

## ACKNOWLEDGEMENTS

First of all, the author wanted to express her gratitude to Jesus Christ who has made all things possible for this thesis report to be completed. Without his grace and guidance, this thesis report could not be finished well. This thesis report with the title of “STUDY OF ADDITION OF CRUDE EXTRACTS OF AFRICAN BITTER (*VERNONIA AMYGDALINA* DEL.) OR GREEN GRASS JELLY (*CYCLEA BARBATA* MIERS) LEAF TO COWPEA (*VIGNA UNGUICULATA*) YOGHURT” was intended for fulfilling some academic requirements in order to obtain the degree of *Sarjana Teknologi Pertanian Strata Satu*, Faculty of Science and Technology, *Universitas Pelita Harapan*, Tangerang.

The author realized that without the supervision, help, and prayer from various parties, this thesis could not be finished on time. Therefore, the author wanted to express her gratitude to all parties who have helped the author in the process of doing this thesis, which are:

1. Mr. Ir. W. Donald R. Pokatong, M.Sc., Ph.D. as the Head of Food Technology Study Program who provided the opportunity to conduct the research and as thesis supervisor who has supervised, encouraged, and supported the author throughout the whole process of thesis completion.
2. Mr. Eric Jobiliong, Ph.D. as Dean of Faculty of Science and Technology.
3. Ms. Dela Rosa, M.M., M.Sc., Apt. as Vice Dean of Faculty of Science and Technology.
4. Mr. Laurence, M.T., as Director Administration and Student Affair of Faculty of Science and Technology.
5. Mr. Dr. Tagor M. Siregar, M.Si., Mr. Dr. A. Parhusip, Ms. Natania, M.Eng., Ms. Yuniwaty Halim, M.Sc., as the Head of Chemistry, Microbiology, Food Processing, Quality Control, and Research Laboratory who had given permission to the writer to conduct the research in the laboratories.
6. Mr. Darius, Mr. Yosafat, Mr. Adi and Mr. Adzie for the guidance and support during the research in the laboratories.

7. All lecturers of Food Technology Study Program of Universitas Pelita Harapan for the knowledge given, which was very useful during the research and thesis report completion.
8. All staff of Faculty of Science and Technology for the help and support.
9. Beloved father (Yahya Soewandono), beloved mother (Lindawati Sukendro), and sister (Feby Angelica) for the love, help, and support during the completion of this final project.
10. Danielle Linggar, Irving Ekaputra, Elvina Suteja, Natasha Collins, Ledy Essen, Tiffany Nathalie and Miki Ocha as friends under the same supervisor for the supports and companion throughout the thesis process.
11. Naomi Nerisha, Gabriella Permata Heru, Cynthia Saputra, Angeline Vania, Frieska Darwis and Teresa for cheering and supporting the author throughout the thesis process.
12. Jane Ivena for the knowledge shared, guidance and help in Microbiology Laboratory.
13. Ellen Tjakrakusuma and Karina Indriani for sharing their thesis experience and knowledge.
14. All friends of Food Technology Study Program batch 2015 Universitas Pelita Harapan that cannot be mentioned one by one who had supported and shared their knowledge and experiences.
15. Everyone who gave support and motivation to the author during the thesis process who cannot be mentioned one by one.

The author acknowledges that there are still many shortcomings in this thesis report and still require much improvement. Therefore, any criticism and suggestions of the reader to improve this report are gladly welcomed. Lastly, the author hope that this report can be useful for the readers. Thank you.

Tangerang, February 2019

Author

## TABLE OF CONTENTS

	page
COVER PAGE	
STATEMENT OF THESIS AUTHENTICITY	
APPROVAL BY THESIS SUPERVISOR	
APPROVAL BY THESIS EXAMINATION COMMITTEE	
ABSTRACT .....	v
<i>ABSTRAK</i> .....	vi
ACKNOWLEDGEMENTS .....	vii
TABLE OF CONTENTS .....	ix
LIST OF FIGURES.....	xii
LIST OF TABLES .....	xiv
LIST OF APPENDICES .....	xv
CHAPTER I INTRODUCTION .....	1
1.1 Research Background.....	1
1.2 Research Problem.....	3
1.3 Objectives.....	4
1.3.1 General Objectives .....	4
1.3.2 Specific Objectives.....	5
CHAPTER II LITERATURE REVIEW .....	6
2.1 Cowpea .....	6
2.2 African Bitter Leaf Plant .....	8
2.2.1 Functions of African Bitter Leaf .....	9
2.3 Green Grass Jelly Plant .....	10
2.3.1 Functions of Green Grass Jelly Leaf .....	11
2.4 Oxidation and Antioxidant Mechanism.....	12
2.4.1 Antioxidant Activity Determination.....	16
2.5 Flavonoid .....	17
2.6 Cowpea Yoghurt.....	18

2.7 Starter Culture and Fermentation .....	20
2.7.1 Types and Mechanisms of Lactic Acid Fermentation .....	21
CHAPTER III RESEARCH METHODOLOGY .....	24
3.1 Materials and Equipment.....	24
3.2 Research Stage I .....	25
3.3 Research Stage II.....	30
3.4 Experimental Design .....	31
3.4.1 Research Stage I .....	31
3.4.2 Research Stage II.....	33
3.5 Method of Analysis .....	35
3.5.1 Antioxidant Assay (Mardjuki, 2018).....	35
3.5.2 Total Phenolic Content Analysis (Lamien-Meda <i>et al.</i> , 2008) .....	35
3.5.3 Total Flavonoid Content Analysis (Mardjuki, 2018) .....	36
3.5.4 Total Microbial Count (Mardjuki, 2018) .....	37
3.5.5 Total Lactic Acid Bacteria (Mardjuki, 2018).....	37
3.5.6 Total Titratable Acidity (AOAC, 2005) .....	37
3.5.7 pH (AOAC, 2005) .....	38
3.5.8 Sensory Evaluation (Lawless & Heymann, 2010) .....	38
3.6 Proximate Analysis Procedures .....	39
3.6.1 Moisture Content Analysis (AOAC, 1995).....	39
3.6.2 Protein Content Analysis (AOAC, 1995).....	40
3.6.3 Fat Content Analysis (BSN, 1992).....	41
3.6.4 Ash Content Analysis (BSN, 1992) .....	42
3.6.5 Carbohydrate Content Analysis (AOAC, 2005).....	42
CHAPTER IV RESULTS & DISCUSSION .....	44
4.1 Taxonomical Verification of African Bitter Leaf, Green Grass Jelly Leaf and Cowpea .....	44
4.2 Lactic Acid Bacteria Identification .....	44
4.3 Yield and Antioxidant Characteristics of Leaf Extracts.....	46

4.3.1 African Bitter Leaf Crude Extract .....	46
4.3.2 Green Grass Jelly Leaf Crude Extract .....	48
4.4 Effect of Concentration of African Bitter Leaf Crude Extract and Fermentation Time on the Cowpea Yoghurt Characteristics .....	49
4.4.1 Antioxidant Characteristics .....	49
4.4.2 pH and Total Titratable Acidity .....	54
4.4.3 Microbial Characteristics .....	57
4.4.4 Selected Formulation of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract for Further Analysis.....	59
4.4.5 Sensory Characteristics .....	59
4.4.6 Proximate Composition of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract.....	63
4.5 Effect of Concentration of Green Grass Jelly Leaf Crude Extract and Fermentation Time on the Cowpea Yoghurt Characteristics .....	64
4.5.1 Antioxidant Characteristics .....	64
4.5.2 pH and Total Titratable Acidity .....	69
4.5.3 Microbial Characteristics .....	71
4.5.4 Selected Formulation of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract for Further Analysis.....	73
4.5.5 Sensory Characteristics .....	74
4.5.6 Proximate Composition of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract.....	77
 CHAPTER V CONCLUSIONS & SUGGESTIONS .....	 79
5.1 Conclusions .....	79
5.2 Suggestions.....	80
 BIBLIOGRAPHY .....	 81
APPENDICES.....	86

## LIST OF FIGURES

	page
Figure 2.1 Taxonomy of Cowpea .....	7
Figure 2.2 Lipid oxidation reaction .....	14
Figure 2.3 Antioxidant Mechanism of Phenolic Compounds (Hydroquinone) .....	16
Figure 2.4 Basic Flavonoid Structure .....	18
Figure 2.5 Procedure of making cowpea milk.....	20
Figure 2.6 Procedure of making cowpea yoghurt.....	21
Figure 2.7 Lactic acid fermentation pathways: (a) Homofermentative; (b) Heterofermentative .....	23
Figure 3.1 Procedure for African bitter leaf extraction .....	26
Figure 3.2 Procedure for green grass jelly leaf extraction.....	27
Figure 3.3 Culture stock preparation .....	28
Figure 3.4 Working culture preparation .....	28
Figure 3.5 Starter culture preparation.....	29
Figure 3.6 Cowpea milk preparation .....	29
Figure 3.7 African bitter or Green grass jelly leaf extract cowpea yoghurt preparation.....	30
Figure 4.1 Lactic acid bacteria identification of <i>Lactobacillus bulgaricus</i> (a) <i>Lactobacillus bulgaricus</i> from experiment; (b) <i>Lactobacillus bulgaricus</i> from Stephens & Turner (2015) .....	45
Figure 4.2 Lactic acid bacteria identification of <i>Streptococcus thermophilus</i> (a) <i>Streptococcus thermophilus</i> from experiment; (b) <i>Streptococcus thermophilus</i> from Stephens & Turner (2015) .....	45
Figure 4.3 Effect of different concentration of African bitter leaf crude extract and fermentation time on antioxidant activity of cowpea yoghurt .....	50
Figure 4.4 Effect of different concentration of African bitter leaf crude extract and fermentation time on total phenolic content of cowpea yoghurt .....	51
Figure 4.5 Effect of different concentration only of African bitter leaf crude extract on total flavonoid content of cowpea yoghurt.....	53

Figure 4.6	Effect of different fermentation time only of African bitter leaf crude extract on total flavonoid content of cowpea yoghurt .....	54
Figure 4.7	Effect of different concentration of African bitter leaf crude extract and fermentation time on pH of cowpea yoghurt.....	55
Figure 4.8	Effect of different concentration of African bitter leaf crude extract and fermentation time on total titratable acidity of cowpea yoghurt .....	56
Figure 4.9	Effect of different concentration of African bitter leaf crude extract and fermentation time on total lactic acid bacteria of cowpea yoghurt .....	57
Figure 4.10	Effect of different concentration of African bitter leaf crude extract and fermentation time on total microorganism of cowpea yoghurt .....	58
Figure 4.11	Effect of different concentration of green grass jelly leaf crude extract and fermentation time on antioxidant activity of cowpea yoghurt.....	65
Figure 4.12	Effect of different concentration of Green grass jelly leaf crude extract and fermentation time on total phenolic content of cowpea yoghurt.....	66
Figure 4.13	Effect of different concentration of Green grass jelly leaf crude extract on total flavonoid content of cowpea yoghurt.....	68
Figure 4.14	Effect of different fermentation time on total flavonoid content of cowpea yoghurt .....	69
Figure 4.15	Effect of different concentration of Green grass jelly leaf crude extract and fermentation time on pH value of cowpea yoghurt .....	70
Figure 4.16	Effect of different concentration of Green grass jelly leaf crude extract and fermentation time on total titratable acidity of cowpea yoghurt.....	71
Figure 4.17	Effect of different concentration of Green grass jelly leaf crude extract and fermentation time on total lactic acid bacteria of cowpea yoghurt .....	72
Figure 4.18	Effect of different concentration of Green grass jelly leaf crude extract and fermentation time on total microorganism of cowpea yoghurt.....	73

## LIST OF TABLES

	page
Table 2.1 Nutritional Composition of Cowpea Mature Seeds in 100g edible portion.....	7
Table 2.2 Categories of Antioxidant Capacity.....	10
Table 2.3 Taxonomy of Green Grass Jelly.....	11
Table 3.1 Formulation of 100g Cowpea Yoghurt .....	29
Table 3.2 Experimental Design of Research Stage I using African Bitter Leaf Crude Extract.....	31
Table 3.3 Experimental Design of Research Stage I using Green Grass Jelly Leaf Crude Extract.....	32
Table 3.4 Experimental Design of Research Stage II .....	34
Table 3.5 Sensory parameter for scoring test.....	39
Table 3.6 Sensory parameter for hedonic test.....	39
Table 4.1 Scoring test result of selected cowpea yoghurt formulation with different African bitter leaf crude extract and fermentation time.....	61
Table 4.2 Hedonic test result of selected cowpea yoghurt formulation with different African bitter leaf crude extract and fermentation time.....	62
Table 4.3 Proximate Composition of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract .....	64
Table 4.4 Scoring test result of selected cowpea yoghurt formulation with different concentration of Green grass jelly leaf crude extract and fermentation time.....	75
Table 4.5 Hedonic test result of selected cowpea yoghurt formulation with different concentration of Green grass jelly leaf crude extract and fermentation time.....	77
Table 4.6 Proximate Composition of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract .....	78



## LIST OF APPENDICES

	page
Appendix A	
Result of Identification of Bitter Leaf and Green Grass Jelly .....	A-1
Statement Letter for Plant Material Identification Result .....	A-2
Appendix B	
Moisture Content of African Bitter Leaf Powder.....	B-1
Moisture Content of Green Grass Jelly Leaf Powder.....	B-1
Moisture Content of African Bitter Leaf Crude Extract.....	B-2
Moisture Content of Green Grass Jelly Leaf Crude Extract .....	B-2
Extraction Yield of African Bitter Leaf Crude Extract .....	B-3
Extraction Yield of Green Grass Jelly Leaf Crude Extract.....	B-3
Appendix C	
Antioxidant Activity of African Bitter Leaf Crude Extract .....	C-1
Total Phenolic Content of African Bitter Leaf Crude Extract.....	C-2
Total Flavonoid Content of African Bitter Leaf Crude Extract .....	C-3
Appendix D	
Antioxidant Activity of Green Grass Jelly Leaf Crude Extract .....	D-1
Total Phenolic Content of Green Grass Jelly Leaf Crude Extract .....	D-2
Total Flavonoid Content of Green Grass Jelly Leaf Crude Extract .....	D-2
Appendix E	
Antioxidant Activity of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract and Fermentation Time .....	E-1
Statistical Analysis of Antioxidant Activity of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract and Fermentation Time.....	E-11
Appendix F	
Table F.1 Antioxidant Activity of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract with Different Fermentation Time .....	F-1

Statistical Analysis of Antioxidant Activity of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract with Different Fermentation Time .....	F-10
--	------

#### Appendix G

Total Phenolic Content of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract with Different Fermentation Time .....	G-1
Statistical Analysis of Total Phenolic Content of Cowpea Yoghurt Added with Different Concentrations of African Bitter Leaf Crude Extract .....	G-5

#### Appendix H

Total Phenolic Content of Cowpea Yoghurt Added with Different Green Grass Jelly Leaf Crude Extract with Different Fermentation Time .....	H-1
Statistical Analysis of Total Phenolic Content of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract.....	H-5

#### Appendix I

Total Flavonoid Content of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract .....	I-1
Statistical Analysis of Total Flavonoid Content of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract .....	I-5

#### Appendix J

Total Flavonoid Content of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract with Different Fermentation Time.....	J-1
Statistical Analysis of Total Flavonoid Content of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract.....	J-5

#### Appendix K

pH value of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract.....	K-1
pH value of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract .....	K-2
Statistical Analysis of pH Value of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract .....	K-4
Statistical Analysis of pH Value of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract .....	K-5

## Appendix L

Total Titratable Acidity of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract .....	L-1
Total Titratable Acidity of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract .....	L-2
Statistical Analysis of Total Titratable Acidity of Cowpea Yoghurt Added with Different Concentration of African Bitter Leaf Crude Extract .....	L-4
Statistical Analysis of Total Titratable Acidity of Cowpea Yoghurt Added with Different Concentration of Green Grass Jelly Leaf Crude Extract.....	L-5

## Appendix M

Total Lactic Acid Bacteria and Total Microorganism of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract.....	M-1
Statistical Analysis of Total Lactic Acid Bacteria of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract.....	M-2
Statistical Analysis of Total Microorganism of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract .....	M-3

## Appendix N

Total Lactic Acid Bacteria and Total Microorganism of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract.....	N-1
Statistical Analysis of Total Lactic Acid Bacteria of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract.....	N-2
Statistical Analysis of Total Microorganism of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract .....	N-3

## Appendix O

Sensory Questionnaire of Scoring Test .....	O-1
Sensory Questionnaire of Hedonic Test.....	O-2

## Appendix P

Scoring Test Result of Selected Formulation of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract .....	P-1
Scoring Test Result of Selected Formulation of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract .....	P-5
Statistical Analysis of Scoring Test Result of the Selected Formulation of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract .....	P-8

Statistical Analysis of Scoring Test Result of the Selected Formulation of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract.....	P-11
--	------

Appendix Q

Hedonic Test Result of Selected Formulation of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract .....	Q-1
Hedonic Test Result of Selected Formulation of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract .....	Q-4
Statistical Analysis of Hedonic Test Result of Selected Formulation of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract.....	Q-7
Statistical Analysis of Hedonic Test Result of Selected Formulation of Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract .....	Q-10

Appendix R

Moisture Content of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control .....	R-1
Ash Content of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control .....	R-2
Protein Content of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control .....	R-3
Fat Content of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control .....	R-4
Carbohydrate Content of Best Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control .....	R-5
Statistical Analysis of Proximate Composition of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control.....	R-6

Appendix S

Moisture Content of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract and Control.....	S-1
Ash Content of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract and Control.....	S-2
Protein Content of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract and Control.....	S-3
Fat Content of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract and Control .....	S-4

Carbohydrate Content of Best Cowpea Yoghurt Added with Green Grass Jelly Leaf Crude Extract and Control.....	S-5
Statistical Analysis of Proximate Composition of Cowpea Yoghurt Added with African Bitter Leaf Crude Extract and Control .....	S-6
Appendix T	
Total colony of Lactobacillus bulgaricus calculation .....	T-1
Growth curve of Lactobacillus bulgaricus .....	T-1
Total colony of Streptococcus thermophilus calculation .....	T-2
Growth curve of Streptococcus thermophilus .....	T-2
Appendix U	
Verification of Cowpea Taxonomy .....	U-1