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PRODUCTION AND PROPERTIES OF SHELF-STABLE SPRAY-DRIED POWDER FROM ENZYME-TREATED SOURSOP (Annona muricata L.) FRUIT

CHANG LEE SIN

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By

CHANG LEE SIN

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Chair: Prof. Hasanah Mohd Ghazali, PhD
Faculty: Food Science and Technology

In Malaysia, the production of soursop (*Annona muricata* L.) fruit has increased dramatically due to a strong consumer demand because of its nutritional and health protective values. However, one of the apparent features of this fruit is that it softens easily after harvested due to a high respiration rate, and this leads to a short shelf life. Thus, in this study, fresh soursop fruit was preserved by spray-drying it into a shelf-stable powder that may serve the food industry as a value-added intermediate product, and also in the health industry as a food supplement. In the first part of the study, commercially available enzyme preparations, Pectinex® Ultra SP-L (pectinase), Fungamyl® 800 L (α-amylase) and Celluclast® 1.5 L (cellulase) (Novozymes, Denmark), were used in a pre-treatment step to liquefy soursop mesocarp and 1.5 % (v/w) pectinase was selected as the primary enzyme because it produced soursop puree effectively. Besides, when pectinase was combined with cellulase, a liquefied puree with a significant ($p \leq 0.05$) reduction of viscosity of up to 50 % within a shorter incubation time (75 minutes) was produced. Apart from determining many physicochemical properties such as pH, titratable acidity, total soluble solid, sugar, and organic acid profiles, the volatile compounds of soursop fruit and puree were compared using a zNose (Ultrafast GC, USA). Principal Component Analysis (PCA) results indicated six important volatile compounds generated the highest total variance (92.9 %) which classifies the aroma profiles into three groups: raw soursop fruit, soursop fruit treated with pectinase, and liquefied puree from combined enzyme treatment. Two ester compounds, namely methyl hexanoate and methyl *trans*-2-hexenoate, were identified as the major volatile compounds present in soursop.

In an attempt to optimize the production conditions for soursop powder, Response Surface Methodology (RSM) was applied to study the effect of cellulase concentration (0 to 2 % v/w) at fixed pectinase concentration (1.5 % v/w), the addition of maltodextrin (20 to 40 % w/w), and spray-drying inlet temperature (130

to 160 ºC) on the properties of spray-dried soursop powder. Results showed that the polynomial model was significantly fitted ($p \leq 0.05$) for process yield, moisture content, water activity, hygroscopicity, and stickiness. However, the bulk density, true density, porosity, particle size and water adsorption index did not fit significantly ($p > 0.05$) into the model. The color of the spray-dried soursop powder appeared slightly creamy, most probably due to the combination of soursop puree (cream) and maltodextrin (white). The glass transition temperature (T_g) was found to range between 46.53 and 58.25 °C, indicating the spray-dried soursop powder is an amorphous material. Surface morphology of powder, viewed by Scanning Electron Microscopy (SEM), showed that the particles exhibited the general morphology of amorphous powder: spherical shape and possessed a continuous wall (crust) without surface cracks. Based on multiple responses optimization of the process, puree that is pre-treated with 1.3 % (v/w) cellulase and incorporated with 37 % w/w maltodextrin and then spray-dried at an inlet temperature of 156 ºC may be transformed into soursop fruit powder that had optimal physicochemical properties.

At the optimum spray drying conditions, the soursop powder obtained had residual stickiness when stored. Thus, the effect of addition of different types [tricalcium phosphate (TCP) and calcium silicate (CS)] and concentrations (0 - 1.5 % w/w) of anticaking agents, and storage temperatures (conventional storage at 25 ± 1 ºC and accelerated storage at 38 ± 1 ºC) on properties of the powder heat-sealed in aluminum laminated polyethylene (ALP) pouches was examined. Statistically, results showed the addition of either of the anticaking agent significantly ($p \leq 0.05$) increased the process yield of powder. The physicochemical properties of the powder were also significantly ($p \leq 0.05$) affected by the presence of an anticaking agent, and by storage time and temperature. The critical moisture content (X_c) for the powder was ranged from 0.069 to 0.072 g H_2O/g ds. Kinetic modelling for color change (ΔE) showed zero order degradation reaction. The lowest kinetic constant was recorded for 1.5 % CS which had the highest activation energy (Ea) (17.26 kJ/mol), indicating powder added with CS had less tendency to undergo color change. Powder added with 1.0 % TCP or 1.5 % CS were optimal for the production of soursop powder, and for estimation of shelf life.

Shelf life of powder optimized under the conditions obtained above was estimated based on the moisture sorption isotherm (MSI) of soursop powder which was determined gravimetrically. Results indicated that the equilibrium relative humidity (ERH), the type of anticaking agents, and storage temperatures had significant ($p \leq 0.05$) effects on the equilibrium moisture content (EMC) of soursop powder. The Guggenheim, Anderson, and de Boer (GAB) model which was used to fit sorption data reflected a sorption curve which followed Type III Brunauer's classification. The monolayer moisture content (M_o) of the powder varied from 0.0221 to 0.0243 g H_2O/g ds. The longest shelf life was predicted for powder treated with 1.5 % TCP (316 days). Prediction of shelf life using the kinetic model was reasonably adequate as the calculated mean relative percent deviation modulus was less than 10 % (2.3 - 6.9 %). Hedonic test of five sensory attributes, namely aroma, color, mouthfeel, taste, and overall acceptability, for all reconstituted soursop drink showed mean scores that were higher than six from a maximum of nine, indicating high acceptability.

Overall, the production of shelf-stable soursop powder was made possible by having a spray drying feed that was puree-like that had low viscosity due to enzymatic treatment of fruit pulp. Soursop powder produced by spray drying exhibited general properties and morphology of an amorphous powder. The addition of food additives demonstrated anticaking effect by reducing moisture adsorption of powder to prevent caking phenomenon. Both anticaking agents prolonged the shelf life of powders possibly by forming a protective barrier on the sample particle to improve powder stability over time.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN DAN PENCIRIAN SERBUK KERING-SEMBUR DARIPADA BUAH DURIAN BELANDA (*Annona muricata* L.) YANG TERAWAT ENZIM

Oleh

CHANG LEE SIN

Mei 2017

Pengerusi: Prof. Hasanah Mohd Ghazali, PhD
Fakulti: Sains dan Teknologi Makanan

Pengeluaran buah durian belanda (*Annona muricata* L.) telah meningkat secara mendadak di Malaysia disebabkan permintaan pengguna kerana nilai khasiat buah ini. Walau bagaimanapun, salah satu ciri buah ini adalah ia melembut dengan mudah kerana ia menpunyai kadar respirasi yang tinggi menyebabkan jangka hayat yang pendek. Jadi, dalam kajian ini, buah durian belanda ditransformasi menjadi serbuk yang stabil dengan teknik pengeringan sembur supaya menjadikan produk pertengahan yang mempunyai nilai tambah yang sihat untuk industri makanan. Dalam bahagian pertama, enzim komersial iaitu Pectinex® Ultra SP-L (pectinase), Fungamyl® 800 L (α-amilase) dan Celluclast® 1.5 L (selulase) (Novozymes, Denmark), telah digunakan dalam langkah pra-rawatan untuk mencairkan mesokarpa durian belanda dan 1.5 % (v/w) pectinase telah dipilih sebagai enzim utama kerana ia menghasilkan puri durian belanda dengan berkesan. Selain itu, apabila pectinase digabungkan dengan selulase, puri yang signifikan ($p \leq 0.05$) lebih cair dengan pengurangan kelikatan sebanyak 50 % dihasilkan dalam jangka pengeraman yang lebih pendek (75 minit). Selain menentukan ciri-ciri fizikokimia seperti pH, keasidan tertitrat, jumlah larut pepejal, gula, dan profil asid organik, kompaun meruap daripada buah dan puri dibandingkan dengan menggunakan zNose (Ultrafast GC, US). Keputusan Principal Component Analysis (PCA) menunjukkan bahawa terdapat enam sebatian meruap utama menghasilkan jumlah varians yang paling tinggi (92.9 %) untuk mengklasifikasikan profil aroma kepada tiga kumpulan: buah durian belanda, puri dihasilkan daripada pectinase, dan puri cair yang dihasilkan daripada gabungan enzim. Dua kompaun ester, iaitu metil hexanoate dan metil *trans*-2-hexenoate, adalah kompaun meruap utama yang terdapat dalam buah durian belanda.

Untuk mengoptimumkan keadaan penghasilan serbuk durian belanda, Metodologi Response Surface (RSM) telah digunakan untuk mengkaji kesan kandungan selulase (0 – 2 % v/w) pada kandungan pectinase yang tetap (1.5 % v/w), penambahan

maltodekstrin (20 – 40 % w/w) dan suhu pengeringan sembur (130 – 160 °C) terhadap sifat-sifat serbuk durian belanda. Hasil kajian menunjukkan bahawa model polinomial adalah bersignifikan ($p \leq 0.05$) terhadap hasil proses, kandungan kelembapan, aktiviti air, hygroscopicity, dan kelekitan. Walau bagaimanapun, ketumpatan pukal, kepadatan sebenar, keliangan, saiz zarah dan index serapan air tidak bersignifikan ($p > 0.05$) untuk model polinomial. Warna serbuk durian belanda adalah berkrim mungkin disebabkan oleh gabungan puri (krim) dengan maltodekstrin (putih). Suhu transition (T_g) adalah di antara 46.53 dan 58.25 °C, menunjukkan serbuk durian belanda adalah bahan amorfus. Morfologi permukaan serbuk diperiksa menggunakan Mikroskop Imbasan Elektron (SEM) menunjukkan bahawa zarah mempamerkan morfologi umum serbuk amorfus yang bentuk bulat dan mempunyai dinding berterusan dengan ketiadaan permukaan yang retak. Berdasarkan keputusan pengoptimuman, puri yang ditambah dengan 1.3 % (v/w) selulase digabungkan dengan 37 % w/w maltodekstrin dan dikering sembur pada suhu 156 °C dapat menghasil serbuk durian belanda yang mempunyai ciri-ciri fizikokimia optimum.

Pada keadaan pengeringan sembur yang optimum, serbuk durian belanda yang dihasil mempunyai sifat kelekitan apabila disimpan. Oleh itu, kesan penambahan ejen anticaking [fosfat trikalsium (TCP) dan kalsium silikat (CS)] dan kandungan (0 – 1.5 % w/w), dan suhu penyimpanan (penyimpanan konvensional pada 25 ± 1 °C dan penyimpanan memecut pada 38 ± 1 °C) ke atas sifat-sifat serbuk dibungkus dalam aluminium berlapis polietilena (ALP) telah dikaji. Keputusan statistik menunjukkan bahawa penambahan egen anticaking meningkatkan hasil proses secara signifikan ($p \leq 0.05$). Sifat-sifat fizikokimia serbuk dijejaskan secara signifikan ($p \leq 0.05$) dengan penambahan egen anticaking, tempoh dan suhu penyimpanan. Kandungan lembapan kritikal (X_c) adalah di antara 0.069 dan 0.072 g H_2O /g ds. Model kinetik untuk perubahan warna (ΔE) menunjukkan tindak balas degradasi sifar. Pemalar kinetik terendah dicatatkan pada serbuk ditambah dengan 1.5 % w/w CS yang mempunyai tenaga pengaktifan yang paling tinggi (Ea) (17.26 kJ/mol), menunjukkan serbuk ditambahkan dengan CS kurang mengalami perubahan warna. Serbuk ditambah dengan 1.0 % w/w TCP dan 1.5 % w/w CS adalah kandungan optimum untuk menghasilkan serbuk durian belanda dan anggaran jangka hayat.

Jangka hayat serbuk yang dioptimumkan dalam keadaan yang diperolehi di atas dianggarkan berdasarkan isoterma kelembapan penyerapan (MSI) yang ditentukan secara gravimetrik. Keputusan menunjukkan bahawa kelembapan keseimbangan relatif (ERH), jenis egen anticaking, dan suhu penyimpanan mempamerkan kesan signifikan ($p \leq 0.05$) terhadap kandungan kelembapan keseimbangan (EMC) serbuk durian belanda. Model Guggenheim, Anderson, dan de Boer (GAB) telah digunakan untuk menyesuaikan data penyerapan dan menunjukkan bentuk sigmoidal yang mengikuti klasifikasi Brunauer jenis III. Kandungan lembapan monolayer (M_o) serbuk adalah di antara 0.0221 dan 0.0243 g H_2O/g ds. Jangka hayat yang paling lama telah dianggar dalam serbuk ditambah dengan 1.5 % w/w TCP (316 hari). Jangka hayat dianggar menggunakan model kinetik adalah sesuai sebab min relative peratus sisihan modulus adalah kurang daripada 10 % (2.31 - 6.93 %). Ujian hedonik untuk lima sifat deria iaitu aroma, warna, perasaan dalam mulut, rasa, dan penerimaan keseluruhan untuk semua minuman durian belanda menunjukkan skor min lebih daripada enam, menunjukkan penerimaan yang tinggi.

Secara keseluruhan, serbuk kering sembur durian belanda boleh dihasil dengan puri yang mempunyai kelikatan yang rendah dengan menggunakan rawatan enzim. Serbuk durian belanda yang dihasilkan dengan operasi pengeringan sembur mempamerkan sifat umum dan morfologi serbuk amorfus. Penambahan aditif makanan menunjukkan anticaking berkesan dengan mengurangkan penyerapan kelembapan untuk mengelakkan fenomena caking. Kedua-dua ejen anticaking memanjangkan jangka hayat serbuk mungkin dengan membentuk pelindung pada sampel zarah untuk meningkatkan kestabilan serbuk.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Hasanah Mohd Ghazali, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Roselina Karim, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Abdulkarim Sabo Mohammed, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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Name of Member of Supervisory Committee: ______________________________

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iv
ACKNOWLEDGEMENTS		vii
APPROVAL		viii
DECLARATION		x
LIST OF TABLES		xvii
LIST OF FIGURES		xx
LIST OF ABBREVIATIONS		xxiv
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	4
	2.1 Soursop	4
	2.1.1 General	4
	2.1.2 Nutrient composition and nutritional value	5
	2.1.3 Harvest and handling issue	5
	2.1.4 Soursop puree	6
	2.2 Enzymatic extraction	7
	2.2.1 Application of enzyme in food industry	7
	2.2.2 Pectolytic enzyme	9
	2.2.3 Pectinase	10
	2.2.4 Enzymatic maceration and liquefaction process	10
	2.3 Drying	11
	2.3.1 Properties of water in food	12
	2.3.2 Spray drying	13
	2.3.3 Spray drying features and process	14
	2.3.4 Spray drying parameters	15
	2.3.5 Spray drying of fruit powders	16
	2.3.6 Measures to overcome spray drying problem	17
	2.4 Response Surface Methodology (RSM)	18
	2.5 Storage of food powder	20
	2.5.1 Kinetic of quality attributes change and Arrhenius model	20
	2.5.2 Packaging of food powder	22
	2.5.3 Caking of food powder and application of anticaking agent	23
	2.5.4 Shelf life of food powder	23
	2.6 Sorption phenomena and sorption isotherms	25
	2.6.1 General	25
	2.6.2 Mathematical models of sorption isotherm	28

2.7 Sensory attribute of food powder 29

3 APPLICATION OF ENZYMES FOR LIQUEFACTION OF SOURSOP PULP TO PRODUCE PUREE 30

3.1 Introduction 30
3.2 Materials and Methods 31
3.2.1 Materials 31
3.2.2 Liquefaction of soursop fruit with a single enzyme 32
3.2.3 Liquefaction of soursop fruit using combination of two enzymes 33
3.2.4 Analysis of samples 33
A. Proximate composition 33
B. pH and titratable acidity 34
C. Total soluble solid 34
D. Ascorbic acid content 34
E. Color analysis 35
F. Sugar composition and content 35
G. Organic acid composition and content 36
H. Aroma profile 36
I. Viscosity 37
3.2.5 Experimental design and statistical analysis 37
A. One-way ANOVA analysis 37
B. Principal Component Analysis (PCA) 38
3.3 Results and Discussion 38
3.3.1 Proximate composition and physicochemical properties of soursop fruit 38
3.3.2 Properties of soursop puree treated with a single enzyme 42
A. Physicochemical properties of puree from pectinase-treated soursop 44
B. Sugar composition and content 45
C. Organic acid composition and content 48
3.3.3 Properties of soursop puree treated with combination of enzymes 48
A. Viscosity 51
B. Physicochemical properties of liquefied puree treated with combined enzymes treatments 52
C. Sugar composition and content 53
D. Organic acid composition and content 55
3.3.4 Color 55
3.3.5 Aroma profile 58
3.4 Summary and Conclusion 63

4 OPTIMIZATION OF SPRAY DRYING INLET TEMPERATURE, CARRIER AND FEED CONCENTRATION FOR THE PREPARATION OF SOURSOP FRUIT POWDER 65

4.1 Introduction 65
4.2 Materials and Methods 66
4.2.1 Materials 66
4.2.2 Liquefaction of soursop puree with pectinase 66
4.2.3 Preparation and spray drying of spray drying feed 67
4.2.4 Experiment design 68
4.2.5 Analysis of spray-dried soursop powder 69
A. Process yield 69
B. Moisture content 70
C. Water activity (Aw) 70
D. Hygroscopicity 70
E. Stickiness 70
F. Bulk density, true density and porosity 71
G. Particle size 71
H. Water Solubility Index (WSI) and Water Adsorption Index (WAI) 72
I. Glass transition temperature (T_g) 72
J. Surface morphology 72
K. Color 73
4.2.6 Experimental design and statistical analysis 74
A. One-way ANOVA analysis 74
B. Response Surface Methodology (RSM) 74
4.3 Results and Discussion 74
4.3.1 Effects of independent variables on the physicochemical properties of powder 75
A. Process yield 79
B. Moisture content 80
C. Water activity (Aw) 83
D. Hygroscopicity 83
E. Stickiness 85
F. Bulk density (BD), true density (TD) and porosity 88
G. Particle size 91
4.3.2 Optimization procedure 94
4.3.3 Verification of model 95
4.3.4 Effects of other independent variables on soursop powder properties 96
A. Water solubility index (WSI) and water adsorption index (WAI) 96
B. Glass transition temperature (T_g) 98

C. Surface morphology 103
D. Color 107
4.4 Summary and Conclusion 108

5 **EFFECTS OF ANTICAKING AGENTS, STORAGE TIME, TEMPERATURE, AND HUMIDITY ON THE QUALITY OF SPRAY-DRIED SOURSOP POWDER** 110
5.1 Introduction 110
5.2 Materials and Methods 111
5.2.1 Materials 111
5.2.2 Preparation of spray drying feed 112
5.2.3 Packaging and storage of soursop powder 112
5.2.4 Assessment of spray-dried soursop powder 113
A. Yield 114
B. Moisture content 114
C. Water activity (Aw) 114
D. Glass transition temperature (T_g) 115
E. Density 115
F. Color 115
G. Kinetic modeling of total color difference (ΔE) 116
H. Water Solubility Index (WSI) 117
I. Surface morphology 118
5.2.5 Statistical analysis 118
5.3 Results and Discussion 118
5.3.1 Effect of anticaking agents and storage conditions on process yield of soursop powder 118
5.3.2 Effect of anticaking agents and storage conditions on properties of soursop powder 119
A. Moisture content 119
B. Water activity (Aw) 122
C. Glass transition temperature 124
D. Density 127
E. Color 129
E1. Color of spray-dried powder 129
E2. Total color difference (ΔE) 131
E3. Kinetics of color change in spray-dried soursop powder during storage 131
F. Water Solubility Index (WSI) 134
G. Surface morphology 136
5.4 Summary and Conclusion 142

6 SHELF LIFE PREDICTION AND SENSORY QUALITY OF SPRAY-DRIED SOURSOP POWDER PACKED IN ALUMINUM LAMINATED POLYETHYLENE POUCH 144
6.1 Introduction 144
6.2 Materials and Methods 145
6.2.1 Materials 145
6.2.2 Preparation and packaging of spray-dried powder 146
6.2.3 Permeability of packaging material 146
6.2.4 Moisture sorption isotherm (MSI) 147
6.2.5 Guggenheim-Anderson-de Boer (GAB) model 148
6.2.6 Shelf-life prediction 149
6.2.7 Sensory Evaluation 150
6.2.8 Statistical analysis 151
6.3 Results and Discussion 151
6.3.1 Permeability of ALP pouch 151
6.3.2 Moisture sorption characteristic of spray-dried soursop powder 153
6.3.3 Shelf life prediction 157
6.3.4 Validation of model 159
6.3.5 Sensory quality of soursop powder 160
6.4 Summary and Conclusion 162

7 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 163
7.1 Summary and Conclusion 163
7.2 Recommendation for future research 165

REFERENCES 166
APPENDICES 186
BIODATA OF STUDENT 219
LIST OF PUBLICATIONS 220

LIST OF TABLES

Table		Page
2.1	Physicochemical properties of soursop fruit and puree	7
2.2	Application of enzyme in fruit and beverages industry	8
2.3	Conditions and applications of enzyme in fruits and vegetables	11
2.4	Water activity range and water binding relationship	12
2.5	Conditions used to produce different powders	16
2.6	Spray drying variables studied in different samples	17
2.7	Application of RSM in spray drying of powders	19
2.8	Quality parameters and kinetic order of different types of food powders	21
2.9	Application of Accelerated Shelf Life Testing (ASLT) in food powder	24
2.10	Types of adsorption isotherm for different food materials	27
2.11	Water activity exhibited by saturated salt solutions	27
3.1	Coded number of soursop fruit and soursop puree from different enzyme treatments used in zNose analysis	37
3.2	Physical composition of soursop fruit	38
3.3	Proximate composition of soursop fruits	39
3.4	Physicochemical properties of soursop fruit	40
3.5	Physicochemical properties of soursop puree produced at different concentrations of pectinase	44
3.6	Physicochemical properties of soursop puree produced using combined enzyme treatments	52
3.7	Sugar content of liquefied soursop puree produced using combined enzyme treatments	53
3.8	Organic acid content of liquefied soursop puree produced using combined enzyme treatments	57
3.9	Color of soursop fruit and soursop puree at different concentrations of enzymes	58

4.1	Independent variables designed from central composite design (CCD)	68
4.2	Matrix of the central composite design (CCD)	69
4.3	Responses on properties of soursop fruit powder for twenty runs of experimental design	76
4.4	Regression coefficient, coefficient of determination (R^2), R^2 (adj), F and p-value, and lack-of-fit test value for independent variables from response surface design analysis (based on Minitab ver. 16.1.0)	77
4.5	p-value and F-value for independent variables in the polynomial response surface models (Minitab 16.1.0)	78
4.6	Experimental data for bulk density, true density, porosity, and particle size responses	89
4.7	RSM analysis of the dependent variables	90
4.8	p-value and T-value for response variables 2 sample t-test	95
4.9	Comparison of experimental and predicted value of optimized spray-dried soursop powder	96
4.10	Effect of independent variables on WSI and WAI of spray-dried soursop powder	97
4.11	Effect of independent variables on color parameter of spray-dried powder	107
5.1	Lag time, level of increase, and critical moisture content (X_c) in moisture content of soursop powder stored at RT and ET at the end of storage	121
5.2	Glass transition temperature (T_g) for samples obtained from different treatments on Day 0 and percent decrease (%) at the end of the storage period	126
5.3	Density (g/cm^3) for spray-dried soursop powder from different treatments over storage day at RT	128
5.4	Density (g/cm^3) for spray-dried soursop powder from different treatments over storage day at ET	128
5.5	Kinetic parameters of soursop powder stored at RT and ET by fitting into zero and first order degradation equation	133
5.6	Activation energy (Ea) of soursop powder obtained from different treatments	134

6.1	List of salt solutions used to generate equilibrium relative humidity (ERH) and equivalent water activity (Aw)	147
6.2	GAB constant (M_o, C_g, and K_g), E, SSR and R^2 values for soursop powder stored under RT and ET	156
6.3	Parameters used for shelf life determination and calculated shelf life	158
6.4	Mean score (%) for sensory attributes of the reconstituted soursop drink	161

LIST OF FIGURES

Figure		Page
1	Soursop fruit	1
2.1	Soursop fruit with stalk, skin with thorns, cottony flesh, and seeds	4
2.2	Deteriorated fruits caused by poor handling procedure	6
2.3	Different pectolytic enzymes and the reaction sites	9
2.4	Pectin structure	10
2.5	Rate of chemical and biochemical reaction across water activity	13
2.6	Schematic diagram of single stage spray drying system	14
2.7	Sorption isotherms of food product	25
2.8	Types of adsorption isotherms	26
3.1	Sample preparation (a) Soursop fruit (b) Skin removal and (c) Vacuum-packed fruit	31
3.2	Preparation of soursop fruit sample	32
3.3	Preparation of soursop puree	33
3.4	Sugar profiles of soursop fruit and standard sugars	40
3.5	Organic acid profiles of raw soursop fruit	41
3.6	Liquefaction effects of different enzymes on soursop fruit after incubation at 50 ºC for 120 minutes	43
3.7	Sugar profile of soursop puree after liquefaction with pectinase	46
3.8	HPLC profiles of glycerol and glycerol-spiked puree sample	47
3.9	Sugar content of soursop puree produced at different concentrations of pectinase	47
3.10	Organic acid profile of soursop puree following liquefaction with pectinase	49
3.11	Organic acid content of soursop puree produced from different concentrations of pectinase	50
3.12	Liquefaction effects of combination enzymes on soursop fruit after incubation at 50 ºC for 120 minutes	50

3.13	Viscosity of puree produced using combined enzyme treatments	51
3.14	Sugar profile of soursop puree produced using combined enzyme treatments	54
3.15	Organic acid profile of liquefied puree at different concentrations of pectinase and cellulase	56
3.16	Aroma profiles of raw soursop fruit	59
3.17	Aroma profiles of pure methyl hexanoate and methyl *trans*-2-hexenoate	60
3.18	Aroma profile of soursop puree treated with pectinase	61
3.19	Loading plot of all 18 variables (A-R)	62
3.20	Scree plot of all variables	62
3.21	Score plot of six important variables for all samples	63
4.1	Spray dryer Mini Büchi B-290	67
4.2	Sample preparation for SEM analysis	73
4.3	Response surface plot (3D) of the interaction effect of cellulase concentration with maltodextrin concentration or inlet temperature	82
4.4	Interaction effect of maltodextrin with inlet temperature on water activity of powder at holding concentration of cellulase 1 % (v/w)	84
4.5	Interaction effect of cellulase concentration with maltodextrin on hygroscopicity of powder at holding inlet temperature of 145 ºC	85
4.6	Stickiness plot of soursop powder at different cellulase concentration at fixed maltodextrin level (30 %) and inlet temperature (145 °C)	86
4.7	Stickiness plot of soursop powder at different maltodextrin level at fixed cellulase concentration (1 %) and inlet temperature (145 °C)	87
4.8	Stickiness plot of soursop powder at different inlet temperature at fixed cellulase concentration (1 %) and maltodextrin level (30 %)	88
4.9	Interaction effect of maltodextrin with inlet temperature on stickiness of powder at holding cellulase concentration of 1% (v/w)	88
4.10	Regression plot of correlation	91

4.11	Unimodal distribution of soursop particles	92
4.12	Effect of independent variables on particle size of soursop powder	93
4.13	Regression plot of correlation between bulk density and particle size of soursop powder	94
4.14	Multiple optimization responses plot	95
4.15	Surface plots of interaction effect on WSI of soursop powder	99
4.16	Thermogram of soursop powder produced at different concentrations of cellulase at fixed maltodextrin level (30 %) and inlet temperature (145 °C)	100
4.17	Thermogram of soursop powder produced at different maltodextrin levels at fixed cellulase concentration (1 %) and inlet temperature (145 °C)	101
4.18	Thermogram of soursop powder produced at different inlet temperatures at fixed cellulase concentration (1 %) and maltodextrin level (30 %)	102
4.19	Thermogram of soursop powder produced under optimized condition	103
4.20	Scanning electron micrographs of soursop powder at different magnifications produced from different concentrations of cellulase at fixed maltodextrin level (30 %) and inlet temperature (145 °C)	104
4.21	Scanning electron micrographs of soursop powder at different magnifications produced from different maltodextrin levels at fixed cellulase concentration (1 %) and inlet temperature (145 °C)	105
4.22	Scanning electron micrographs of soursop powder at different magnifications produced from different inlet temperatures at fixed cellulase concentration (1 %) and maltodextrin level (30 %)	106
4.23	Scanning electron micrographs of soursop powder produced under optimized condition	106
5.1	Packaging and storage of powder packed in ALP pouch	112
5.2	A wooden stick was used to hold the pouches vertically at 15 mm interval space	113
5.3	Pouches stored in a desiccator maintained at 93 ± 2 % RH at room temperature, RT (25 ± 1 ºC) and elevated temperature, ET (38 ± 1 ºC)	114

5.4	Gas Pycnometer (AccuPyc II 1340) for measurement of true density	116
5.5	Process yield of soursop powder from different treatments	119
5.6	Moisture content of soursop fruit powder obtained from different treatments at different storage temperatures	120
5.7	Water activity (Aw) of soursop powder from different treatments and stored at different temperatures	123
5.8	A typical DSC thermogram of control sample at Day 0	124
5.9	Glass transition temperatures (T_g) of soursop fruit powder obtained from different treatments	125
5.10	Color of spray-dried soursop powder from different treatments over storage period	130
5.11	Total color difference (ΔE) for soursop powder obtained from different treatments and stored at different temperatures	132
5.12	WSI (%) of soursop fruit powder obtained from different treatments and stored at different temperatures	135
5.13	Scanning electron micrographs of soursop powder obtained from different treatment (A to E) and stored at RT on Day 0 (1), 49 (2), and 91 (3) at magnification level of 2000x	138
5.14	Scanning electron micrographs of soursop powder obtained from different treatment (A to E) and stored at ET on Day 0 (1), 49 (2), and 91 (3) at magnification level of 2000x	140
6.1	Samples placed in different controlled RH environments generated from different saturated salt solutions	148
6.2	Sensory evaluation	151
6.3	Weight gained by silica gel (kg) packaged in ALP pouches over storage day	152
6.4	Experimental equilibrium moisture content (EMC) of soursop powder. GAB denotes Guggenheim-Anderson-de Boer model trendline	154
6.5	Relative percent deviation modulus, E (%) of soursop powder	160
6.6	Relationship of experiment moisture content (Exp) and calculated values (Pred) over storage days	160

LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
°	Degree
° C	Degree celcius
° Brix	Degree Brix
%	Percentage
θ	Day
∞	Infinity
λ	Lambda
±	Plus minus
+	Positive
-	Negative
~	Approximate
ΔE	Color change
ΔC_p	Heat capacity changes
µg	Microgram
µL	Microliter
µM	Micromole
µm	Micrometer
α	Alpha
ABTS	3-ethylbenzothiazoline-6-sulphonic acid
ALP	Aluminum laminated polyethylene
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASLT	Accelerated shelf life testing

Aw	Water activity
BD	Bulk density
BET	Brunauer-Emmett-Teller
BOPP	Biaxially oriented polypropylene
BOPP/MCPP	Metallized co-extruded biaxially oriented polypropylene
C	Chroma
CCD	Central Composite Design
C_g	Monolayer adsorption constant
cm	Centimeter
cm^3	Centimeter cube
Cp	Centipoise
CO_2	Carbon dioxide
d	Diameter
DE	Dextrose Equivalency
db	Dry basis
ds	Dry solid
DSC	Differential Scanning Calorimetry
E	Mean relative percent derivation modulus
Ea	Activation energy
e.g.	Example gratia (for example)
EMC	Equilibrium moisture content
Eq.	Equation
ERH	Equilibrium relative humidity
et al.	And others
FAO	Food and Agriculture Organization
FAU-F	Fungal Alpha-Amylase unit
FDA	Food and Drug Administration

FW	Fresh weight
g	Gram
GAB	Guggenheim-Anderson-de Boer
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrophotometry
h	Height
hr	Hour
H	Hydrogen
H °	Hue angle
H_m	Monolayer molar sorption enthalpies
H_n	Multilayer molar sorption enthalpies
H_1	Bulk liquid molar sorption enthalpies
HDPP	High-density polypropylene
HPLC	High Performance Liquid Chromatography
HP-SPME	Headspace Solid-Phase Microextraction
i.e.	Id est (that is)
IU	International Unit
IUB	International Union of Biochemistry
IUPAC	Internaltion Union of Pure and Applied Chemistry
k	Kinetic constant
K	Kelvin
K_g	Multilayer adsorption constant
Kp	Permeability of packaging
kcal	Kilocalories
kg	Kilogram
kJ	Kilojoule
kV	Kilovolt

l	Length
L	Liter
LCMS	Liquid Chromatography-Mass Spectrometry
LLDPE	Linear low density polyethylene
m	Meter
M_0	Monolayer moisture content
m^2	Meter square
m^3	Meter cube
mg	Milligram
min	Minute
mL	Milliliter
mol	Mole
mm	Millimeter
MSI	Moisture sorption isotherm
MW	Molecular weight
n	Reaction order
N	Nitrogen
NA	No available information
ND	Not detected
nm	Nanometer
No.	Number
O	Oxide
p	Probability
Pa	Pascal
PCA	Principal Component Analysis
PET	Polyethylene terephthalate
PGNU	Polygalacturonase unit

PP	Polypropylene
ppm	Part per million
psig	Per square inch gauge
PTFE	Polytetrafluoroethylene
Q	Heat of adsorption
QDA	Quantitative Descriptive Analysis
R	Universal gas constant
R^2	Determination coefficient
R^2 adj	Adjusted R-square
RE	Relative error
RH	Relative humidity
RID	Refractive Index Detector
rpm	Rate per minute
RSEX	Reflectance Specular-Excluded
RSM	Response Surface Methodology
s	Second
SAW	Surface Acoustic Wave
SD	Standard deviation
SEM	Scanning Electron Microscopy
SSR	Sum of square residual
t	Time
T	Temperature
TA	Titratable acidity
TD	True density
TEs	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
T_g	Glass transition temperature

TS	Total solid
$T_{surface}$	Temperatures of particles surfaces
TSS	Total soluble solid
USDA	United State Department of Agriculture
UV	Ultraviolet
v	Volume
w	Weight
wd	Width
WAI	Water adsorption index
wb	Wet basis
W_s	Dry weight
WSI	Water solubility index
x	Times
X_c	Critical moisture content
X_i	Initial moisture content

CHAPTER 1

INTRODUCTION

Annona muricata L. is commonly known as soursop in English and *durian belanda* in Malay. It is fairly a large fruit with a generally heart-shaped to oval with dark green, rough and thin skin (Figure 1). The flesh is a white cottony pulp with soft pithy core connecting the juicy pulp (Nakasone and Paull, 1998). In Malaysia, the production of soursop fruit has increased from 887.4 metric tonnes in 2011 to 1483.7 metric tonnes in 2015 (Fruit Crops Statistic, 2015). In recent years, soursop has received considerable attention from the public because of the nutritional and health protective values of this fruit. Numerous studies have been done concerning the nutritional and health-benefit properties. Umme *et al.* (1997) reported that a hundred gram of soursop contains 20.9 mg of ascorbic acid and 7.59 g/ of sugar which indicates soursop is nutritionally high in carbohydrates and contains significant amounts of vitamins. Soursop also has many therapeutic properties such as antibacterial, anticancer, sedative, and other properties (Asprey and Thornton, 1995). Soursop fruit is recognized as one of the plant based therapies in United States National Center for Complementary and Alternative Medicine (NCCAM). A study by Diaz *et al.* (2012) showed 40.5 % of breast cancer patients out of 149 respondents agreed that soursop radically improved their health status. High antioxidant content (6.09 ± 0.13 µM TEAC/g) in soursop fruit is able to reduce the risk of heart diseases and prevent cardiovascular diseases (De Souza *et al.*, 2012b). This suggests soursop can be processed and incorporated into human diet in order to improve the nutritional status of consumer.

Figure 1: Soursop fruit

One of the apparent features of soursop fruit is it softens and bruises easily, and releases an unpleasant strong smell when ripe (Janick and Paull, 2008; Allen, 1967). These are associated with a rapid respiration rate of soursop fruit that has a double sigmoidal growth pattern (two maximum respiratory), making it highly susceptible to physical damages, thus soursop is very perishable and has a short shelf life (Worrell *et al.*, 1994). Hence, raw soursop should be processed into intermediate products such as puree, which can then be value-added into commercial products such as jam, jelly, sweets and beverages (De Souza *et al.*, 2012a; Gratão *et al.*, 2007; Umme *et al.*, 1999). Soursop puree is usually prepared by blending fruit pulp (without seed) with distilled water at a ratio of 1:2 (Onyechi *et al.*, 2012; Abbo *et al.*, 2006; Umme *et al.*, 1997). However, excessive energy is required to remove water during further processing such as drying operation to produce powder. Thus, incorporation of enzymatic method has become an important and essential part of processes used in the modern food industries because water is often not incorporated either during puree or juice production.

In the food processing industry, enzymatic mash treatment is a well-known modern process to obtain higher yield of juice extraction, increase press capacity, improve clarification and liquefaction, and reduce viscosity (Cerreti *et al.*, 2016; Lima *et al.*, 2015; Sagu *et al.*, 2014; Schols *et al.*, 2009). Food grade pectinase hydrolyses pectin and produced low viscosity juice that brings extra advantages for the subsequent filtration process (Croak and Corredig, 2006; Demir *et al.*, 2001). The addition of another cell wall degrading enzyme, cellulase, enhances the breakdown of cell wall matrix that is composed of cellulose and hemicellulose, thus releasing more juice. For optimum liquefaction of fruit pulp to produce low-viscosity puree, preliminary studies must always be conducted to determine the optimal conditions (contact time, concentration of enzyme, temperature of incubation and pH) for reaction which depends on the degree of liquefaction required and the type of fruits (Landbo and Meyer, 2004).

Spray drying is a commercial and well known drying technique used to transform liquid raw material into a solid product (Bhandari *et al.*, 2008). This operation is favorable because the outcome of spray drying process is fine particles with free flowing properties. Spray drying has been widely used in the production of fruit powders (Patil *et al.*, 2014; Ferrari *et al.*, 2012; Chin *et al.*, 2010). In order to obtain spray-dried products with better characteristics and process yield, it is important to optimize the drying process. Response Surface Methodology (RSM) is applied as a statistical method to design the experiment and optimize responses (Myers and Montgomery, 1995). In addition, an anticaking agent such as tricalcium phosphate or calcium silicate is often incorporated into the powders which are then packed in suitable packing materials such as aluminum laminated polyethylene (ALP) in order to further improve the stability of the powder.

In this study, soursop puree was produced from soursop pulp with the aid of enzymes in order for liquefaction to take place. The most effective enzyme in the preparation of soursop puree which can be used as a base feed of spray drying process was then selected. For effective powder production, the effects of maltodextrin concentration and spray drying inlet temperatures on powder production were examined by the RSM experiment design. The effect of packing the powder in heat-sealed aluminum laminated polyethylene (ALP) pouch on storage stability was also evaluated. In addition, the effects of types and concentrations of anticaking agents, and storage time and temperature were investigated and kinetic modeling was determined. The powder which showed the best storage stability was selected to predict the potential shelf life. Thus, the specific objectives of this study were:

1. To prepare soursop puree that is suitable to be used as spray drying feed through enzymatic liquefaction of soursop pulp.
2. To investigate the effect of concentration of cellulase, maltodextrin level, and inlet temperature on process yield and physicochemical properties of the spray-dried soursop powder.
3. To optimize spray drying inlet temperature, carrier and feed concentration for the preparation of soursop fruit powder.
4. To investigate the effects of anticaking agents, storage time, and temperature on process yield and physicochemical properties of spray-dried soursop powder.
5. To predict the shelf life and sensory quality of spray-dried soursop powder packed in aluminum laminated polyethylene pouch using an accelerated shelf life testing (ASLT) approach.

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