

TABLE OF CONTENTS

CHAPTERS	PAGE NO.
1. Introduction	1
2. Review of Literature	7
2.1 Saccharification process	7
2.2 Microbial enzyme production	8
2.3 Free amino nitrogen	11
2.4 Fermentation process	12
2.5 Use of probiotic organisms	14
2.6 Use of ultrasonication in extraction	16
2.7 Use of microwave in extraction	17
3. Materials and Methods	18
3.1 Standardization of the Saccharification Process	18
3.1.1 Design of experiment	19
3.1.2 Saccharification process	20
3.1.3 Estimation of reducing sugar content	22
3.1.4 Estimation of Free amino nitrogen	22
3.1.5 Analysis for Total phenolic Compounds.	22
3.1.6 Acidity	23
3.2. Fermentation of the wort established yeast.	23
3.2.1 Design of experiment	23
3.2.2 Fermentation process	25
3.2.3 Alcohol content estimation	26
3.2.4 Colour measurement of the products	26
3.2.5 Fortification of the drink with probiotic <i>Lactobacillus</i> species.	27
3.2.6 Fortification of the wine	27
3.2.7 Sensory evaluation of the products	28
3.3 Optimization of the extraction of polyphenols from husk of black rice using sonication and microwave	28
3.3.2 Extraction process	29

3.3.3 Analysis for Total phenolic compounds	31
3.3.4 HPLC analysis for Total phenolic compounds.	31
3.3.5 Determination of antioxidant activity	32
3.3.6 Estimation of anthocyanins	32
3.3.7 Estimation of flavonoids	32
3.3.8 GC-MS analysis for volatile aromatic compounds	33
4. Results and Discussion	34
4.1. Modeling of the saccharification process from black rice.	34
4.1.1 Effect of process variables on reducing sugar.	34
4.1.2 Effect of process variables on total phenol content	35
4.1.3 Effect of process variables on free amino nitrogen.	35
4.1.4 Effect of process variables on acidity.	36
4.2. Modeling of the fermentation process from black rice.	44
4.2.1 Effect of process variables on ethanol concentration.	44
4.3 Colour measurement of the products	50
4.3.1 Fortification of the wine with probiotic <i>Lactobacillus</i> species and sensory evaluation of the products	50
4.3.2 GC-MS analysis for volatile aromatic compounds	51
4.3.3 HPLC analysis for phenolic compounds in black rice.	52
4.3.4 Sensory evaluation of the products	54
4.4 Modeling of the extraction process from rice husk.	55
4.4.1 Effect of process variables on extraction of total phenolic compounds.	56
4.4.2. Effect of process variables on extraction of flavonoid	56
4.4.3 Effect of process variables on extraction of the anthocyanin contents.	57
4.4.4 Effect of process variables on antioxidant activity.	58
4.4.5 HPLC analysis for phenolic compounds black rice husk	66
5. Summary and Conclusion	68
6. Bibliography	70
7. Appendix	79
8. Publications	80

LIST OF TABLES

TABLES	PAGE NO.
4.1 The design of process variable and their levels	37
4.2 Experimental design of the three factors and four variable Box Behken design	38
4.3 Analysis of variance showing the linear, quadratic interaction and lack of fit of the response variables	38
4.4 Estimated regression coefficients of the fitted second order polynomial for response variables.	39
4.5 Estimated optimum conditions	39
4.6 Estimated optimum conditions, predicted, and experimental values of responses under the following conditions	39
4.7 Independent variables in the experimental plan	45
4.8 Experimental design matrix with experimental and predicted value of ethanol yield.	46
4.9 Analysis of variance showing the linear, quadratic interaction and lack of fit of the response variables	47
4.10 Estimated regression coefficients of the fitted second order polynomial for response variables.	47
4.11 Estimated optimum conditions	48
4.12 Estimated optimum conditions, predicted, and experimental values of responses under the following conditions	48
4.13 Colour measurement of the product	50
4.14 Volatile aromatic compounds detected by GC-MS analysis	51
4.15 Amount of phenolic compound in black rice and black rice wine	53
4.16 sensory score of different type of wine	54
4.17 Independent variables and their levels employed in a Box Behken design for optimization of rice husk extracts	59
4.18: Experimental design of the five factors and four variable Box Behken design	60
4.19 Analysis of variance showing the linear, quadratic interaction and lack of fit of the response variables	61
4.20 Estimated regression coefficients of the fitted second order polynomial for response variables	61
4.21 Estimated optimum conditions	62
4.22 Estimated optimum conditions, predicted, and experimental values of responses under the following conditions	62
4.23: Amount of phenolic compound in black rice husk and at optimal condition by HPLC analysis	67

LIST OF FIGURES

FIGURES	PAGE NO.
1.1. Conventional Starch Fermentation	2
3.1 Microbial strain	20
3.2 Black rice	20
3.3 Black rice powder	20
3.4 Flow chart of saccharification	21
3.5 After saccharification filtration of wort	21
3.6 Wort obtained after saccharification	21
3.7 <i>Saccharomyces cerevisiae</i> ATCC9763	24
3.8 Flowchart of fermentation	25
3.9 Incubator used for fermentation	26
3.10 Lyophilized cultures of <i>Lactobacillus acidophilus</i> ATCC 4356 and <i>Lactobacillus sakei</i> ATCC 15521	27
3.11 Black paddy husk	29
3.12 Husk powder	29
3.13: Ultrasonic bath	30
3.14: Microwave oven	30
3.15: Flow chart of extraction of polyphenol by ultrasound and microwave	30
3.16: Extracted samples under different conditions	31
4.1 Effects of varying temperature and pH on reducing sugar at constant incubation period.	40
4.2 Effects of varying temperature and incubation period on reducing sugar constant pH	40
4.3 Effects of varying incubation period and pH on reducing sugar at constant temperature.	40
4.4 Effects of varying temperature and pH on Total phenol content at constant incubation period	41
4.5 Effects of varying Temperature and Incubation time on Total phenol content at constant pH.	41
Fig.4.6 Effects of varying Incubation time and pH on Total phenol content at constant Temperature	41
4.7 Effects of varying temperature and pH on free amino nitrogen at constant incubation period.	42
4.8 Effects of varying temperature and incubation period on free amino nitrogen at constant pH.	42
4.9 Effects of varying incubation period and pH on free amino nitrogen at constant temperature.	42
4.10 Effects of varying temperature and pH on acidity at constant incubation period	43
4.11 Effects of varying temperature and incubation period on acidity at constant pH.	43
4.12 Effects of varying incubation period and pH on acidity at constant temperature.	43
4.13 Effect of varying, temperature and incubation period on ethanol yield at constant pH.	49

4.14 Effect of varying, pH and incubation period on ethanol yield at constant temperature	49
4.15. Effect of varying, pH and temperature on ethanol yield at constant incubation period	49
4.16 <i>Lactobacillus acidophilus</i>	50
4.17 <i>Lactobacillus sakei</i>	50
4.18 <i>Lactobacillus sakei</i> ATCC 1552 and <i>Lactobacillus acidophilus</i> ATCC 4356	50
4.19 Type of products was prepared	51
4.20 GC-MS chromatogram of the black rice wine for volatile aromatic compounds	52
4.21 HPLC chromatogram of black rice before fermentation	52
4.22 HPLC chromatogram of black rice after fermentation	53
4.23 Effect of ethanol concentration and solvent solute ratio of factor on yields of total polyphenol	63
4.24. Effect of microwave time and sonication temperature of factor on yields of flavonoid	63
4.25. Effect of microwave time and solvent solute ratio of factor on yields of flavonoid	63
Fig 4.26 Effect of Solvent solute ratio and sonication time of factor on yields of flavonoid	64
4.27. Effect of solvent solute ratio and sonication temperature of factor on yields of anthocyanin.	64
4.28. Effect of ethanol concentration and sonication temperature of factor on yields of anthocyanin	64
4.29 Effect of ethanol concentration and solvent solute ratio of factor on yields of anthocyanin	65
4.30. Effect of Ethanol Concentration and Sonication time of factor on yields of anthocyanin	65
4.31. Effect of ethanol concentration and sonication temperature of factor on yields of antioxidant activity	65
4.32. Effect of ethanol concentration and solvent solute ratio of factor on yields of antioxidant activity	65
4.33 HPLC chromatogram of black rice husk	66
4.34 HPLC chromatogram of black rice husk at optimal condition	66

LIST OF ABBREVIATIONS

UAE	Ultrasound-assisted extraction
LAB	Lactic acid bacteria
RSM	Response surface methodology
ATCC	American type cell culture
RPM	Rotation per minutes
RCCD	Rotatable central composite design
FAN	Free amino nitrogen
BBD	Box-Behnken designs
PB	Plackett-Burman
MAE	Microwaves assisted extraction
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
GC-MS	Gas chromatography mass spectrometry
RSA	Radical scavenging activity
TPC	Total phenolic content