 20mins treatment	Reduction VC (CFU/ml)	% Kill	% Survival
Candida krusei	2.3×10^9	2.05×10^7	2.1×10^9	96.13	3.87
C. lusitaniae	1.81×10^9	1.5×10^7	1.66×10^9	99.17	0.83
C. norvegensis	2.1×10^9	2.0×10^7	1.9×10^9	99.05	0.95
C. parapsilosis	2.44×10^9	2.0×10^7	2.1×10^9	99.19	0.81
C. zeylanoides	2.2×10^9	2.0×10^7	2.0×10^9	99.09	0.91
G. capitatum	2.23×10^9	1.90×10^7	2.04×10^9	99.14	0.86
K. ohmeri	2.2×10^9	2.0×10^7	2.0×10^9	99.09	0.91
Rhodo. minuta	2.15×10^9	1.80×10^7	1.97×10^9	99.16	0.84

Key: VC= Viable count (CFU/ml), values are means of duplicate count.

Appendix VI: Fermentation of 5% Sorghum Starch with Wild And Brewing Yeast Strains for Ethanol Production (PH 6.5).

	CDW(g/100ml)/isolates						RS(g/100ml)/isolates						CND(cells/l00ml)/isolates						ETY%(v/v)/isolates					
FMT (Days)	**INS**	**2F36**	**7F8**	**7F9**	**DS6**	**DS20**	**2F36**	**7F8**	**7F9**	**DS6**	**DS20**	**INS**	**2F36**	**7F8**	**7F9**	**DS6**	**DS20**	**INS**	**2F36**	**7F8**	**7F9**	**DS6**	**DS20**	**INS**
1	0.06	0.12	0.12	0.06	0.14	0.06	1.02	1.16	1.18	0.73	1.50	1.25	0.17	0.17	0.21	0.20	0.16	0.26	2.25	3.40	3.75	9.09	3.75	6.82
2	0.26	0.18	0.20	0.12	0.16	0.12	0.68	0.96	0.81	0.71	1.20	1.12	0.23	0.27	0.23	0.40	0.39	0.61	2.27	3.40	5.00	7.95	5.00	6.82
3	0.26	0.24	0.24	0.14	0.20	0.14	0.60	0.94	0.59	0.57	0.45	0.55	0.47	0.26	0.37	0.31	0.50	0.64	7.95	6.82	8.33	5.68	8.33	7.95
4	0.48	0.28	0.28	0.22	0.30	0.22	0.57	0.94	0.51	0.51	0.38	0.53	0.22	0.20	0.18	0.25	0.41	0.41	9.09	7.95	9.09	7.95	9.09	17.05
5	1.18	0.32	0.36	0.22	0.32	0.22	0.51	0.39	0.38	0.39	0.35	0.45	0.21	0.19	0.14	0.24	0.24	0.22	17.05	13.63	9.09	11.36	9/09	18.18
6	0.34	0.40	0.42	0.36	0.40	0.36	0.38	0.26	0.38	0.26	0.33	0.41	0.21	0.16	0.12	0.18	0.19	0.20	13.89	14.78	9.09	5.68	9.09	7.95
7	0.38	0.65	0.57	0.49	0.48	0.49	0.38	0.22	0.26	0.23	0.30	0.37	0.19	0.15	0.08	0.10	0.15	0.17	3.91	14.06	10.93	4.00	10.93	4.64

Results are means of triplicate determination

INS = *Saccharomyces carlbergensis*

2F36 = *Kodam aeaohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichumscapitatum*

DS6 = *Rhodotorulaminuta*

DS20 = *Candida zeylanoides*

FMT = Fermentation time (days)

CDW = Cell dry weight

RS = Reducing sugar

CND = Cell number determination

ETY = Ethanol yield.

Appendix VII: Fermentation of 5% Sorghum Starch with Mutant (nitrous acid treated) and Brewing Yeast Strainsfor Ethanol Production at 20 Minutes.

FMT		CDW(g/100ml)/isolates						CND(cells/100ml)/isolates						RS(g/100ml)isolates						ETY%(v/v)/isolates					
(days)	pH	INS	2F36	7F8	7F9	DS6	DS20	INS	2F36	7F8	7F9	DS6	DS20	INS	2F36	7F8	7F9	DS6	DS20	INS	2F36	7F8	7F9	DS6	DS20
1	6.5	0.06	0.12	0.12	0.20	0.10	0.10	0.26	0.17	0.14	0.15	0.16	0.12	1.25	1.36	0.63	0.68	1.48	1.01	6.82	1.14	4.55	6.82	1.14	4.55
2	6.5	0.26	0.20	0.20	0.26	0.18	0.30	0.61	0.17	0.16	0.30	0.63	0.42	1.12	1.34	0.62	0.62	0.93	0.89	6.82	4.55	11.36	9.09	5.68	6.82
3	6.5	0.26	0.28	0.28	0.26	0.20	0.42	0.64	0.47	0.19	0.37	0.33	0.50	0.55	0.80	0.52	0.59	0.62	0.86	7.95	4.55	14.78	13.64	6.82	7.95
4	6.5	0.48	0.40	0.34	0.44	0.34	0.44	0.41	0.35	0.32	0.31	0.31	0.42	0.53	0.77	0.41	0.55	0.42	0.77	17.05	13.64	13.64	14.78	6.82	6.82
5	6.5	1.18	0.42	0.40	0.56	0.44	0.50	0.22	0.26	0.24	0.30	0.29	0.31	0.45	0.71	0.35	0.54	0.41	0.43	18.18	17.05	13.64	17.05	9.09	14.78
6	6.5	0.34	0.44	0.42	0.54	0.44	0.58	0.20	0.21	0.20	0.23	0.29	0.20	0.41	0.50	0.26	0.44	0.41	0.38	7.95	14.06	15.91	14.06	13.64	14.78
7	6.5	0.38	0.50	0.44	0.81	0.57	0.62	0.17	0.17	0.14	0.19	0.16	0.20	0.37	0.19	0.17	0.25	0.29	0.28	4.64	11.76	16.16	11.36	16.40	12.50

Results are means of triplicate determination

INS = *Saccharomyces carlbergensis*

2F36 = *Kodameaohmeri*

7F8 = *Candida norvegensis*

7F9 = *Geotrichumcapitatum*

DS6 = *Rhodotorulaminuta*

DS20 = *Candida zeylanoides*

FMT = Fermentation time (days)

CDW = Cell dry weight

RS = Reducing sugar

CND = Cell number determination

ETY = Ethanol yield.

Appendix VIII: Assessment of Leavening Capacities of Wild and Baker's Yeast Strains in Sweetened Dough

Yeast strain	Dough volume (ml) at Diffirnet Incubation Times (mins)								
	0mins	**30mins**	**60mins**	**90mins**	**120mins**	**150mins**	**180mins**	**24hrs**	**48hrs**
S. cerevisiae (instant yeast)	98	165	245	280	280	280	280	165	130
C. norvegensis	86	86	86	86	86	86	86	100	200
C. zeylanoides	84	84	84	84	84	84	84	135	152
G. capitatum	90	90	90	90	90	90	90	122	150
K. ohmeri	82	82	82	82	82	82	82	160	275
Rhodo. Minuta	75	75	75	75	75	75	75	80	102

Values are means of duplicate readings.

Appendix IX: Assessment of Leavening Capacities of Mutant Yeast Strains (nitrous acid) Treatment in Sweetened Dough

Yeast strain	Dough volume (ml) at Diffirnet Incubation Times (mins)								
	0mins	**30mins**	**60mins**	**90mins**	**120mins**	**150mins**	**180mins**	**24hrs**	**48hrs**
NAM7F8	90	90	90	90	90	90	90	260	275
NAMDS20	84	84	84	84	84	84	84	95	155
NAM7F9	90	90	90	90	90	90	90	130	181
NAM2F36	90	90	90	90	90	90	90	100	160
NAMDS6	92	92	92	92	92	92	92	99	137

Values are means of duplicate readings

Appendix X: Assessment of Leavening Capacities of Wild and Baker's Yeast Strains in Unsweetened Dough

Yeast strain	Dough volume (ml) at Different Incubation Times (mins)								
	0mins	**30mins**	**60mins**	**90mins**	**120mins**	**150mins**	**180mins**	**24hrs**	**48hrs**
C. norvegensis	74	74	74	74	74	74	74	160	120
C. zeylanoides	77	77	77	77	77	77	77	185	130
G. capitatum	75	75	75	75	75	75	75	159	130
K. ohmeri	75	75	75	75	75	75	75	198	220
Rhodo. minuta	71	71	71	71	71	71	71	179	184
Saccharomyces cerevisiae*	78	148	210	248	248	248	248	230	140

Values are means of duplicate readings. *Control

Appendix XI: Assessment of Leavening Capacities of Mutant Yeast Strains (nitrous acid) Treatment in Unsweetened Dough

Yeast strain	Dough volume (ml) at Different Incubation Time								
	0mins	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**	**24hrs**	**48hrs**
NAM7F8	72	72	72	72	72	72	72	92	122
NAMDS20	70	71	72	72	72	72	72	111	131
NAM7F9	73	73	73	73	73	73	73	124	140
NAM2F36	70	72	74	74	74	74	74	108	150
NAMDS6	73	73	73	73	73	73	73	159	130
****Control***	98	165	245	280	280	280	280	165	130

Values are means of duplicate readings.

**Saccharomyces cerevisiae*

Appendix XII: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Baker's Yeast.

Time minutes/hours	Dough type / Baker's yeast (IDY)	Mean ± SEM	t	p-value
0 mins	Sweetened Unsweetened	98.00 ± 2.00 78.00 ± 5.00	3.714	0.065
30 mins	Sweetened Unsweetened	165.00 ± 35.00 198.00 ± 1.00	-0.942	0.445
60 mins	Sweetened Unsweetened	245.00 ± 45.00 210.00 ± 10.00	0.759	0.527
90 mins	Sweetened Unsweetened	280.00 ± 5.00 248.00 ± 40.00	0.794	0.511
120 mins	Sweetened Unsweetened	280.00 ± 5.00 248.00 ± 40.00	0.794	0.511
150 mins	Sweetened Unsweetened	280.00 ± 5.00 248.00 ± 40.00	0.794	0.511
180 mins	Sweetened Unsweetened	280.00 ± 5.00 248.00 ± 40.00	0.794	0.511
24 hours	Sweetened Unsweetened	165.00 ± 0.00 230.00 ± 0.00		
48 hours	Sweetened Unsweetened	130.00 ± 0.00 140.00 ± 0.00		

Statistics

$P \geq 0.05$ = No significant difference

-t cannot be computed because the standard deviations of both groups are 0.

IDY = Instant dried yeast

Appendix XIII: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Wild Yeasts

Time minutes/hours	Dough type /Organism	Mean ± SEM	t	P-value
0 – 180 mins	Sweetened *Candida norvegensis* Unsweetened	86.00 ± 14.00 79.00 ± 5.00	0.471	0.684
24 hours	Sweetened *Candida norvegensis* Unsweetened	100.00 ± 4.00 160.00 ± 5.00	-9.370	0.013
48 hours	Sweetened *Candida norvegensis* Unsweetened	200.00 ± 56.00 120.00 ± 20.00	1.345	0.311
0 – 180 mins	Sweetened *Candida zeylanoides* Unsweetened	84.00 ± 1.00 77.00 ± 10.00	0.697	0.558
24 hours	Sweetened *Candida zeylanoides* Unsweetened	135.00 ± 45.00 185.00 ± 5.00	-1.104	0.385
48 hours	Sweetened *Candida zeylanoides* Unsweetened	152.00 ± 52.00 130.00 ± 30.00	0.366	0.749
0-180 mins	Sweetened *Geotrichum capitatum* Unsweetened	90.00 ± 8.00 75.00 ± 5.00	1.590	0.253
24 hours	Sweetened *Geotrichum capitatum* Unsweetened	122.00 ± 28.00 159.00 ± 10.00	-1.224	0.339

48 hours	Sweetened *Geotrichum capitatum* Unsweetened	149.50 ± 35.50 130.00 ± 30.00	0.420	0.719
0-180 mins	Sweetened *Kodamaea ohmeri* Unsweetened	82.00 ± 2.00 75.00 ± 5.00	1.300	0.323
24 hours	Sweetened *Kodamaea ohmeri* Unsweetened	160.00 ± 5.00 198.00 ± 8.00	-4.028	0.056
48 hours	Sweetened *Kodamaea ohmeri* Unsweetened	270.00 ± 14.50 220.00 ± 20.00	2.044	0.178
0-180 mins	Sweetened *Rhodotorula minuta* Unsweetened	75.00 ± 5.00 71.00 ± 1.00	0.784	0.515
24 hours	Sweetened *Rhodotorula minuta* Unsweetened	80.00 ± 0.00 178.50 ± 6.50	-15.154	0.004
48 hours	Sweetened *Rhodotorula minuta* Unsweetened	106.00 ± 4.00 184.00 ± 6.00	-10.817	0.008

Statistics: $P > 0.05$ = No significant difference
$P \leq 0.05$ = Significant difference exist.

Appendix XIV: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Mutants Yeast Strains of Nitrous Acid Treatment

Time minutes/hours	Dough type /Organisms	Mean ± SEM	t-value	P-value
0 – 180 mins	Sweetened *Candida norvegensis* Unsweetened	90.00 ± 5.00 72.00 ± 2.00	3.343	0.079
24 hours	Sweetened *Candida norvegensis* Unsweetened	260.00 ± 20.00 92.00 ± 0.00	8.400	0.014
48 hours	Sweetened *Candida norvegensis* Unsweetened	275.00 ± 10.00 122.00 ± 0.00	15.300	0.004
0 mins	Sweetened *Candida zeylanoides* Unsweetened	84.00 ± 1.00 70.00 ± 1.00	9.899	0.010
30 mins	Sweetened *Candida zeylanoides* Unsweetened	84.00 ± 1.00 71.00 ± 0.00	13.000	0.006
60 – 180 mins	Sweetened *Candida zeylanoides* Unsweetened	84.00 ± 1.00 71.50 ± 0.50	11.180	0.008
24 hours	Sweetened Candida zeylanoides Unsweetened	97.50 ± 1.50 111.00 ± 17.00	-791	0.512
48 hours	Sweetened *Candida zeylanoides* Unsweetened	155.00 ± 4.00 131.00 ± 11.00	2.050	0.177
0-180 mins	Sweetened *Geotrichum capitatum* Unsweetened	90.00 ± 0.00 73.00 ± 1.00	17.000	0.003
24 hours	Sweetened *Geotrichum capitatum* Unsweetened	130.00 ± 20.00 124.00 ± 26.00	0.183	0.872
48 hours	Sweetened *Geotrichum capitatum* Unsweetened	181.00 ± 11.00 140.00 ± 2.00	3.667	0.067
0 mins	Sweetened *Kodamaea ohmeri* Unsweetened	90.00 ± 5.00 70.00 ± 0.00	4.000	0.057
30 mins	Sweetened *Kodamaea ohmeri* Unsweetened	90.00 ± 5.00 70.00 ± 0.00	3.600	0.067
60-180 mins	Sweetened *Kodamaea ohmeri* Unsweetened	90.00 ± 5.00 72.00 ± 0.00	3.200	0.085

24 hours	Sweetened *Kodamaea ohmeri* Unsweetened	100.00 ± 2.00 108.00 ± 0.00	-4.000	0.057
48 hours	Sweetened *Kodamaea ohmeri* Unsweetened	160.00 ± 20.00 150.00 ± 0.00	0.500	0.667
0-180 mins	Sweetened *Rhodotorula minuta* Unsweetened	92.00 ± 2.00 73.00 ± 5.00	3.528	0.072
24 hours	Sweetened *Rhodotorula minuta* Unsweetened	99.00 ± 1.00 159.00 ± 1.00	-42.426	0.001
48 hours	Sweetened *Rhodotorula minuta* Unsweetened	137.00 ± 3.00 130.00 ± 10.00	0.670	0.572

Statistics: $P \leq 0.05$ = Significant difference exist.

$P \geq 0.05$ = No Significant difference exist.

Appendix XV: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Mutant Yeast Strains (UV Light- Irradiated) Candidanorvegensis.

Time (mins/hours)	Dough type	Mean ± SEM	T	P – value
0mins	Sweetened	67.29 ± 2.23	- 2.596	0.034
	Unsweetened	73.43 ± 0.78		
30mins	Sweetened	70.71 ± 1.32	- 1.767	0.103
	Unsweetened	73.43 ± 0.78		
60mins	Sweetened	72.14 ± 1.03	- 0.992	0.341
	Unsweetened	73.43 ± 0.78		
90mins	Sweetened	72.14 ± 1.03	0.992	0.341
	Unsweetened	73.43 ± 0.78		
120mins	Sweetened	72.14 ± 1.03	0.992	0.341
	Unsweetened	73.43 ± 0.78		
150mins	Sweetened	76.43 ± 1.67	1.623	0.341
	Unsweetened	73.43 ± 0.78		
180mins	Sweetened	76.43 ± 1.67	1.623	0.141
	Unsweetened	73.43 ± 0.78		
24hrs	Sweetened	76.71 ± 1.686	-3.661	0.008
	Unsweetened	99.14 ± 5.89		
48hrs	Sweetened	82.00 ± 2.28	-2.144	0.073
	Unsweetened	113.00 ± 14.28		

$P \geq 0.05$ (0 – 180mins), No significant difference exist.

$P \leq 0.05$ (24 – 48hrs), significant difference exist.

Appendix XV Continued: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Mutant Yeast Strains (UV Light- Irradiated) ***Candida zeylanoides.***

Time (mins/hours)	Dough type	Mean ± SEM	T	P – value
0mins	Sweetened	76.86 ± 1.100	3.012	0.011
	Unsweetened	73.00 ± 0.655		
30mins	Sweetened	76.86 ± 1.00	3.012	0.011
	Unsweetened	73.00 ± 0.655		
60mins	Sweetened	76.86 ± 1.100	3.012	0.011
	Unsweetened	73.00 ± 0.655		
90mins	Sweetened	76.86 ± 1.100	3.012	0.011
	Unsweetened	73.00 ± 0.655		
120mins	Sweetened	76.86 ± 1.100	3.012	0.011
	Unsweetened	73.00 ± 0.655		
150mins	Sweetened	76.86 ± 1.100	3.012	0.011
	Unsweetened	73.00 ± 0.655		
180mins	Sweetened	76.86 ± 1.100	3.012	0.011
	Unsweetened	73.00 ± 0.655		
24hrs	Sweetened	77.43 ± 1.020	-4.674	0.003
	Unsweetened	114.43 ± 7.849		
48hrs	Sweetened	78.86 ± 1.033	-5.765	0.001
	Unsweetened	145.00 ± 11.427		

$P \geq 0.05$ (0 – 180mins), No significant difference exist.
$P \leq 0.05$ (24 – 48hrs), significant difference exist.

Appendix XV Continued: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Mutant Yeast Strains (UV Light- Irradiated) *Geotrichum capitatum*

Time (mins/hours)	Dough type	Mean ± SEM	T	P – value
0mins	Sweetened	73.29 ± 3.364	0.501	0.633
	Unsweetened	71.57 ±0.612		
30mins	Sweetened	73.29 ±3.364	0.501	0.633
	Unsweetened	71.57 ± 0.612		
60mins	Sweetened	74.14 ± 3.240	0.780	0.463
	Unsweetened	71.57 ± 0.612		
90mins	Sweetened	74.14 ± 3.240	0.780	0.463
	Unsweetened	71.57 ± 0.612		
120mins	Sweetened	74.43 ± 3.131	0.896	0.403
	Unsweetened	71.57 ± 0.612		
150mins	Sweetened	74.43 ± 3.131	0.896	0.403
	Unsweetened	71.57 ± 0.612		
180mins	Sweetened	74.14 ± 3.131	0.896	0.403
	Unsweetened	71.57 ± 0.612		
24hrs	Sweetened	78.29 ± 3.550	-4.273	0.004
	Unsweetened	141.43 ± 14.344		
48hrs	Sweetened	89.57 ± 5.911	-5.787	0.000
	Unsweetened	166.43 ± 11.894		

$P \geq 0.05$ (0 – 180mins), No significant difference exist.
$P \leq 0.05$ (24 – 48hrs), significant difference exist.

Apendix XV Continued: Comparisonof Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Mutant Yeast Strains (UV Light-Irradiated) *Kodamaea ohmeri*

Time (mins/hours)	Dough type	Mean ± SEM	t	P – value
0mins	Sweetened	84.00 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
30mins	Sweetened	84.57 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
60mins	Sweetened	84.00 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
90mins	Sweetened	84.00 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
120mins	Sweetened	84.00 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
150mins	Sweetened	84.00 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
180mins	Sweetened	84.00 ± 2.390	4.264	0.001
	Unsweetened	72.57 ± 1.212		
24hrs	Sweetened	86.57 ± 2.534	-3.224	0.007
	Unsweetened	132.00 ± 13.860		
48hrs	Sweetened	111.71 ± 8.434	-3.058	0.014
	Unsweetened	168.00 ± 16.361		

$P \leq 0.05$, significant difference exists between sweetened and unsweetened dough.

Appendix XV Continued: Comparison of Mean Values of Leavening Capacities of Sweetened and Unsweetened Dough of Mutant Yeast Strains (UV Light -Irradiated) *Rhodotorula minuta*

Time (mins/hours)	Dough type	Mean ± SEM	t	P – value
0mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
30mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
60mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
90mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
120mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
150mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
180mins	Sweetened	73.71 ± 0.565	0.317	0.759
	Unsweetened	73.29 ± 1.229		
24hrs	Sweetened	77.86 ± 1.405	-3.434	0.014
	Unsweetened	152.57 ± 21.711		
48hrs	Sweetened	82.14 ± 1.831	-5.775	0.001
	Unsweetened	141.43 ± 10.102		

$P \geq 0.05$ (0 – 180mins), No significant difference exist.
$P \leq 0.05$ (24 – 48hrs), significant difference exist.

Appendix XVI: Assessment of the Mean Performance of the Leavening Capacities of the Mutant Yeast Strains (UV Light-Irradiated) of Sweetened Dough using Analysis of Variance (ANOVA).

	Mean ± SEM				
Yeast strains	**0mins**	**30mins**	**60mins**	**90mins**	**120mins**
Candida norvegensis	67.29 ± 2.23[c]	70.71 ± 1.32[b]	72.14 ± 1.03[b]	72.14 ± 1.03[b]	72.14 ± 1.03[b]
Candida zeylanoides	76.86 ± 1.00[b]	76.86 ± 1.00[b]	76.86 ± 1.00[b]	76.86 ± 1.00[b]	76.86 ± 1.00[b]
Geotrichumcapitatum	73.29± 3.36[b]	73.29 ± 3.36[b]	74.14 ± 3.24[b]	74.14± 3.24[b]	74.43 ± 3.13[b]
Kodamaeaohmeri	84.00 ± 2.39[a]	84.00 ± 2.39[a]	84.00± 2.39[a]	84.00± 2.39[a]	84.00± 2.39[a]
Rhodotorulaminuta	73.71± 0.57[b]	73.71 ± 0.57[b]	73.71± 0.57[b]	73.71± 0.57[b]	73.71± 0.57[b]
	150mins	**180mins**	**24hrs**	**48hrs**	
Candida norvegensis	76.43± 1.67[b]	76.43± 1.67[b]	76.71± 1.69[b]	82.00± 2.28[b]	
Candida zeylanoides	76.86± 1.00[b]	76.86± 1.00[b]	77.43± 1.02[b]	78.86± 1.03[b]	
Geotrichumcapitatum	74.43± 3.13[b]	74.43± 3.13[b]	78.29± 3.55[b]	89.57± 5.91[b]	
Kodamaeaohmeri	84.00± 2.39[a]	84.00± 2.39[a]	86.57± 2.53[a]	111.71 ± 8.43[a]	
Rhodotorulaminuta	73.71± 0.57[b]	73.71± 0.57[b]	77.86± 1.41[b]	82.14± 1.83[b]	

DMRT values ($P \leq 0.05$) $F = 6.453$, $P = 0.001$
Significant difference exists at $P \leq 0.05$.
Values are mean of duplicate readings. Mean values with different superscripts are significantly different.
Means values were separated using Ducan multiple range test (DMRT).

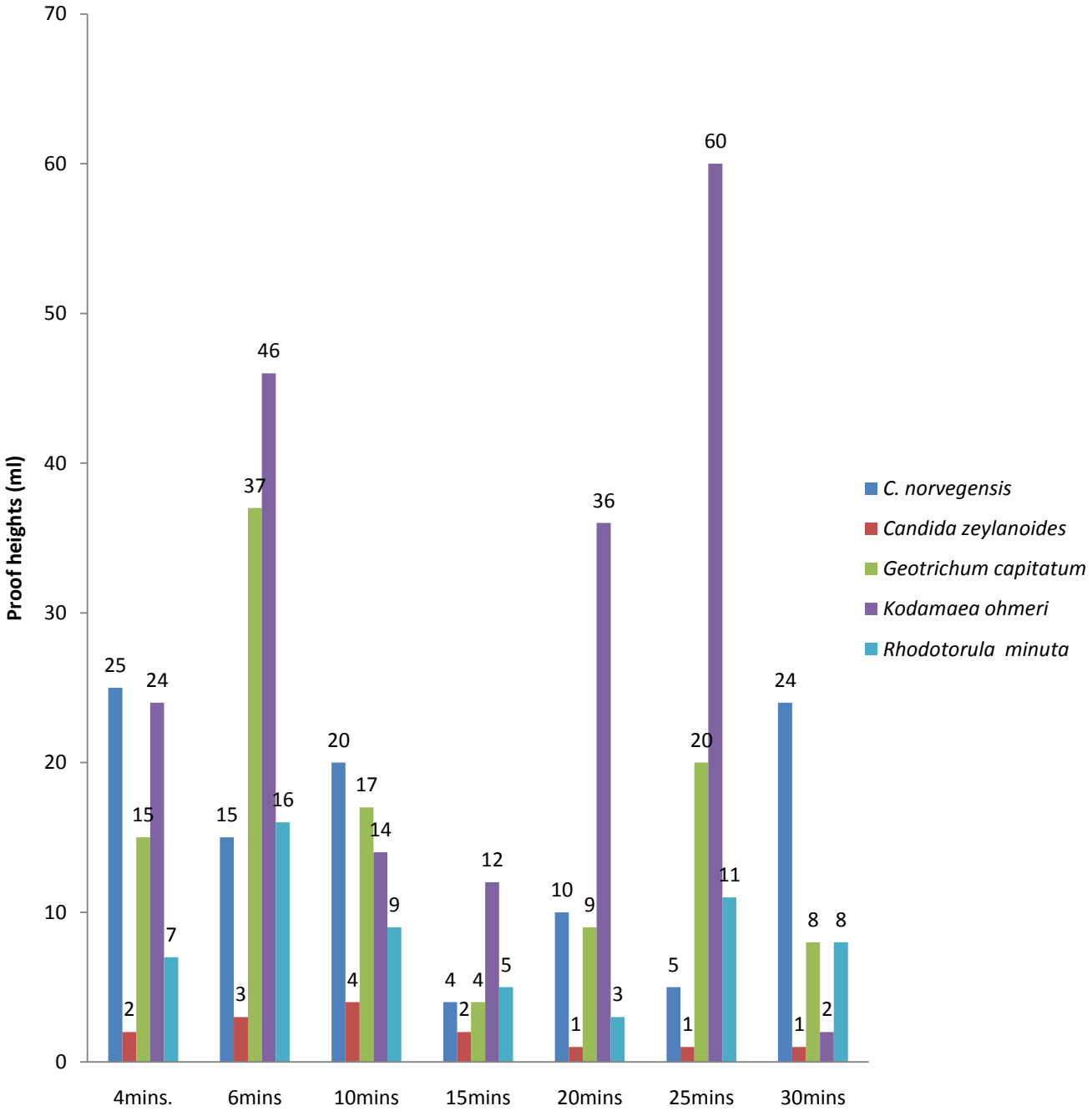
Appendix XVII: Assessment of the Mean Performance of the Leavening Capacities of the Mutant Yeast Strains (UV Light- Irradiated) of Unsweetened Dough using Analysis of Variance (ANOVA).

	Mean ± SEM				
Yeast strains	**0mins**	**30mins**	**60mins**	**90mins**	**120mins**
Candida norvegensis	73.43 ± 0.78 [a]	73.43 ± 0.78 [a]	73.43 ± 0.78 [a]	73.43 ± 0.78 [a]	73.43 ± 0.78 [a]
Candida zeylanoides	73.00 ± 0.66 [a]	73.00 ± 0.66 [a]	73.00 ± 0.66 [a]	73.00 ± 0.66 [a]	73.00 ± 0.66 [a]
Geotrichumcapitatum	71.57 ± 0.61 [a]	71.57 ± 0.61 [a]	71.57 ± 0.61 [a]	71.57 ± 0.61 [a]	71.57 ± 0.61 [a]
Kodamaeaohmeri	72.57 ±1.21 [a]	72.57 ±1.21 [a]	72.57 ±1.21 [a]	72.57 ±1.21 [a]	72.57 ±1.21 [a]
Rhodotorulaminuta	73.29 ± 1.23 [a]	73.29 ± 1.23 [a]	73.29 ± 1.23 [a]	73.29 ± 1.23 [a]	73.29 ± 1.23 [a]
	150mins	**180mins**	**24hrs**	**48hrs**	
Candida norvegensis	73.43 ± 0.78 [a]	73.43 ± 0.78 [a]	99.14 ± 5.89 [b]	113.00 ± 14.23 [b]	
Candida zeylanoides	73.00 ± 0.66 [a]	73.00 ± 0.66 [a]	114.43 ± 7.85[ab]	145.00 ± 11.43[ab]	
Geotrichumcapitatum	71.57 ± 0.61 [a]	71.57 ± 0.61 [a]	141.43 ± 14.34[ab]	166.43 ± 11.89 [a]	
Kodamaeaohmeri	75.57 ± 1.21 [a]	75.57 ± 1.21 [a]	132.00 ± 13.86[ab]	168.00 ± 16.36 [a]	
Rhodotorulaminuta	73.29 ± 1.23 [a]	73.29 ± 1.23 [a]	152.57 ± 21.71 [a]	141.43 ± 10.10[ab]	

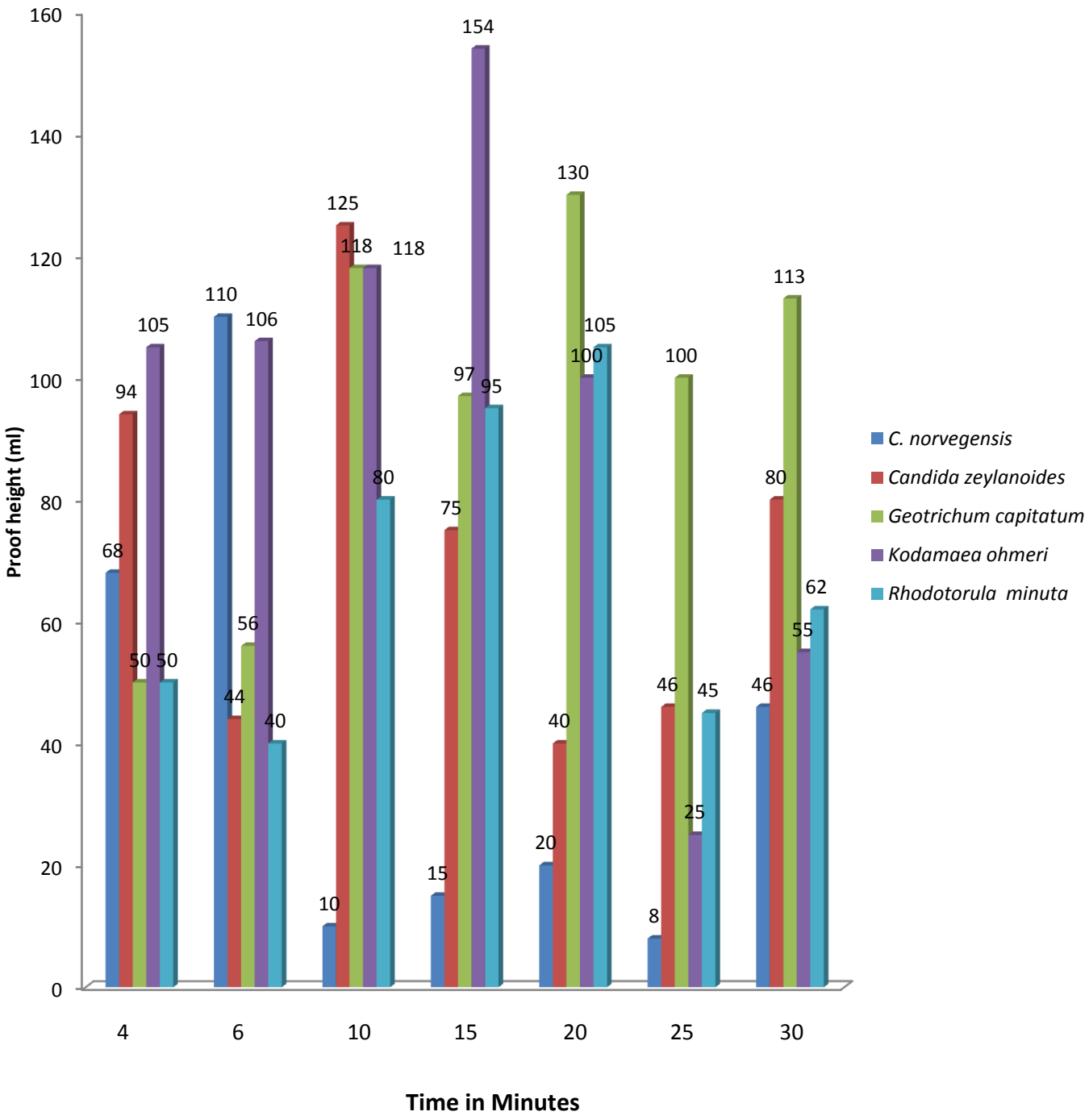
DMRT values ($P \leq 0.05$)

Statistics $F = 2.970$, $P = 0.035$

Significant differences exist at $P \leq 0.05$. Values are means of duplicate reading. Mean values with different superscripts are significantly different. Mean values were separated using Ducan multiple range test



Appendix XVIII: Proof Heights of UV Mutants 4 to 30 Mins of Sweetened Dough



Appendix XIX: ProofHeights of UV Mutants 4 to 30 Mins of Unsweetened Dough