

# CONTENTS

	Page
Acknowledgement	d
Abstract in Thai	e
Abstract in English	g
List of Tables	k
List of Figures	n
Statement of Originality in Thai	p
Statement of Originality in English	q
Chapter 1 Introduction	1
1.1 Rationale	1
1.2 Research objectives	2
1.3 Education/application advantages	2
1.4 Research design, scope and method	3
Chapter 2 Literature Reviews	4
2.1 Soy and soy germ isoflavones	4
2.2 Isoflavones extraction	8
2.3 The role of isoflavones on health	10
2.4 The researched of isoflavone aglycones production	13
2.5 Isoflavone aglycones purification	13
2.6 Bacteria associated with soy fermentation and $\beta$ -glucosidase production	15
2.7 Sensory Evaluation	18
Chapter 3 Materials and Methodology	20
Research design	20

Chapter 4	Results and Discussion	31
4.1	The optimal isoflavone glucosides extraction for precursor to isoflavone aglycones production	31
4.1.1	Study on isoflavone glucosides extraction from soy germ	31
4.1.2	The optimal concentration of ethanol on efficiency isoflavone glucosides extraction	34
4.1.3	Effect of extraction time and temperature on efficiency of isoflavone glucosides from soy germ	35
4.1.4	Effect of extraction time investigation on efficiency of isoflavone glucosides from soy germ	40
4.2	Study of $\beta$ -glucosidase production from <i>B. coagulans</i> PR03	41
4.2.1	Study of suitable formulas <i>B. coagulans</i> PR03 for $\beta$ -glucosidase production	41
4.2.2	Study on optimal conditions affecting the $\beta$ -glucosidase production from beef extract and acidification	42
4.2.3	Kinetics of production $\beta$ -glucosidase from <i>B. coagulans</i> PR03	46
4.3	The optimal condition of isoflavone aglycones production from soy germ	52
4.3.1	Preliminary the optimal time of isoflavone aglycones production	52
4.3.2	Optimization of time and temperature on isoflavone aglycones production	53
4.3.3	Study on isoflavone purification using amberlite XAD-4	58
4.4	Development of health supplements beverage from isoflavone aglycones	63
4.4.1	Development of prototype for isoflavone aglycones beverage	63
4.4.2	Study on the quality of isoflavone aglycones beverage products from soy germ	70
Chapter 5	Conclusion	74
	References	76
	Appendix	
	Appendix A	86
	Appendix B	90
	Curriculum Vitae	96

## LIST OF TABLES

	Page
Table 2.1 <i>Bacillus</i> spp. detected in Thai native fermented soybean	16
Table 3.1 Experimental design of 2 <sup>2</sup> factorial experiment in central composite design on extraction time and temperature for isoflavone glucosides extraction	23
Table 3.2 The levels of factors for the composition of <i>B. coagulans</i> PR03 cultural media and incubation condition at low (-) and high (+) levels affecting $\beta$ -glucosidase production	24
Table 3.3 Plackett and Burman design for modifying condition affecting the $\beta$ -glucosidase activity	24
Table 3.4 2 <sup>2</sup> factorial experiments in central composite design for find optimal $\beta$ -glucosidase production	25
Table 3.5 2 <sup>2</sup> factorial central composite design for studying time and temperature of isoflavone aglycones production	27
Table 3.6 2 <sup>2</sup> factorial experiment in central composite design for study amount of passion fruit juice and fructose syrup	29
Table 4.1 Isoflavone glucoside content from soy germ by different extraction method	31
Table 4.2 Isoflavone glucosides content extracted from ethanol	34
Table 4.3 2 <sup>2</sup> factorial in central composite design for studied temperature and time of isoflavone glucosides extraction from soy germ	35
Table 4.4 Isoflavone glucosides content of soy germ extract with 80% ethanol at different temperatures and times extraction	36

Table 4.5 The relationship between temperature and time of extraction of isoflavone glucosides	37
Table 4.6 Scopes of study factors and required features	39
Table 4.7 The amount of isoflavone glucosides at different extraction time	40
Table 4.8 $\beta$ -glucosidase activity using Plackett and Burman design (n=8)	41
Table 4.9 Effect of <i>B. coagulans</i> PR03 on $\beta$ -glucosidase activity	42
Table 4.10 The $\beta$ -glucosidase activities from varied beef extract and pH value	43
Table 4.11 Relationship of beef extract and pH value on $\beta$ -glucosidase activity	43
Table 4.12 Scope of factors and required features	45
Table 4.13 Optical density, number of colony and $\beta$ -glucosidase activities at different incubation times of <i>B. coagulans</i> PR03	47
Table 4.14 Isoflavone glucosides content at different incubation time	52
Table 4.15 Isoflavone aglycones content at different incubation time	53
Table 4.16 2 <sup>2</sup> factorial with central composite design for studying the effect of production time and temperature on isoflavone aglycones production.	54
Table 4.17 Isoflavone aglycones production at different times and temperatures	54
Table 4.18 The relationship between production time and temperature of isoflavone aglycones composition	55
Table 4.19 Scope of studied factors and properties	57
Table 4.20 Percentage of isoflavone aglycones purity at amberlite XAD-4 50 g per 100 ml isoflavone aglycones solution	59
Table 4.21 Percentage of isoflavone aglycones purity at amberlite XAD-4 100 g per 100 ml isoflavone aglycones solution	60
Table 4.22 Percentage of isoflavone aglycones purity at amberlite XAD-4 150 g per 100 ml isoflavone aglycones solution	61

Table 4.23 Percentage of isoflavone aglycones purity at amberlite XAD-4 200 g per 100 ml isoflavone aglycones solution	62
Table 4.24 Percentage yield and percent purity of isoflavone aglycones at different Amberlite XAD-4 resin	63
Table 4.25 Nutritional value of isoflavone aglycones beverage	64
Table 4.26 2 <sup>2</sup> factorial in central composite design for studied of passion fruit juice and fructose syrup	65
Table 4.27 Sensory evaluation of isoflavone aglycones beverage varied of passion fruit juice and fructose syrup	66
Table 4.28 Relationship between the amount of passion fruit juice and fructose syrup	67
Table 4.29 Scope of studied factors and desired features	69
Table 4.30 Nutritional value, chemical properties and microbiological properties of developed products	70
Table 4.31 Data of tester	72
Table 4.32 Consumer acceptance test	73
Table B-1 Concentration <i>p</i> -nitrophenol The results showed absorption at 405 nm	93

## LIST OF FIGURES

	Page
Figure 1.1 Scope of isoflavone aglycone production using $\beta$ -glucosidase	3
Figure 2.1 Soy germ	6
Figure 2.2 Structural characteristics of isoflavones	7
Figure 2.3 Isoflavones purification	14
Figure 3.1 Isoflavone extraction with supercritical carbon dioxide	21
Figure 3.2 Isoflavone extraction with supercritical fluid extraction	21
Figure 3.3 Isoflavone extraction with high-power ultra sonication	21
Figure 3.4 Isoflavone extractions from soy germ by variation ethanol concentration	22
Figure 3.5 The study of the kinetics of $\beta$ -glucosidase production	26
Figure 3.6 Amberlite XAD-4 preparations	28
Figure 4.1 Pulp of soy germ after extracted various method	32
Figure 4.2 Scanning electron microscope (SEM) of soy germ structure	33
Figure 4.3 Response surfaces of the equation; temperature and time of isoflavone glucosides extraction	38
Figure 4.4 Response area of temperature and time extraction on isoflavone glucosides	39
Figure 4.5 Response area of the correlation between beef extract and pH value on $\beta$ -glucosidase activity	44
Figure 4.6 Response area of beef extract and pH value on $\beta$ -glucosidase activity	45
Figure 4.7 The study of the kinetics of $\beta$ -glucosidase production	46
Figure 4.8 Optical densities of <i>B. coagulans</i> PR03 at different incubation times	47
Figure 4.9 Number of colonies of <i>B. coagulans</i> PR03 at different incubation time	48
Figure 4.10 $\beta$ -glucosidase activity at different incubation time	48
Figure 4.11 Response surfaces of correlation production time and temperature on isoflavone aglycones	56

Figure 4.12 Response surfaces of production time and temperature on isoflavone aglycones	57
Figure 4.13 Response surface of the relationship of passion fruit juice and fructose syrup for sweetness, sourness and overall acceptance	68
Figure 4.14 Response surfaces of passion fruit juice and fructose syrup	69
Figure A-1 The external appearance of isoflavone glucosides extract	86
Figure A-2 Soy germ extracted	86
Figure A-3 Isoflavone glucosides extract at different extraction temperature and time	87
Figure A-4 <i>B. coagulans</i> PR03 on optimal media	87
Figure A-5 The package of isoflavone aglycones beverage from soy germ (Box)	88
Figure A-6 The package of isoflavone aglycones beverage from soy germ (Bottle)	88
Figure A-7 Isoflavone aglycones beverage	89
Figure B-1 Chromatogram of isoflavones standard	92
Figure B-2 Standard curve of p-nitrophenol	93
Figure B-3 Nutrition Fact (English version)	94
Figure B-4 Nutrition Fact (Thai version)	95

## ข้อความแห่งการริเริ่ม

- 1) วิทยานิพนธ์นี้ได้นำเสนอวิธีการพัฒนากระบวนการผลิตไอโซฟลาโวนชนิดอะไกลโคนด้วยเทคโนโลยีเอนไซม์ บนพื้นฐานของแนวความคิดของ การลดกลิ่นอันไม่พึงประสงค์ของผลิตภัณฑ์ รวมถึงพัฒนาเป็นผลิตภัณฑ์เสริมอาหารในรูปแบบเครื่องดื่มไอโซฟลาโวนชนิดอะไกลโคน ที่เหมาะกับกลุ่มผู้บริโภคในวัยสูงอายุเพื่อลดความเสี่ยงที่เกิดจากโรคต่างๆ
- 2) เพื่อเพิ่มประสิทธิภาพของกระบวนการผลิตไอโซฟลาโวนชนิดอะไกลโคนด้วยเทคโนโลยีเอนไซม์ และกระบวนการพัฒนาผลิตภัณฑ์เครื่องดื่มไอโซฟลาโวนอะไกลโคน ได้นำเสนอในวิทยานิพนธ์นี้

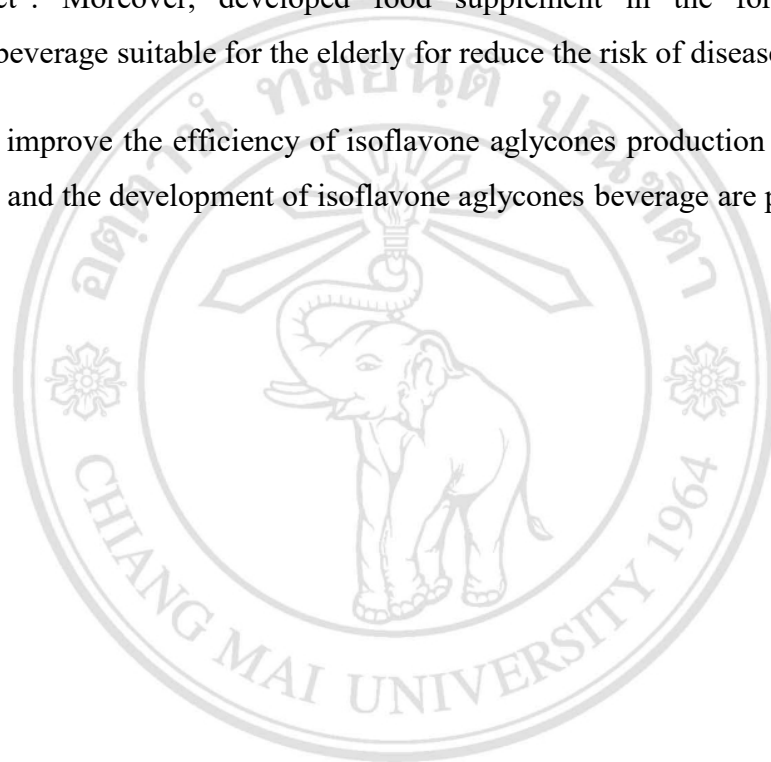


ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved



## STATEMENTS OF ORIGINALITY

- 1) This thesis demonstrates a method of development of isoflavone aglycones production using enzyme technology based on the concepts of “Reduce the odor of the product”. Moreover, developed food supplement in the form isoflavone aglycones beverage suitable for the elderly for reduce the risk of disease.
- 2) In order to improve the efficiency of isoflavone aglycones production using enzyme technology and the development of isoflavone aglycones beverage are proposed.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved