

UNIVERSITI PUTRA MALAYSIA

FACTORS AFFECTING BROWNING OF SAGO (METROXYLON SAGU ROTTB.) PITH AND THEIR EFFECTS ON SAGO STARCH

SHIRLENE MARIA ANTHONYSAMY

FSMB 2002 16

FACTORS AFFECTING BROWNING OF SAGO (*METROXYLON SAGU* ROTTB.) PITH AND THEIR EFFECTS ON SAGO STARCH

Ву

SHIRLENE MARIA ANTHONYSAMY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of Requirement for the Degree of Master of Science

October 2002



Dedication

Specially dedicated to my late father, S. Anthonysamy

and

To my beloved mother Elizabeth Thevasagayam

You have made me what I am today.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

FACTORS AFFECTING BROWNING OF SAGO (*METROXYLON SAGU* ROTTB.) PITH AND THEIR EFFECTS ON SAGO STARCH

By

SHIRLENE MARIA ANTHONYSAMY

October 2002

Chairman : Assoc. Prof. Dr. Nazamid Saari

Faculty : Food Science and Biotechnology

The effect of four parameters on the browning of sago pith was evaluated i.e. maturity of the sago palm, and holding time, pH and temperature of the sago pith slurry. The effect of sago palm maturity on the browning of sago pith was determined using sago pith slurries from three maturity stages i.e. young, premature and mature. The sago palms were sectioned into top and bottom to examine the effect of trunk growth on the concentrations of soluble phenolic compounds. Two soluble phenolic compounds were found to be present in the sago pith i.e. (+)-catechin and (-)-epicatechin. Concentrations of (+)-catechin and (-)-epicatechin were higher in mature sago palms while distribution of these compounds with trunk gowth did not show a clear correlation. Soluble polyphenol oxidase (SPPO) activity increased while latent polyphenol oxidase (LPPO) activity decreased with increase in maturity of the sago palms. Colour development in the slurry monitored in terms of lightness (L) and redness (a) using the HunterLab Ultrascan Spectrocolorimeter was more intense (darker and redder) in mature sago palms.



The effect of holding time, pH and temperature of the sago pith slurry was studied using a mature sago palm. Browning was evaluated based on concentrations of (+)-catechin and (-)-epicatechin and colour development in the sago pith slurry. The effect of holding time (duration for which sago pith slurries were held) on the browning of sago pith slurry was studied for 1, 6, 12 and 24 hours. An increase in holding time resulted in significant (P<0.05) decreases of (+)-catechin and (-)-epicatechin. Colour development in the slurry was significantly (P<0.05) more intense with increased holding time but was not significant (P<0.05) after six hours. The effect of pH on the browning of sago pith was studied using sago pith slurries with pH from 3.5 to 7.0 with a holding time of 24 hours. Concentrations of (+)-catechin and (-)-epicatechin decreased significantly (P<0.05) with an increase in pH particularly between pH 5.0 and 6.5 which resulted in significant (P<0.05) colour development. The effect of temperature on the browning of sago pith was investigated using sago pith slurries with temperatures from 10°C to 50°C with a holding time of 24 hours. The amount of (+)-catechin and (-)-epicatechin decreased significantly (P<0.05) with the increase in temperature while colour development increased significantly (P<0.05) with temperatures particularly above 40°C.

The quality of sago starch extracted from slurries with different pH and temperatures and held for 24 hours was determined. Starch quality was determined in terms of colour development, granule morphology and pasting profile. Significant (P<0.05) colour development was noted in starches extracted from slurries of different pH and temperatures though they were washed twice during extraction. Granule morphology of the starches was observed using the Scanning Electron Microscope. Starches from slurries of strong acidic (pH 3.5 to 4.5) conditions exhibited fissures on the surface of granules while those from weaker acidic (pH 5.0 to 7.0) conditions had

minor protrusions. Starches from slurries at different temperatures had only minor modifications on the surface of the granules. The pasting profile of the starches was examined using the Brabender Viskograph. Starches from strong acidic conditions showed lower viscosities while starches from high temperatures (30°C to 50°C) were not greatly affected.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat ijazah Master Sains

FAKTOR YANG MEMPENGARUHI PEMERANGAN DALAM SLURI SAGU (METROXYLON SAGU ROTTB.) DAN KESANNYA TERHADAP KANJI SAGU

Oleh

SHIRLENE MARIA ANTHONYSAMY

Oktober 2002

Pengerusi : Prof. Madya Dr. Nazamid Saari

Fakulti : Sains Makanan dan Bioteknologi

Empat parameter utama pemerangan dalam sagu telah dikaji iaitu kematangan batang sagu, dan jangka masa penyimpanan, pH serta suhu sluri sagu. Kesan kematangan batang sagu terhadap pemerangan sagu dilakukan dengan menggunakan sluri sagu dari tiga peringkat kematangan iaitu muda, pramatang dan matang. Pokok sagu dibahagikan mengikut bahagian atas dan bawah untuk mengkaji kesan pertumbuhan batang sagu terhadap kepekatan bahan fenolik terlarut. Dua bahan fenolik terlarut didapati hadir dalam sagu iaitu (+)-katekin dan (-)-epikatekin. Kepekatan (+)-katekin dan (-)-epikatekin didapati lebih tinggi dalam pokok sagu yang matang sementara taburan bahan ini dengan pertumbuhan batang sagu tidak menunjukkan korelasi yang nyata. Aktiviti enzim polifenol oksida terlarut meningkat manakala aktiviti enzim polifenol oksida tidak terlarut menurun dengan meningkatnya kematangan batang sagu. Perkembangan warna sluri sagu yang diukur berdasarkan keterangan (L) dan kemerahan (a) dengan menggunakan HunterLab Scan menunjukkan bahawa batang sagu yang matang menghasilkan warna yang lebih ketara (lebih gelap dan merah).

Kesan jangka masa penyimpanan, pH dan suhu sluri sagu dikaji dengan menggunakan batang sagu yang matang. Pemerangan dikaji berdasarkan kepekatan (+)-katekin dan (-)-epikatekin serta kewujudan warna. Kesan jangka masa penyimpanan (tempoh sluri sagu disimpan) terhadap pemerangan sagu diperhatikan untuk 1, 6, 12 dan 24 jam. Peningkatan jangka masa pemerangan menyebabkan penurunan dalam kepekatan bahan fenolik secara signifikan (P<0.05). Kewujudan warna lebih ketara secara signifikan (P<0.05) dengan meningkatnya jangka masa pemerangan tetapi tidak signifikan (P<0.05) selepas enam jam. Kesan pH terhadap pemerangan sluri sagu dikaji dengan menggunakan sluri sagu pada pH antara 3.5 hingga 7.0 serta jangka masa pemerangan selama 24 jam. Kepekatan (+)-katekin dan (-)-epikatekin menurun secara signifikan (P<0.05) dengan meningkatnya pH terutamanya antara pH 5.0 dan 6.5 yang menyebabkan kewujudan wama sluri sagu yang signifikan (P<0.05). Kesan suhu terhadap pemerangan sluri sagu diperhatikan dengan menggunakan sluri sagu pada suhu antara 10°C hingga 50°C serta jangka masa pemerangan selama 24 jam. Amaun (+)-katekin dan (-)-epikatekin menurun secara signifkan (P<0.05) dengan meningkatnya suhu sementara kewujudan warna sluri meningkat secara signifikan (P<0.05) dengan meningkatnya suhu terutamanya pada suhu melebihi 40°C.

Kualiti kanji sagu yang diekstrak daripada sluri yang berlainan pH dan suhu serta jangka masa penyimpanan selama 24 jam dikaji. Kualiti kanji sagu ditentukan dari segi kewujudan warna, morfologi granul dan profil pemasakan. Kewujudan warna yang signifikan (P<0.05) dikesan pada kanji sagu yang diekstrak daripada sluri yang berlainan pH dan suhu walaupun telah dibasuh sebanyak dua kali semasa pengekstrakan. Morfologi granul kanji sagu diteliti menggunakan *Scanning Electron*



Microscope. Granul kanji sagu daripada sluri dengan pH asid kuat (pH 3.5 hingga 4.5) mempunyai kesan rekahan pada permukaannya manakala granul sagu dari sluri dengan pH asid lemah (pH 5.0 hingga 7.0) hanya mempunyai sedikit bonjolan. Kanji sagu daripada sluri pada suhu berlainan hanya menunjukkan sedikit modifikasi pada permukaan granul. Profil pemasakan kanji ditentukan dengan menggunakan *Brabender Viskograph*. Kanji sagu daripada sluri dengan pH asid kuat mempunyai viskositi yang lebih rendah manakala kanji sagu daripada sluri dengan suhu lebih tinggi (30°C hingga 50°C) pula tidak banyak berubah.



ACKNOWLEDGEMENTS

Writing up a research thesis is no easy task. It is made more stressful when you do not have the right people around you. Among the many souls that have helped me along the way, my foremost gratitude goes to my Project Supervisor Assoc. Prof. Dr Nazamid Saari. He has been very very patient with me despite my shortcomings. His encouragement and valuable advice has been my motivation all the while. Special appreciation must also be extended to Assoc. Prof. Dr Sharifah Kharidah Muhammad who has been really generous with her time and guidance. Not forgetting my other co-supervisors Dr Fatimah Abu Bakar for her kind assistance and motivation and Assoc. Prof. Dr Radzali Muse for his fruitful opinions and invaluable time. I would also like extend my recognition to Professor Dr Hasanah Mohd Ghazali who has been gracious with her time and advice in assessing my thesis.

For all those staff of FSMB who have helped me during the course of my research: Mr Chan, Kak Jam, Encik Azman, Encik Halim, Kak Rauna, Kak Siti, Kak Husaini; words are not enough to thank you for the help you have given me. My labmates Yetty, Galila, Kala, Mei Chee and Mahanum Mahmud; thank you for your companionship and help, life would have been millions of minutes slower without you around. My mom, my mentor, as always, thank you so much for putting up with me. Not forgetting my sister; Selvi Margaret, my brothers; Justin Joseph, Isaac Stephen and my in-laws. Thank you for your support, your encouragement, your prayers and your love that kept me going and still is. Lastly, to the one above, your mysterious ways of answering prayers is always an amazement. Nothing would have been possible without you!



TABLE OF CONTENTS

DEDICATION		2
ABSTRACT	0	3
ABSTRAK		6
ACKNOWLEDGEMENTS		9
APPROVAL		10
DECLARATION		12
LIST OF TABLES		16
LIST OF FIGURES		17
LIST OF PLATES		20
LIST OF ABBREVIATIONS		23

CHAPTER

1	INTRODUCTION	25
2	LITERATURE REVIEW The Sago Palm (<i>Metroxylon sagu</i>) History Characteristics of the Sago Palm Properties of Sago Pith Properties of Sago Starch Significance of Sago Starch in Industrial Application Processing of Sago Starch Traditional Extraction Method Modern Extraction Method Problems Encountered during the Processing of Sago Starch Enzymatic Browning Phenolic Compounds Polyphenol Oxidase and Peroxidase Factors that Influence Browning Maturity of Plant Material Effect of Processing Conditions Evaluation of Browning Identification and Separation of Phenolic Compounds Measurement of Colour Development Evaluation of Starch Quality as Affected by pH and temperature	29 29 31 33 41 43 45 46 48 49 52 56 57 60 61 64 66
3	Granule Morphology Pasting Profile EFFECT OF SAGO (<i>METROXYLON SAGU</i>) PALM MATURITY ON TH	66 67 HE
5	BROWNING OF SAGO PITH Introduction Materials and Methods Plant Material Chemicals and Reagents	70 70 71 71 71

	Extraction of Soluble Phenolic Compounds Separation of Soluble Phenolic Compounds using HPLC Measurement of Colour Extraction of SPPO and LPPO Measurement of SPPO and LPPO Activities Measurement of Protein Concentration Statistical Analysis Results and Discussion Soluble Phenolic Compounds in Sago Pith Effect of Sago Palm Maturity in the Browning of Sago Pith Slurry Concentration of Soluble Phenolic Compounds Activities of SPPO and LPPO Colour Development Conclusion	72 73 74 75 75 76 77 77 82 82 84 86 88
4	 EFFECT OF HOLDING TIME, pH and TEMPERATURE OF SAGO PITH SLURRY ON ITS BROWNING Introduction Materials and Methods Plant Material Chemicals and Reagents Preparation of Sago Pith Slurry Separation of Soluble Phenolic Compounds using HPLC Measurement of Colour Statistical Analysis Results and Discussion Effect of Holding Time of Sago Pith Slurry on the Browning of Sago Concentration of Soluble Phenolic Compounds Oxidation of Soluble Phenolic Compounds 	 89 89 90 90 91 91 91 92 92 93 94 97 97 97 97 97 97 97 97 101 101 102 104
5	EFFECT OF HOLDING TIME, pH AND TEMPERATURE OF SAGO PITH SLURRY ON THE QUALITY OF SAGO STARCH Introduction Materials and Methods Plant Material Chemicals and Reagents Preparation of Sago Pith Slurry Starch Extraction Determination of Colour Determination of Starch Structure	106 106 107 107 107 107 108 108 108



Determination of Pasting Profile	109
Statistical Analysis	109
Results and Discussion	110
Effect of pH and Holding Time of Sago Pith Slurry on the Quality	
of Sago Starch	110
Colour of Sago Starch	110
Granule Morphology	116
Pasting Profile of Sago Starch	121
Effect of Temperature and Holding Time of Sago Pith Slurry on	123
the Quality of Sago Starch	
Colour of Sago Starch	123
Granule Morphology	127
Pasting Profile of Sago Starch	131
Conclusion	133
6 GENERAL CONCLUSION AND RECOMMENDATIONS	134
BIBLIOGRAPHY	
APPENDICES	
BIODATA OF THE AUTHOR	150



LIST OF TABLES

TABLE		Page
1	Composition of Sago Pith	34
2	Comparison of granule sizes of various starches	35
3	Comparison of amylose content in various starches	36
4	Various uses of sago starch in small industries	41
5	Relative substrate specificities of three polyphenol oxidases	54
6	Concentration of (+)-catechin and (-)-epicatechin in theTop and Botton Sections of the Mature Sago Palm	m 81
7	Concentration of (+)-catechin and (-)-epicatechin after different holding times of the sago pith slurry	94



,

LIST OF FIGURES

FIGURE		Page
1	Uses of Sago Palm	38
2	Flow chart of modem extraction of sago starch	46
3	Commonly occurring Phenolic Compounds	50
4	Optical Isomers of Catechin	52
5	HPLC Chromatogram of Standard Phenolics	78
6	HPLC Chromatogram of Sago Pith Slurry from Top section of the Mature Sago Palm	79
7	HPLC Chromatogram of Sago Pith Slurry from Bottom section of the Mature Sago Palm	80
8	Concentration of (+)-catechin and (-)-epicatechin in Sago Pith at at at different stages of Sago Palm Maturity	83
9	SPPO and LPPO Activities from different stages of Sago Palm Maturity	85
10	L values of Sago Pith Slurry from different stages Sago Palm Maturity	86
11	a values of Sago Pith Slurry from different stages of Sago Palm Maturity	87
12	Absorbance of Sago Pith Slurry Supernatant from different stages of Sago Palm Maturity	87



13	Amount of (+)-catechin and (-)-epicatechin in the Sago Pith Slurry after different holding times	93
14	L and a values of Sago Pith Slurry after different holding times	95
15	Absorbance of Sago Pith Slurry Supernatant after different holding times	96
16	Amount of (+)-catechin and (-)-epicatechin in the Sago Pith Slurry after 24 hours of holding time at different pH	98
17	L and a values of Sago Pith Slurry after 24 hours of holding time at different pH	100
18	Absorbance of Sago Pith Slurry supernatant after 24 hours of holding time at different pH	100
19	Amount of (+)-catechin and (-)-epicatechin in the Sago Pith Slurry after 24 hours of holding time at different temperature	102
20	L and a values of Sago Pith Slurry after 24 hours of holding time at different temperature	103
21	Absorbance of Sago Pith Slurry Supernatant after 24 hours of holding time at different temperature	104
22	L values of Sago Starch extracted from Sago Pith Slurry after 24 hours of holding time at different pH	111
23	a values of sago starch extracted from sago pith slurry after hours of holding times at different pH	111
24	Pasting profile of Starches extracted at different pH	122
25	L values of Sago Starch after 24 hours holding time at different temperature	123



26	a values of Sago Starch after 24 hours of holding time at different temperature	
27	Pasting profile of Starches extracted at different temperature	132



LIST OF PLATES

PLATE		Page
1	Sago Palms	30
2	Mature Sago Palm	42
3	Sago logs ready to be towed to the factory	42
4	Logs being fed into a rasper	43
5	Shaker sieves with water added simultaneously	44
6	Long wooden troughs for starch sedimentation	44
7	Sedimented starch	45
8	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 3.5	112
9	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 4.0	112
10	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 4.5	113
11	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 5.0	113
12	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 5.5	114



13	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 6.0	114
14	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 6.5	115
15	Starch extracted from Sago Pith Slurry after 24 hours of holding time at pH 7.0	115
16	Starch granules from Sago Pith Slurry for pH control	117
17	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 3.5	117
18	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 4.0	118
19	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 4.5	118
20	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 5.0	119
21	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 5.5	119
22	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 6.0	120
23	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 6.5	120
24	Starch granules from Sago Pith Slurry after 24 hours of holding time at pH 7.0	121
25	Starch extracted from Sago Pith Slurry after 24 hours of holding time at 10 °C	125



26	Starch extracted from Sago Pith Slurry after 24 hours of holding time at 20 °C	125
27	Starch extracted from Sago Pith Slurry after 24 hours of holding time at 30 $^{\circ}\mathrm{C}$	126
28	Starch extracted from Sago Pith Slurry after 24 hours of holding time at 40 $^{\circ}\mathrm{C}$	126
29	Starch extracted from sago pith slurry after 24 hours of holding time at 50 °C	127
30	Starch granules from Sago Pith Slurry for temperature control	128
31	Starch granules from Sago Pith Slurry after 24 hours of holding time at 10°C	129
32	Starch granules from Sago Pith Slurry after 24 hours of holding time at 20°C	129
33	Starch granules from Sago Pith Slurry after 24 hours of holding time at 30 °C	130
34	Starch granules from Sago Pith Slurry after 24 hours of holding time at 40 $^{\circ}\mathrm{C}$	130
35	Starch granules from Sago Pith Slurry after 24 hours of holding time at 50°C	131



LIST OF ABBREVIATIONS

Absorbance Unit	AU
Brabender Unit	BU
Carbon eighteen	C ₁₈
Carbon six	C ₆
Carbon three	C ₃
High Performance Liquid Chromatography	HPLC
Kilogram	kg
Latent Polyphenol Oxidase	LPPO
Mature Bottom	MB
Mature Top	MT
Microgram	μg
Microgram per millilitre	μg/L
Microlitre	μL
Micrometre	μ m
Milli molar	mM
Millilitre	ml
Millimetre	mm
Millitre per minute	ml/min
Minutes	min
Molar	Μ
Nanometre	nm
Normality	Ν
Polyphenol oxidase	PPO



Premature Bottom	PMB
Premature Top	PMT
Relative centrifugal force unit	RCF
Retention time	Rτ
Scanning Electron Microscope	SEM
Soluble Polyphenol Oxidase	SPPO
Ultraviolet	UV
Volume per volume	v/v
Weight per volume	w/v
Young Bottom	YB
Young Top	ΥT

