PUSAT PENGAJIAN TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN DISERTASI
MUTAKHIR SATU (1) NASKAH

Nama penyelia: **Dr. Tan Thuan Chew**

Bahagian: **Teknologi Makanan**

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh
~~Encik/Puan/~~Cik Nur Thaherah Binti Mior Azman

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

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

Sekian, terima kasih.

06 August 2020

(Tandatangan dan cop) Tarikh

Dr. Tan Thuan Chew
Senior Lecturer
School of Industrial Technology
Universiti Sains Malaysia

PHYSICOCHEMICAL PROPERTIES AND ENZYME ACTIVITY OF POLYPHENOL OXIDASE AND PEROXIDASE FROM FRESH POMELO (*Citrus maxima*) JUICE

by

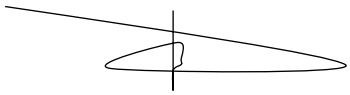
NUR THAHERAH BINTI MIOR AZMAN

Dissertation submitted in partial fulfillment of the requirements for the degree of
Bachelor of Technology (B. Tech) in the field of Food Technology
School of Industrial Technology
Universiti Sains Malaysia

July 2020

DECLARATION BY AUTHOR

I am the author of the above title paper hereby declare that this thesis is belonged to my original work and contained no material previously published or written by another author except the references have been made in text. I also declare that the content of this thesis comprising of the result which have been carried out during my research project and has been submitted as the part of requirement of any other academic degree program.



NUR THAHERAH BT MIOR AZMAN

JULY 2020

ACKNOWLEDGEMENTS

Foremost, I would like to express my deepest gratitude to my supervisor, Dr. Tan Thuan Chew for his convincingly guided, persistent feedback and encouraged me to do the right things which relevant to the goal of this project. Despite of being busy with his duty, he was still willing to sacrifice his time to help me in all the time of project and writing of this thesis. Without his persistent guidance, this project could not have reached its goal.

Besides that, I would like to bear my deepest gratitude to all the laboratory staffs who guide me dealing with the instruments and equipment. Without their guidance I would not manage to complete my laboratory experimental analysis of my project.

I would also to express my deepest thanks to my partnerships who kept provide motivational support and always help me through thick and thin. They also encouraged me to keep going on until this project completed. It would not be possible to write this thesis without support from them.

Last but not least, I would also to express my deepest gratitude to my family who always provide great supported and encouraged me get through until this project completed.

NUR THAHERAH BT MIOR AZMAN

JULY 2020

TABLE OF CONTENTS

	Page
DECLARATION OF AUTHOR	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
LIST OF SYMBOLS	viii
ABSTRAK	ix
ABSTRACT	x
CHAPTER 1 INTRODUCTION	
1.1 Research background	1
1.2 Rationale of the study	3
1.3 Objectives	3
CHAPTER 2 LITERATURE REVIEW	
2.1 Citrus fruit	4
2.2 Pomelo (*Citrus maxima*)	5
2.2.1 Origin and distribution	5
2.2.2 Nomenclature	5
2.2.3 Fruit characteristics	6
2.2.4 Properties of fruit	8
2.2.5 Fruit usage	10
2.3 Regulation of fruit juice	10
2.3.1 Regulation 237	10

2.3.2 CODEX General Standard For Fruit Juices 11
2.4 Browning 13
2.4.1 Enzymatic browning 13
2.4.1.1 Polyphenol oxidase 14
2.4.1.2 Peroxidase 15
2.4.2 Non enzymatic browning 16
2.5 Factor affecting enzyme activity 16
2.5.1 pH 16
2.5.2 Temperature 17
2.5.3 Maturity and composition of fruit 18
2.5.4 Variety of fruit 18
CHAPTER 3 MATERIALS AND METHODS
3.1 Materials 20
3.1.1 Pomelo (*Citrus maxima*) 20
3.1.2 Chemical reagents 20
3.2 Methods 20
3.2.1 Sample preparation 20
3.2.2 Yield 21
3.2.3 Physicochemical properties analysis 21
3.2.3.1 pH 21
3.2.3.2 Titratable acidity 21
3.2.3.3 Total soluble solids 22
3.2.3.4 Total phenolic content 22
3.2.3.5 Vitamin C content 23
3.2.4 Enzyme activity 24

3.2.4.1 Determination of polyphenol oxidase 24

3.2.4.2 Determination of peroxidase activity 25

3.2.5 Statistical analysis 25

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 Visual appearance 26

4.2 Yield 26

4.3 Physicochemical properties of pomelo juice 27

4.3.1 pH 27

4.3.2 Titratable acidity 28

4.3.3 Total soluble solids 29

4.3.4 Total phenolic content 30

4.3.5 Vitamin C content 30

4.4 Enzyme activity 32

4.4.1 Polyphenol oxidase activity of pomelo juice 32

4.4.2 Peroxidase activity of pomelo juice 32

4.4.2.1 Effect of pH on peroxidase activity 33

4.4.2.2 Effect of temperatures on peroxidase activity 35

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.5 Conclusions 38

5.2 Recommendations 38

REFERENCES 40

APPENDICES 48

LIST OF TABLES

Table	Caption	Page
2.1	Botanical classification of pomelo.	6
2.2	Common names of pomelo.	6
2.3	Nutrient compositions of pomelo (*Citrus maxima*) fruit.	8
2.4	Minimum Brix level for reconstituted juice based on Annex.	13
2.5	Optimum pH for polyphenol oxidase and peroxidase in various fruits.	17
2.6	Optimum temperature for polyphenol oxidase and peroxidase in various fruits.	18
3.1	List of chemicals and reagents used for analysis.	20
4.1	Physicochemical properties of pomelo juice.	31
4.2	Effect of pH on the activity of peroxidase in pomelo juice.	35
4.3	Effect of temperature on the activity of peroxidase in pomelo juice.	37

LIST OF FIGURES

Table	Caption	Page
2.1	Cross section of pomelo fruit.	7
2.2	White and pink cultivar pomelo.	8
2.3	Schematic reaction catalysed by polyphenol oxidase.	14
2.4	Schematic reaction catalysed by peroxidase.	15
4.1	Visual appearance of pomelo pulp.	26
4.2	Visual appearance of pomelo juice	26

LIST OF ABBREVIATIONS

Abbreviations	Definition
ANOVA	Analysis of Variance
DF	Dilution factor
GAE	Gallic acid equivalents
PPO	Polyphenol oxidase
POD	Peroxidase
TA	Titratable acidity
TSS	Total soluble solids
TPC	Total phenolic content

LIST OF SYMBOLS

Symbols	Definition
hr	Hour
L	Litre
min	Minute
s	second
mL	Millilitre
N	Concentration in normality (g/L)
P	Significant level (dimensionless)
%	Percentage
°C	Degree Celsius

SIFAT-SIFAT FIZIKOKIMIA DAN AKTIVITI ENZIM POLIFENOL OKSIDASE DAN PEROKSIDASE DARIPADA JUS LIMAU BALI (*Citrus maxima*) SEGAR

ABSTRAK

Limau bali (*Citrus maxima*) ialah buah asli Asia Tenggara dan berpotensi untuk dikomersialkan dalam bentuk jus buah. Kajian mengenai sifat-sifat fizikokimia daripada jus limau bali segar dapat diukur dengan keasidan, keasidan boleh titrat, jumlah pepejal larut, jumlah kandungi fenolik dan kandungan vitamin C untuk memperoleh maklumat yang berguna di dalam industri makanan. Jus limau bali memenuhi kriteria yang dinyatakan di dalam Peraturan-peraturan Makanan 1985, Peraturan 237 dengan mengandungi jumlah pepejal terlarut 9.83 ± 0.12 °Brix yang mewakili kemanisan jus buah. Keasidan jus limau bali dicatatkan 6.24 ± 0.01yang dianggap sebagai makanan yang kurang berasid dengan nilai keasidan tertitrat 0.09 ± 0.01% adalah rendah. Tambahan pula, jus limau bali berkeupayaan untuk dikomersialkan sebagai jus buah yang menyegarkan kerana mengandungi banyak kandungan fenolik dan vitamin larut air 90.09 ± 6.74 mg GAE/100 g dan 65.59 ± 2.00 mg/100 mL. Peroksidase (POD) didapati hadir dan mempunyai aktiviti yang lebih tinggi daripada polifenol oksidase (PPO) di mana aktiviti enzim tidak boleh dikesan pada keasidan 5.5 dan suhu 25 °C yang ditetapkan dengan beberapa pengubahsuaian terhadap isi padu sampel oleh itu, POD digunakan dalam kajian ini. Aktiviti POD telah didapati maksimum pada pada keasidan 5.5 dan 6.0 manakala suhu optima dapat dilihat pada 60 °C dan 70 °C. Justeru itu, jus limau bali dicadangkan untuk menjalani suhu rendah masa panjang di dalam industri makanan kerana rawatan terma yang tinggi boleh menggalakkan aktiviti peroksidase di dalam jus limau bali.

PHYSICHOCHEMICAL PROPERTIES AND ENZYME ACTIVITY OF POLYPHENOL OXIDASE AND PEROXIDASE FROM FRESH POMELO (*Citrus maxima*) JUICE

ABSTRACT

Pomelo (*Citrus maxima*) is a native of Southeast Asia and has the potential of being commercialized in form of fruit juice. A study of physicochemical properties of fresh pomelo juice was determined its pH, titratable acidity, total soluble solids, total phenolic content, and vitamin C content in order to obtain useful information in food industries. The pomelo juice fulfilled the requirement as stated in the Food Regulation 1985, Regulation 237 with 9.83 ± 0.12 °Brix which represented the sweetness of the fruit. The pH of pomelo juice was recorded to be 6.24 ± 0.01 which considered as less acidic food with low (0.09 ± 0.01%) titratable acidity value. Furthermore, pomelo juice has the potential to be commercialized as thirst quenching fruit juice due to a considerable amount of total phenolic content and vitamin C 90.09 ± 6.74 mg GAE/100 g and 65.59 ± 2.00 mg/100 mL. The peroxidase (POD) was observed to be present and actively higher than polyphenol oxidase (PPO) whereby the activity of enzyme cannot be detected at pH 6.0 and temperature 25 °C with some modifications on the sample volume thus, POD was found to be useful in this study. The activity of POD was found to be optimum at pH 5.5 and pH 6.0 while the optimum temperature was observed at 60 °C and 70 °C. Hence, pomelo juice is suggested to undergo low temperature long time or LTLT in food industries because high thermal treatment can promote the activity of POD in pomelo juice