



**PUSAT PENGAJIAN TEKNOLOGI INDUSTRI  
UNIVERSITI SAINS MALAYSIA**

**BORANG PENYERAHAN DISERTASI MUTAKHIR  
SATU (1) NASKAH**

Nama penyelia: \_\_\_\_\_ Dr. Uthumporn Utra @ Sapina Abdullah \_\_\_\_\_

Bahagian: \_\_\_\_\_ Teknologi Makanan \_\_\_\_\_

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh

Encik/Puan/Cik: \_\_\_\_\_ Chin Wen Yi \_\_\_\_\_

mengenai disertasinya sebagaimana yang dipersetujui oleh Panel Pemeriksa di *Viva Voce*-nya.

2. Saya ingin mengesahkan bahawa saya berpuas hati dengan pembetulan/ pindaan yang dilaksanakan oleh calon.

Sekian, terima kasih.

\_\_\_\_\_  
(Tandatangan dan cop)

\_\_\_\_\_  
7 OGOS 2020

(Tarikh)



# **DEVELOPMENT OF FERMENTED WATER KEFIR AMARANTH DRINK**

by

**CHIN WEN YI**

A dissertation submitted in partial fulfillment of the requirement for the degree of  
Bachelor of Technology (B. Tech) in the field of Food Technology  
School of Industrial Technology  
University Science Malaysia

July 2020

## DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

*Chin*

---

CHIN WEN YI

JULY 2020

## **ACKNOWLEDGEMENT**

This thesis will not succeed without the help of certain individuals throughout the period of this study.

I would like to express my sincere gratitude to my supervisor, Dr. Uthumporn Utra @ Sapina Abdullah for giving me useful advices and guidance from the start to the end of my final year project. Secondly, I would like to thank my examiner, Dr Tan Thuan Chew for giving me comments and suggestion for improvements of my study.

I would also like to acknowledge all the laboratory assistants in School of Industrial Technology, En Abdul Rahim Md Sari, En Maarof Salleh, En Abdul Ghoni Ruslan and En Mohamad Firdaus Mohd Adnan for their guidance and expertise given along the study. Next, I must acknowledge all the postgraduate students, Koh Wee Yin, PhD student, Lim Xiao Xian and Syuzeliana, MSc student under supervision of Dr. Uthumporn Utra @ Sapina Abdullah for giving me helps and guidance along the journey of completing my study.

Last but not least, I would like to thank my dear family and friends for their motivation, understanding and supports in making the study success.

CHIN WEN YI

JULY 2020

## TABLE OF CONTENTS

|  | Page |
|--|------|
| ACKNOWLEDGEMENT  | ii   |
| TABLE OF CONTENTS  | iii  |
| LIST OF TABLES   | viii |
| LIST OF FIGURES  | ix   |
| LIST OF ABBREVIATIONS  | x    |
| LIST OF APPENDICES   | xiii |
| ABSTRAK  | xiv  |
| ABSTRACT   | xv   |
| CHAPTER 1 INTRODUCTION   | 1    |
| 1.1 Research Background  | 1    |
| 1.2 Problem Statement  | 3    |
| 1.3 Objectives   | 4    |
| CHAPTER 2 LITERATURE REVIEW  | 5    |
| 2.1 Amaranths  | 5    |
| 2.1.1 General Description of Amaranths   | 5    |
| 2.1.2 Health benefits of Vegetable Amaranth  | 6    |
| 2.1.3 Nutritional Composition and Health Benefit of Vegetable Amaranths<br>( <i>Amaranthus tricolor</i> and <i>Amaranthus blitum</i> ) | 8    |
| 2.2 Functional Drinks  | 9    |
| 2.3 Probiotics   | 11   |
| 2.4 Water kefir  | 12   |
| 2.4.1 Water Kefir Grains   | 12   |

|   |    |
|---|----|
| 2.4.2 Water kefir drinks  | 15 |
| 2.4.3 Health Benefits of Water Kefir  | 16 |
| 2.4.4 Fermentation Process of Water Kefir   | 18 |
| CHAPTER 3 MATERIALS AND METHOD  | 20 |
| 3.1 Design of Experiment  | 20 |
| 3.2 Preliminary Study   | 21 |
| 3.3 Materials   | 21 |
| 3.4 Preparation of Red and Green Amaranth Vegetable Juice                                 | 21 |
| 3.5 Fermentation of Water Kefir Grains(Process to Activate Water Kefir Grains)            | 22 |
| 3.6 Preparation of Fermented Water Kefir Amaranth Drinks                                  | 22 |
| 3.7 Physicochemical Analysis  | 23 |
| 3.7.1 pH Value  | 23 |
| 3.7.2 Total Soluble Solids  | 23 |
| 3.7.3 Sugar Composition   | 23 |
| 3.7.3a Sample Preparation   | 24 |
| 3.7.3b Standard Solution Preparation  | 24 |
| 3.7.3c Determination of Sugar Composition Using High Performance<br>Liquid Chromatography | 24 |
| 3.7.4 Total Phenolic Content  | 25 |
| 3.7.4a Sample Extracts Preparation  | 25 |
| 3.7.4b Standard Solutions Preparation   | 25 |
| 3.7.4c Folin-Ciocalteu Assay  | 26 |
| 3.7.5 Antioxidant Activity  | 26 |
| 3.7.5a Sample Extracts Preparation  | 26 |

|  |    |
|--|----|
| 3.7.5b DPPH Assay  | 27 |
| 3.7.6 Titratable Acidity   | 27 |
| 3.8 Microbiological Analysis   | 28 |
| 3.8.1 Serial Dilution  | 28 |
| 3.8.2 Preparation of Agar Medium   | 28 |
| 3.8.2a Plate Count Agar (PCA)  | 28 |
| 3.8.2b Potato Dextrose Agar (PDA)  | 28 |
| 3.8.2c De Man, Rogosa, Sharpe (MRS) Agar   | 29 |
| 3.8.2d Eosin Methylene Blue (EMB) Agar   | 29 |
| 3.8.3 Preparation of Broth Medium  | 29 |
| 3.8.3a Lauryl Tryptose (LST) Broth   | 29 |
| 3.8.3b Brilliant Green Bile (BGLB) Broth   | 29 |
| 3.8.3c EC ( <i>Escherichia coli</i> ) Broth  | 29 |
| 3.8.4 Bacteria Enumeration and Plate Counting  | 30 |
| 3.8.4a Colony Forming Unit and Calculation   | 30 |
| 3.8.4b Total Plate Count   | 30 |
| 3.8.4c Yeast and Mould Count   | 30 |
| 3.8.4d Lactobacilli count  | 31 |
| 3.8.5 Isolation and Enumeration of Total Coliforms, Fecal Coliforms and<br><i>Escherichia coli</i> | 31 |
| 3.8.5a Most Probable Number (MPN) Technique  | 31 |
| 3.8.5b Presumptive Test for Total Coliforms and Fecal Coliforms                                    | 32 |
| 3.8.5c Confirmatory Test for Total Coliforms   | 32 |
| 3.8.5d Confirmatory Test for Fecal Coliforms   | 32 |

|  |    |
|--|----|
| 3.8.5e Escherichia coli Test             | 33 |
| 3.9 Storage Studies                      | 33 |
| 3.9.1 Lactobacilli Count                 | 33 |
| 3.9.2 pH Value                           | 33 |
| 3.10 Statistical Analysis                | 34 |
| CHAPTER 4 RESULTS AND DISCUSSIONS        | 35 |
| 4.1 Preliminary Test                     | 35 |
| 4.2 Physicochemical Analysis             | 35 |
| 4.2.1 pH value                           | 35 |
| 4.2.2 Total soluble solids               | 37 |
| 4.2.3 Sugar composition                  | 39 |
| 4.2.4 Total phenolic content             | 43 |
| 4.2.5 Antioxidant activity               | 45 |
| 4.2.6 Titratable acidity                 | 47 |
| 4.3 Microbiological analysis             | 48 |
| 4.3.1 Total plate count                  | 49 |
| 4.3.2 Lactobacilli count                 | 50 |
| 4.3.3 Yeast and mould count              | 52 |
| 4.3.4 Total coliforms                    | 53 |
| 4.3.5 Fecal coliforms                    | 54 |
| 4.4 Storage study                        | 55 |
| 4.4.1 pH                                 | 55 |
| 4.4.2 Lactobacilli count                 | 57 |
| CHAPTER 5 CONCLUSION AND RECOMMENDATIONS | 59 |



REFERENCES

61

APPENDICES

76

## LIST OF TABLES

| <b>Table Caption</b>   | <b>Page</b> |
|--|-------------|
| 3.6 Abbreviation of the fermented water kefir amaranth drinks and their controls   | 23          |
| 4.1 Abbreviation of the fermented water kefir amaranth drinks and their controls   | 35          |
| 4.2.1 pH values of fermented drinks and their respective controls  | 36          |
| 4.2.2 Total soluble solids of fermented samples and their respective controls  | 39          |
| 4.2.3 Sugar composition of fermented amaranth samples and their respective controls  | 40          |
| 4.2.4 Total phenolic contents of fermented samples and their respective controls   | 44          |
| 4.2.5 Antioxidant activity of fermented drinks and their respective controls expressed as percentage of DPPH radical scavenging activity | 46          |
| 4.2.6 Titratable acidity of fermented samples and their respective controls  | 48          |
| 4.3.1 Total plate count of fermented samples and their respective controls   | 49          |
| 4.3.2 Lactobacilli count of fermented samples  | 51          |
| 4.3.3 Yeast and mold count of fermented samples and their respective controls  | 53          |
| 4.3.4 Total coliform count of fermented samples and their respective controls  | 54          |
| 4.3.5 Fecal coliform count of fermented samples and their respective controls  | 55          |

## LIST OF FIGURES

| <b>Figure Caption</b>   | <b>Page</b> |
|---|-------------|
| 3.1 Development of fermented water kefir amaranth drinks  | 20          |
| 4.4.1 pH values of fermented samples and their respective controls during two weeks of storage at 4°C           | 57          |
| 4.4.2 Lactobacilli count of fermented samples and their respective controls during two weeks of storage at 4°C. | 58          |

## LIST OF ABBREVIATIONS

| Abbreviation  | Caption   |
|---------------|---|
| °C            | Degree Celsius  |
| $\alpha$      | alpha   |
| $\beta$       | beta  |
| $\mu\text{m}$ | micrometer  |
| $\mu\text{L}$ | microliter  |
| AOAC          | Association of Official Analytical Chemists                             |
| ANOVA         | Analysis of Variance  |
| BGLB          | Brilliant Green Bile Agar   |
| CaEDTA        | Calcium disodium edetate  |
| CFU           | Colony Forming Unit   |
| CFU/mL        | Colony Forming Units per milliliter                                     |
| EC            | <i>Escherichia coli</i> Broth   |
| EMB           | Eosin Methylene Blue Agar   |
| g             | gram  |
| GC5           | Fermented green amaranth without water kefir grains with 5% brown sugar |
| GC7           | Fermented green amaranth without water kefir grains with 7% brown sugar |
| GK5           | Fermented green amaranth with water kefir grains with 5% brown sugar    |
| GK7           | Fermented green amaranth with water kefir grains with 7% brown sugar    |
| HPLC          | High-performance liquid chromatography                                  |

|                                 |   |
|---------------------------------|---|
| hr                              | hour  |
| L                               | liter   |
| LST                             | Lauryl Tryptose Broth   |
| M                               | molarity  |
| min                             | minute  |
| mg                              | milligram   |
| mg GAE/100g                     | milligram of gallic acid equivalents per 100 g                        |
| mL                              | milliliter  |
| mL/min                          | milliliter per minute   |
| mm                              | millimeter  |
| mmHg                            | millimeter of mercury   |
| MPN                             | Most probable number  |
| MRS                             | De man, Rogosa, Sharpe Agar   |
| Na <sub>2</sub> CO <sub>3</sub> | sodium carbonate  |
| NaOH                            | sodium hydroxide  |
| PCA                             | Plate Count Agar  |
| PDA                             | Potato Dextrose Agar  |
| RC5                             | Fermented red amaranth without water kefir grains with 5% brown sugar |
| RC7                             | Fermented red amaranth without water kefir grains with 7% brown sugar |
| RK5                             | Fermented red amaranth with water kefir grains with 5% brown sugar    |
| RK7                             | Fermented red amaranth with water kefir grains with 7% brown sugar    |

UV-Vis

w/v

Ultraviolet-visible

mass/volume

## **LIST OF APPENDICES**

|            |   |
|------------|---|
| APPENDIX A | Preliminary Test                                  |
| APPENDIX B | Standard Curve of Sugar Analysis                  |
| APPENDIX C | Standard Curve of Total Phenolic Content Analysis |
| APPENDIX D | MPN Index Table                                   |

## PENGHASILAN MINUMAN KEFIR AIR BAYAM

### ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan minuman kefir air dari jus bayam sebagai alternatif untuk vegetarian dan pengguna yang mempunyai intoleransi laktosa bagi menggantikan minuman berasas susu haiwan. Pelbagai analisis telah dijalankan ke atas minuman kefir air bayam merah (*Amaranthus tricolor*) dan bayam hijau (*Amaranthus blitum*) yang difermentasi dengan tahap gula (5% dan 7%) yang terpilih. Kedua-dua minuman kefir air bayam merah dan hijau mencapai penurunan signifikan ( $p < 0.05$ ) dalam nilai pH, jumlah pepejal larut dan tahap sukrosa menunjukkan proses fermentasi yang berkesan. Minuman kefir air bayam merah yang difermentasi menggunakan 5% tahap gula menunjukkan peningkatan signifikan ( $p < 0.05$ ) dalam aktiviti antioksidan dan kandungan jumlah fenolik telah mengenal pasti kandungan nutrisi yang tinggi. Kedua-dua minuman kefir air bayam merah dan hijau dapat klaim properti probiotik disebabkan pencapaian minima  $10^6$  CFU/mL lactobacilli lepas 2 minggu penyimpanan. Keputusan dari analisis mikrobiologi telah menunjukkan lactobacilli ( $13.39 \times 10^6$  CFU/mL) dan yis dan kulat ( $25.23 \times 10^6$  CFU/mL) dalam minuman kefir air bayam merah yang difermentasi menggunakan 7% tahap gula jauh lebih tinggi berbanding bayam hijau. Kedua-dua sampel mempunyai kandungan coliform yang dalam julat selamat dan ketiadaan coliform fecal telah menunjukkan kualiti mikrobiologi yang stabil. Namun begitu, jus bayam merah dan hijau tidak sepenuhnya sesuai sebagai substrat minuman kefir air disebabkan keasidan tertitrat dan kiraan sel boleh hidup yang rendah. Kesimpulannya, minuman kefir air bayam merah dan bayam hijau telah berjaya dihasilkan.



## DEVELOPMENT OF FERMENTED WATER KEFIR AMARANTH DRINK

### ABSTRACT

The aim of this study is to develop fermented water kefir drink from vegetable amaranth juice as an alternative for vegetarians and lactose intolerants to replace dairy beverages. Various analyses were conducted on fermented water kefir red amaranth (*Amaranthus tricolor*) and green amaranth (*Amaranthus blitum*) drinks fermented with selected sugar levels (5% and 7%). Both fermented red and green amaranth samples with 7% sugar achieved significant ( $p < 0.05$ ) decrement in pH value, total soluble solids, and sucrose concentration which represented efficient water kefir fermentation. Fermented red amaranth sample showed significant ( $p > 0.05$ ) increment in antioxidants activity and total phenolic content after fermentation represented higher nutritional value. Both fermented red and green amaranth samples were able to claim probiotic properties due to the achievement of minimum  $10^6$  CFU/mL of lactobacillus after 2 weeks of storage. Results from microbiological analyses showed a significantly ( $p < 0.05$ ) higher lactobacilli count ( $13.39 \times 10^6$  CFU/mL) and yeast and mold count ( $25.23 \times 10^6$  CFU/mL) in fermented red amaranth drink with 7% sugar level compared to green amaranth. Both samples had acceptable range of coliform count and absence of fecal coliforms represented the stable microbial quality. However, red and green amaranth vegetable juice were not fully suitable to use as water kefir substrate due to low titratable acidity and total viable cell count. As an overall, fermented red and green amaranth water kefir drinks were successfully produced.